

# ENCYCLOPEDIA OF GRAIN SCIENCE

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# **FOREWORD**

G rains are the staff of life. Mindful of this, it is impossible to contemplate a world devoid of grain. The efficient production and processing of grain are critical activities that are sustained by science. Indeed, it has been said that efficient technology is essentially the science that works.

Grain science is the driving force behind the efficient use of grain. The beginnings of grain science are lost in antiquity. However, the research literature and the practices used for processing all the grains of the world are wide-ranging, disparate, and complex. The transfer of scientific knowledge that relates to food raw materials is essential and important for the improvement of world health. In order to collate and present current knowledge and the future potential of grain science to the world, a group of distinguished experts were assembled to produce this *Encyclopedia of Grain Science*.

This remarkable and unique work of science and scholarship comes in three volumes and covers all aspects of grain science. It is always important that scientific information is presented in a logical and reader-friendly manner. The *Encyclopedia of Grain Science* is beautifully produced and easy to read. Related chapters are cross-referenced and the authors have presented the science and associated technology of their chapters in a manner that is accurate, informative, and clear.

It is difficult to predict how often this encyclopedia will be consulted for ideas, information, and clarification of any aspect of the production, processing, and use of grains worldwide. However, with regard to a complex and wide-ranging subject such as grain science, a readily available single source of information is an essential requirement for all those who produce and process grains in industries such as food-making, farming, animal-feeding, grain-breeding, baking, brewing, distilling, and marketing. This book is a uniquely important source of reference for teachers and academics involved in teaching, and research and development of grain science and technology. Students, consultants, politicians, historians, and the general public will find this publication useful as regards the accessing of information on grain that would require an extensive search of literature that is not readily available. Those involved in related sciences and industries should regard this book as a valuable source of comparative information on biotechnology and processing, especially as regards foods, drinks, and beverages. The reference section of each chapter is designed to direct users to important articles that will elaborate further on the science, technology, and methodology of summarized information.

Having had the privilege to be involved in grain science and technology for a long time, I have been associated with many books that deal with grain science. The *Encyclopedia of Grain Science*, without doubt, will take its place as one of the great books of the world of grain science. It is written by experts dedicated to the important aim of improving grain quality and availability in a world that regards grains as its primary food source.

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# **PREFACE**

G rains have always been critical to mankind's existence. The word "bread," the most obvious product of grains, has become synonymous with food, as indicated by the motto of the United Nation's Food and Agriculture Organization: "Fiat panis," meaning "Let there be bread." Primitive humans relied on grain found wherever it grew until the dramatic discovery that some of this grain, if planted and nurtured, would produce a crop that could be harvested and stored to sustain the family from one harvest to the next. The cultivation of grains was thus the critical development that changed human from the hunter–gatherer nomad into the settled agriculturalist, leading in turn to opportunities for cultural activities. The accompanying breakthroughs were the discoveries of tools and processes to make the harvested grains more tasty and nutritious, namely, the processes of crushing and sieving, and of mixing the resulting flour with water and baking the dough to make primitive forms of bread or porridge.

Since those early days, there have been great improvements in all aspects of grain science and technology. Today, a wide range of grain species is exploited in all countries of the world. These include the cereal grains, the legumes, and the oilseeds. Their breeding, production, transport, and processing are very big business worldwide, irrespective of whether the country is a net importer or exporter. Although much of world grain production is consumed close to the site of production, large excesses of production over consumption in several regions mean that world trade in grains is also very big business. Methods of processing in today's world cover the full range from traditional methods, handed down through many generations, to very sophisticated large-scale factories. Throughout this diversity of approaches to grain utilization, grains supply a great proportion of the world's food – both as energy and protein sources. Grains also make a major contribution to our diet via grain-based feedstuffs for animals. A significant proportion of grain production goes for industrial uses, thereby contributing to the wider range of our foods (e.g., margarine, sweeteners in drinks, and protein supplements in meats and in synthetic milk drinks), as well as entering an amazing diversity of non-food applications (e.g., adhesives, plastics and paper products).

Our main aim, in compiling this encyclopedia, was to cover everything in the complex range of topics that a true *Encyclopedia of Grain Science* should offer. This diversity is three dimensional:

- 1. One axis covers the wide range of grain species, especially those of economic value to mankind and his general environment. These include the cereal grains, the oilseeds, the pulses (grain legumes), the soybean (which is both a pulse and an oilseed), the pseudocereals, as well as amaranth and quinoa.
- 2. The second dimension covers the sequence of events that is common to all grains, namely, breeding and selection, the production of seed for sowing and the grain for harvest, harvesting, storage, transport and marketing of the grain, and finally processing of the grain to produce food and feed products.
- 3. A third axis also considered involves the diversity of scientific disciplines used to investigate the questions arising from the study of grains at all stages of their production and utilization.

By the use of a comprehensive review process and the careful selection of authors, every effort has been made by the Editors and the distinguished Editorial Advisory Board to ensure that the *Encyclopedia of Grain Science* covers this wide diversity of topics and is accurate, readable, and up-to-date. The work is also extensively cross-referenced and indexed to ensure that the reader is able to easily locate information as needed.

Readers are invited to enjoy using this encyclopedia. We hope that they be rewarded by the discovery of valuable information, authoritative answers to perplexing questions, and pictures – in words and in actual illustrations – pictures that take the reader to the ends of the Earth, or maybe just down the street.

So, bite into a slice of freshly baked bread, spread with canola margarine or peanut butter, washed down with barley-brewed beer and consider that in this encyclopedia all the known answers are provided to tell the story of how these appetizing foods reach your dinner table.

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# **AMARANTH**

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## Introduction

Amaranth is a rediscovered "new" crop. It was an ancient crop that was under cultivation 5000-7000 years ago as a staple food of the ancient Aztecs. Amaranth was long forgotten partially because it was prohibited for food use due to religious reasons after the Spanish conquest of the New World. Nowadays it is still cultivated as a minor crop in Central and South America and some areas of Asia and Africa. Since the late 1970s, it has been consistently observed that amaranth possesses characteristics of fast growth rate, good tolerance to stress (drought, salinity, alkalinity, acidic, or poor soil), and high potential for biomass and grain yield. Amaranth is a pseudocereal crop and produces cereal-like grains that contain high levels of nutritionally favorable protein, unusual quality of starch, and high-quality oil (including squalene). Amaranth grains can be used for foods and its vegetative parts can be used for animal feed (e.g., forage, silage) or green manure. The red-colored vegetative tissue produces high levels of betacyanin pigments that can be used as natural food colorants. Amaranth has been attracting worldwide attention as a highpotential new crop with multiple uses.

# **Amaranth Production and Development**

Although amaranth has long been cultivated as a minor crop in Central and South America and some areas of Asia and Africa, its production has rapidly expanded since the early 1980s, particularly in developing countries. In many countries — especially in Asia and Africa (e.g., China, India, Nepal, Ethiopia, and Kenya) — introduction, cultivation, and development of amaranth have been

undertaken. Since 1982, the Chinese Academy of Agricultural Sciences has introduced hundreds of amaranth lines/varieties from the United States and other countries into China. Some selected superior varieties (e.g., R104 and K112) have been successfully grown in over 20 provinces. Amaranth has attained great popularity in China for use in feed and food and for other purposes. Current rapid mass expansion of amaranth is mainly for use as a feed crop for animal production. Amaranth production in China has now reached  $\sim 300\,000\,\mathrm{ha}$  annually, ranking as the top amaranth producer in the world. The amaranth production in Russia is estimated to be more than 100 000 ha, and some significant production in India and Mexico is expected as well. Despite the fact that in China and Russia there is larger-scale cultivation of amaranth mainly for feed use, there is still appreciable commercial cultivation of amaranth for human nutrition in Mexico, South American countries, China, USA, Poland, Austria, etc.

There is considerable variation in amaranth yield per hectare in different countries. In China, depending on environmental conditions and cultivation systems, field production may yield  $\sim\!2200-5500\,\mathrm{kg}\,\mathrm{ha}^{-1}$  of amaranth grain, and  $\sim\!90\,000-180\,000\,\mathrm{kg}\,\mathrm{ha}^{-1}$  of fresh plants (silage). During cultivation trials,  $\sim\!3000\,\mathrm{kg}\,\mathrm{ha}^{-1}$  is achieved with good agronomic practice and good seed sources, and even a maximum yield of  $4000\,\mathrm{kg}\,\mathrm{ha}^{-1}$  has been obtained in Montana, USA, and  $6000\,\mathrm{kg}\,\mathrm{ha}^{-1}$  in Peru. There was an average yield of  $2250\,\mathrm{kg}\,\mathrm{ha}^{-1}$  in the northwest hills of India and  $2000\,\mathrm{kg}\,\mathrm{ha}^{-1}$  in Austria.

In the USA, extensive research work on amaranth has been conducted, especially under an amaranth research program at the Rodale Research Center, which started in 1976 and lasted for 14 years. Richest genetic resources of *Amaranthus* from many countries and regions in the world were collected and introduced. Special breeding varieties and different products have been developed, which are sometimes sold in health food shops or supermarkets. Although amaranth cultivation in the USA ranges from the Great Plains to the Rocky Mountains in Colorado, production is very limited, with perhaps only a few hundred

acres planted. Notably, through collaboration of scientists from all over the world, initiated by this center, amaranth development has been stimulated worldwide.

Research and development of grain amaranth in China, amaranth biology, chemistry, and processing, and other technology have been summarized and reviewed. A series of studies on *Amaranthus* have been carried out recently at The University of Hong Kong. These references and researches demonstrate the unusual properties of *Amaranthus* starch, protein, oil, and pigments, and reveal their potential for use as food, being helpful to enhance future development and utilization of amaranth.

# Biological and Agronomic Characteristics of Amaranth

Amaranth, a pseudocereal crop, is a dicotyledonous C4 plant belonging to the genus *Amaranthus* (which consists of some 75 species) of the family Amaranthaceae. There are two major types of amaranth: grain amaranth (e.g., *A. cruentus* L., *A. caudatus* L., *A. hybridus* L., and *A. hypochondriacus* L.) and vegetable amaranth (e.g., *A. tricolor* L., *A. dubius* L., and *A. lividus* L.). Grain amaranth is believed to originate from Central and South America, whereas the main vegetable types are believed to originate from Southeast Asia.

Amaranthus plants usually prefer hot, bright sunlight and are distributed from the tropics through the semiarid regions. Cultivated species can grow in the tropics, subtropics, and temperate zones, and grow well even in some cold areas such as Nepal and Chinese Tibet. Amaranth possesses high yield potential and high stress tolerance to drought, salinity, alkalinity, or acidic soil conditions. It typically gives a grain yield of 2250–4500 kg ha<sup>-1</sup> and a fresh leaf-and-stem weight of 30 000–60 000 kg ha<sup>-1</sup>.

The amaranth plant varies from branched to unbranched, prostrate to erect, and dwarf to over 4 m in height. The leaves are normally elliptical, with an acute tip and a cuneate base; leaf size varies significantly between and within species. The flowers are indefinite inflorescences. Amaranth seed morphology is unlike common true cereal grains. Seedcoat color ranges among black, brown, yellow, and white. Seed embryo is campylotropous, i.e., circular, with the ends nearly touching and enclosing the perisperm (Figure 1). The seeds are extremely small, with 0.5-1.2 g per 1000 seeds or 850-1700 seeds per gram (30-70) times smaller than a typical wheat grain). A single plant can produce more than 500 g of grains. However, in actual field production, seed

yield of amaranth varies greatly (15-50 g per plant), depending on the varieties used and soil fertility conditions.

# Composition of Amaranth Grains and Vegetative Tissues

#### **Grains**

Amaranth is one of the few multipurpose plants that can supply grains and leafy vegetables of high nutritional quality for use as food and feed. Amaranth grain is characterized by a relatively higher content of protein, higher lipid content, and lower starch content than those of the major cereals. A comparison of the average chemical compositions between amaranth (*A. hypochondriacus*) and main cereals (maize, rice, and wheat) is shown in **Table 1**. **Table 2** shows the proximate chemical compositions of the grains of several *Amaranthus* species, indicating that there is some variation within an overall pattern among and within species.

Total dietary fiber content (soluble and insoluble) in amaranth grains from A. caudatus, A. cruentus, and A. hypochondriacus ranges between 7.1% and 16.4%. The nonstarch polysaccharides make up  $\sim 6-7\%$  in the light-colored or dark grains. The content of various mineral elements and vitamins in amaranth grains from major species was analyzed. The levels of P, Ca, K, and Mg are usually higher than those found in common cereal grains. The P/Ca ratio varies from 1.9 to 2.6. It is of nutritional interest to find that amaranth grains contain relatively higher level of iron than that in cereal grains. Amaranth grains are relatively high in tocopherol (546 ppm), riboflavin, etc.

#### Leaves

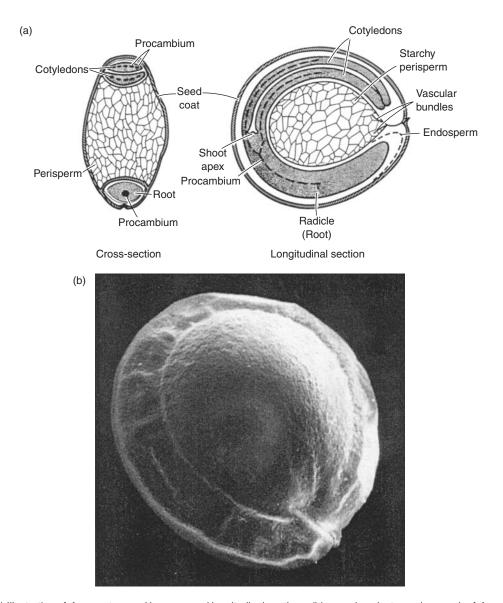
Amaranth leaves contain 27.8–48.6% dry matter as crude protein and, on average, have somewhat more protein than spinach, as shown in Table 3. The fat content ranges from 2.9% to 7.1% and contains linoleic acid as the major concentration of unsaturated fatty acid (40-55%) and palmitic acid as saturated fatty acid (18–25%). Amaranth leaves and stems are a very good source of fiber (11.1–22.9%). Minerals – such as P, Fe, Mg, and Ca - also exist in high concentrations, giving a total of 33.1-40.7% of ash content. Vitamins C and A are in concentrations of nutritionally significant levels, averaging 420 and 250 ppm, respectively. Seedlings, leaves, and stems of amaranth extensively save as tasty vegetables and animal feeds in the numerous places of the world, especially in China, India, and Southeast Asia. The protein isolated from leaves of *Amaranthus* 

species is rich in lysine (6.2-6.8%), higher than that of alfalfa, cabbage, and maize. It also has relatively good amounts of other amino acids. The edible and nutritional quality of vegetable amaranth is almost equal to spinach.

In addition, recently it has been found that redcolored vegetative tissues (seedlings, leaves, inflorescences, etc.) of *Amaranthus* species contain high level of betalain pigments as natural colorants. There has also been much information on other compounds in amaranth (tannin, phytic acid, oxalate, saponins, nitrates, trypsin inhibitor, etc.) with antiphysiological activity in animal performance. Tannins are of importance from the nutritional point of view, since they may influence protein digestibility and reduce mineral bioavailability. Tannins in different amaranth species range between 0.1% and 0.25% and seem to be low enough not to have nutritional impact. The phytate content (0.34-0.61%) in amaranth is higher than that found in rice (0.1-0.14%), but lower than that detected in maize and wheat.

# **Amaranth Starch**

Starch is the main component of amaranth grain, composing ~48-69% of its total dry weight. Starch generally consists of straight-chain and branched-chain molecules, i.e., amylose and amylopectin.



**Figure 1** (a) Illustration of *A. cruentus* seed in cross- and longitudinal sections; (b) scanning electron micrograph of *A. cruentus* seed. (Adapted from Irving DW, Betschart AA, and Saunders RM (1981) Morphological studies on *Amaranthus cruentus*. *Journal of Food Science* 46(4): 1170 and Becker R (1994) Amaranth oil: composition, processing, and nutritional qualities. In: Paredes-López O (ed.) *Amaranth: Biology, Chemistry, and Technology*, p. 135.)

Table 1 Comparison of proximate composition between amaranth grains and some cereals<sup>a</sup>

Composition	Amaranth <sup>b</sup>	Wheat	Corn	Rice
Carbohydrate	59.2	66.9	67.7	75.4
Crude protein	16.6 <sup>c</sup>	14.0 <sup>d</sup>	10.3 <sup>e</sup>	8.5 <sup>e</sup>
Fat .	7.2	2.1	4.5	2.1
Crude fiber	4.1	2.6	2.3	0.9
Ash	3.3	1.9	1.4	1.4
Moisture	9.6	12.5	13.8	11.7

<sup>&</sup>lt;sup>a</sup>Percentage on dry weight basis; data from Saunders RM and Becker R (1984) Amaranthus: a potential food and feed resource. In: Pomeranz Y (ed.) Advances in Cereal Science and Technology, vol. 6, pp. 357-396. St. Paul, MN: American Association of Cereal Chemists; Singhal RS and Kulkarni PR (1988) Composition of the seeds of some Amaranthus species. Journal of the Science of Food and Agriculture 42(4): 325-331; Yue SX, Sun HL, and Tang DF (1993) The Research and Development of Grain and Amaranth in China (in Chinese). Beijing: Chinese Agricultural Science and Technology Press; and Segura-Nieto M, Barba de la Rosa AP, and Paredes-López O (1994) Biochemistry of amaranth proteins. In: Paredes-López O (ed.) Amaranth: Biology, Chemistry, and Technology, pp. 75-106. Boca Raton, FL: CRC Press.

Table 4 shows that amylose makes up from 0% (waxy) to 34.3% of total starch in amaranth grain with the balance being the amylopectin, showing that there is considerable variation in amylose/amylopectin ratio among and within the Amaranthus species. Light-colored grains normally contain more starch (mean 69%) than dark-colored grains. Starches isolated from A. hypochondriacus genotypes contain the lowest amount of amylose (7.8%), whereas A. retroflexus genotypes have the highest amylose (34.3%). The average amylose content of 124 genotypes is 19.2%.

Amaranth starch granules come in polygonal, lenticular, circular, and elliptical shapes. Amaranth starch granules are extremely small, ranging from 0.8 to 2.5 µm in diameter. Comparatively, diameters of the granules in commercial starch range from 3-8 µm for rice starch to 15-100 µm for potato starch. Amaranth starch granule size and its size distribution are characteristics that influence the functional properties of starch.

**Table 2** Chemical composition of amaranth grains from various species<sup>a</sup>

Component	A. caudatus	A. cruentus	A. hybridus	A. hypochondriacus
Carbohydrate	59.6–62.8	60.7–62.6	58.6	57.0
Crude protein <sup>b</sup>	17.6-18.4	13.2-18.2	14.0	17.9
Fat	6.9-8.1	6.3-8.1	6.7	7.7
Crude fiber	3.2-5.8	3.6-4.4	6.6	2.2
Ash	3.1-4.4	2.8-3.9	3.6	4.1
Moisture	9.5-11.6	6.2-8.8	10.5	11.1

<sup>&</sup>lt;sup>a</sup> Percentage on dry weight basis; data from Singhal RS and Kulkarni PR (1988) Composition of the seeds of some Amaranthus species. Journal of the Science of Food and Agriculture 42(4): 325-331; Yue SX, Sun HL, and Tang DF (1993) The Research and Development of Grain Amaranth in China (in Chinese). Beijing: Chinese Agricultural Science and Technology Press; and Segura-Nieto M, Barba de la Rosa AP, and Paredes-López O (1994) Biochemistry of amaranth proteins. In: Paredes-López O (ed.) Amaranth: Biology, Chemistry, and Technology, pp. 75-106. Boca Raton, FL: CRC Press.  $^{b}$  N × 5.85.

**Table 3** Proximate composition of leaves from several *Amaranthus* species<sup>a</sup>

Component	Amaranthus species <sup>b</sup>						Spinach
	1	2	3	4	5	6	
Crude protein <sup>c</sup>	48.6	46.5	33.3	27.8	44.5	32.7	34.4
Fat	3.3	6.8	2.9	3.9	4.6	7.1	3.2
Crude fiber	12.8	11.1	22.9	17.5	14.1		46.2
Ash	35.2	35.4	40.7		33.1		16.1

<sup>&</sup>lt;sup>a</sup> Percentage on dry weight basis; adapted from Saunders RM and Becker R (1984) Amaranthus: a potential food and feed resource. In: Pomeranz Y (ed.) Advances in Cereal Science and Technology, vol. 6, pp. 357-396. St. Paul, MN: American Association of Cereal Chemists; Teutonico RA and Knorr D (1985) Amaranth: composition, properties, and applications of a rediscovered food crop. Food Technology 39(4):49-54; and Segura-Nieto M, Barba de la Rosa AP, and Paredes-López O (1994) Biochemistry of amaranth proteins. In: Paredes-López O (ed.) Amaranth: Biology, Chemistry, and Technology, pp. 75–106. Boca Raton, FL: CRC Press.
<sup>b</sup>(1) A. caudatus; (2) A. cruentus; (3) A. edulis; (4) A. hybridus; (5) A. spinosus; (6) A. tricolor.

<sup>&</sup>lt;sup>b</sup>Mean values of four Amaranthus species (A. cruentus, A. caudatus, A. hypochondriacus, and A. hybridus).

 $<sup>^{</sup>c}$  N  $\times$  5.85.

 $<sup>^{</sup>d}$ N × 5.7.

 $<sup>^</sup>e$  N imes 6.25.

 $<sup>^{</sup>c}$ N $\times$  6.25.

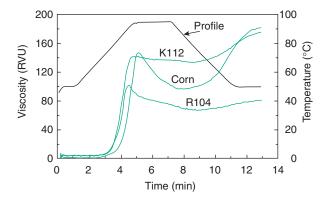
**Table 4** Starch and amylose content of amaranth grains (%)<sup>a</sup>

Species	Starch	Amylose
A. cruentus	48-63	22.1 (n = 48)
A. hybridus	55	21.1 (n = 14)
A. hypochondriacus	52-62	7.8 (n = 6)
A. retroflexus		34.3 (n = 3)
A. spinosus		18.1 (n = 4)
A. tricolor	51	29.0 (n = 8)
A. viridis		12.9 (n = 3)
124 genotypes		$19.2\pm13.9$
Light-colored seeds	$69 \pm 3 \ (n=9)$	

<sup>&</sup>lt;sup>a</sup> n, numbers of genotypes; data from Saunders RM and Becker R (1984) Amaranthus: a potential food and feed resource. In: Pomeranz Y (ed.) Advances in Cereal Science and Technology, vol. 6, pp. 357–396. St. Paul, MN: American Association of Cereal Chemists; Yue SX, Sun HL, and Tang DF (1993) The Research and Development of Grain Amaranth in China (in Chinese). Beijing: Chinese Agricultural Science and Technology Press; and Wu HX and Corke H (1999) Genetic diversity in physical properties of starch from a world collection of Amaranthus. Cereal Chemistry 76: 877–883.

Since the 1970s some interesting findings on physico-chemical and functional properties of amaranth starch have been reported, e.g., stable paste properties and a wide range of viscosity, resistance to shear thinning, and gelatinization temperatures. The focus of such research was restricted to very few Amaranthus species or genotypes, and sometimes gave contradictory results due to the genetic variation of the properties of amaranth starch. A. hypochondriacus starch had a higher amylograph viscosity, a higher gelatinization temperature, a lower swelling power, and a higher solubility than wheat starch. A. paniculatus (synonym: A. cruentus) starch had moderate swelling power, higher solubility, and lower pasting viscosity than foxtail millet starch. Compared to maize starch, A. paniculatus starch had a higher paste viscosity, lower paste clarity, and higher freeze/thaw stability.

A large number of *Amaranthus* germplasm (26 species) were introduced into China for evaluation of amaranth starch properties. Compared to the reference maize, rice, potato, and wheat starches, *Amaranthus* starch tended to have more stable paste, i.e., lower pasting viscosities (lower shear thinning and lower retrogradation), higher gelatinization temperature, and higher energy of enthalpy for gelatinization. Also, *Amaranthus* starch paste was more resistant to cold storage and had softer gel texture, lower changes of hardness, cohesiveness, modulus, and adhesiveness. Figure 2 displays the Rapid Visco Analyzer (RVA) pasting profiles of *Amaranthus* starch compared with maize starch. A wide range of variation was found in the several tested properties of starch



**Figure 2** RVA pasting profiles of two *Amaranthus* genotypes (K112 and R104) compared with corn (Adapted from Wu HX, Yue SX, Sun HL, and Corke H (1995) Physical properties of starch from two genotypes of *Amaranthus cruentus* of agricultural significance in China. *Starch/Stärke* 47: 295–297 and Corke H, Wu HX, Yue SX, and Sun HL (1997) Developing specialty starches from new crops: a case study using grain amaranth. In: Campbell GM, Webb C, and McKee SL (eds.) *Cereals: Novel Uses and Processes*, pp. 91–102. NY: Plenum Press.)

among the *Amaranthus* species and among genotypes within the same species, suggesting that the wide genetic diversity necessitates specific choices for specific uses. Interestingly, it was observed that there were significant effects of environment on the tested properties of *Amaranthus* starch, indicating that starch of the same genotype might have different starch properties if grown in different locations. Additionally, obvious effects on pasting and rheological properties from genotypic, pH, and salt factors were found.

Recently, amaranth starch has attracted increasing attention because of its unusual properties. Possible product uses of amaranth starch, capitalizing on its small granule size, could include food thickeners, dusting powders for cosmetic as well as food uses, laundry starch, and even possible biodegradable plastics. The unique properties of A. caudatus starch, namely, its pasting properties and high susceptibility to amylase, might represent potential uses as food, feed, and fuel. A. hypochondriacus starch had relatively low gelatinization temperature and food freeze-thaw stability, indicating its use in gravies, sauces, and soups. A. paniculatus starch could be used in salad dressings and custard preparations. There were also reports on chemical modification (e.g., succinvlation and hydroxypropylation) of amaranth starches and their properties.

Many studies demonstrated that starches isolated from many *Amaranthus* species could be used as good thickeners, stabilizers in salad dressing, canned food, sauce, frozen food, etc., because of their stable paste, low retrogradation, freeze—thaw resistance,

and heating and cooling resistance. Also, there has been a report that describes amaranth starch as behaving very poorly in breads and cakes from the standpoint of color and appearance, texture, and volume, probably because of its low amylose content. During starch application, it is worth paying attention to a fairly wide variation of amaranth starch properties among different species and genotypes.

#### **Amaranth Protein**

The second most abundant component of amaranth grains is protein. Based on the amino acid composition, amaranth seed protein is known to be of higher quality than most of the major cereal grains. The essential amino acid composition of amaranth grains from different Amaranthus species is summarized in **Table 5.** The lysine content is 2-3 times higher than that of the most common cereals, and sulfur amino acid content is also relatively high as compared with that of the most important legumes (pea, bean, soybeans, etc.). Table 5 also shows clear variation in amino acid composition among different species. Because amaranth protein has a better balance of the essential amino acids than in cereals and legumes, it scores higher than other seeds (e.g., wheat, barley, soybean, and maize) in the FAO/WHO Nutritionist's Protein Value Chart: a score of 100 is the ideal, whereas amaranth protein received the highest score of 75, compared to cow milk (score = 72), soybeans (68), and peanuts (52), indicating that its balance was closer to the optimum required in the human diet (FAO/WHO).

Seed proteins are generally classified into four types based on solubility: albumin, globulin, prolamin, and glutelin. Most studies on amaranth proteins suggested that albumin was the major fraction (48.9-65%), followed by glutelin (22.4-42.3%), globulin (13.7-18.1%), and prolamin (1.0-3.2%). However, there was variation in amaranth protein fractions because of protein extraction and fractionation procedures, and due to genetic variation. For instance, it was reported that the fractionated proteins from five different Amaranthus species averaged albumin (65%), globulin (17%), prolamin (11%), and glutelin (7%). Also, it was reported that the isolated proteins from A. hypochondriacus consisted of 46-49% albumin plus globulin, 3% prolamin, and 30-33% glutelin, and in another study of same species, similar percentages of albumin, globumin, prolamin, and glutelin were 51.0, 15.9, 2.0, and 31.1, respectively.

In the 1980s and 1990s, many researchers isolated major fractions (albumin, globulin, and glutelin) of proteins from main *Amaranthus* species and examined

**Table 5** Essential amino acid composition of grains from three major *Amaranthus* species (g per 100 g protein)<sup>a</sup>

Amino acid	A. caudatus	A. cruentus	A. hypochondriacus
Cystine	2.3	2.0-3.8	2.0-3.9
Isoleucine	3.6-4.1	3.4 - 3.7	2.8-3.8
Leucine	5.9 - 6.3	4.8 - 5.9	5.0-5.8
Lysine	5.7 - 6.4	4.8 - 5.8	3.2-6.0
Methionine	2.4 - 3.3	1.8 - 2.6	0.6-1.6
Phenylalanine	3.4-4.0	3.2 - 4.5	3.8-4.5
Threonine	3.8	3.2 - 4.2	2.6-4.3
Tryptophan	1.1	nd <sup>b</sup>	1.1-4.0
Tyrosine	2.8	2.4 - 4.0	3.1-4.0
Valine	4.1 - 4.7	3.9 - 4.3	3.2-4.2
Met + Cys	4.7	3.8 - 5.4	2.6-5.5
Phe + Tyr	6.2	5.6 - 8.5	6.9-8.5

<sup>a</sup> Data from Saunders RM and Becker R (1984) *Amaranthus*: a potential food and feed resource. In: Pomeranz Y (ed.) *Advances in Cereal Science and Technology*, vol. 6, pp. 357–396. St. Paul, MN: American Association of Cereal Chemists and Segura-Nieto M, Barba de la Rosa AP, and Paredes-López O (1994) Biochemistry of amaranth proteins. In: Paredes-López O (ed.) *Amaranth: Biology, Chemistry, and Technology*, pp. 75–106. Boca Raton, FL: CRC Press.

<sup>b</sup>nd, not determined.

their biochemical and physico-chemical properties and molecular weights. Based on their measurements of its emulsion activity, foaming stability, and surface hydroscopicity, along with its relatively high heat stability, it was concluded that amaranth proteins could be useful as effective emulsifying and foaming agents. On the other hand, there were reports that *A. cruentus* and *A. hypochondriacus* protein fractions had poor emulsifying and foaming properties, limited water absorption, and good gel-foaming properties.

In the past several years, a series of studies on properties and utilization of amaranth protein have been carried out. It was found that protein concentrates made from amaranth grains of five genotypes exhibited better solubility, emulsification, and foaming properties than the commercially available soy proteins (Table 6). The effect of proteins on starch gelatinization and retrogradation was investigated using the hydrolyzed and native protein concentrates of amaranth. The results suggested that native proteins tend to be attracted towards the starch granule and contribute to regulation of swelling and amylose leaching during gelatinization, therefore resulting in a weaker starch gel. However, hydrolyzed proteins showed similar effect, but to a much lesser extent. Furthermore, amaranth protein concentrates were examined for direct application in certain food systems (wheat dough and noodle). It was found that the mixing properties of wheat flour were improved by the addition of the protein concentrates. Addition of protein concentrates to a noodle formulation also showed some positive influence on final product

**Table 6** Functional properties of amaranth protein concentrations, compared to soy protein isolates<sup>a</sup>

Species	Genotypes	Emulsifying activity (%)	Solubility (%)	Foam expansion (ml)	Foam stability (ml)
A. cruentus	K112	55.6	19.9	49	34
A. cruentus	K350	73.3	56.5	91	56
A. cruentus	K459	61.9	42.1	52	26
A. cruentus	R104	56.0	47.2	35	18
A. hybridus	No.3	62.2	60.5	68	26
Soybean	Soy A	50.6	33.3	23	20
Soybean	Soy B	45.7	21.3	28	22
LSD <sub>0.05</sub> <sup>b</sup>	•	3.1	4.5	20	9

<sup>&</sup>lt;sup>a</sup> Data from Bejosano FP and Corke H (1999) Protein quality evaluation of *Amaranthus* wholemeal flours and protein concentrates. *Industrial Crops and Products* 76: 100–106.

**Table 7** Oil and squalene content of amaranth grains and other seeds<sup>a</sup>

Species	Oil (%)	Squalene (%)
A. cruentus (1)	5.57-7.72	4.52-5.44
A. cruentus (2) <sup>a</sup>		5.0 - 8.0
A. hybridus	6.40	5.23
A. hypochondriacus	5.35-7.05	3.62-5.01
A. tricolor	5.08	6.14
Corn	4.0	0.03
Cottonseed	7.0	0.01
Rice	1.0-3.0	0.3
Olive	36.0	0.4
Peanut	47.0	0.03

<sup>&</sup>lt;sup>a</sup> Data from Lyon and Becker (1987), other data in the table from Becker R (1994) and He HP, Cai YZ, Sun M, and Corke H (2002) Extraction and purification of squalene from *Amaranthus* grain. *Journal of Agricultural and Food Chemistry* 50: 368–372.

quality. A few amaranth protein concentrates were also evaluated as fillers for an emulsion-type meat product. Only one of the amaranth protein concentrates (genotype K112) gave some favorable results.

# **Amaranth Oil**

Oil is another chemical component of much interest in amaranth grain, because it contains relatively higher levels of oil (5-8%) than cereal grains; moreover, there is a considerable amount of squalene in amaranth oil  $(\sim 5\%)$  compared with that in oils of cereals and other oil crops (0.01-0.4%) (Table 7).

Palmitic, oleic, and linoleic acids are the major fatty acids in amaranth oil, with the saturated/unsaturated fatty acid (S/U) ratios ranging from 0.12 to 0.50. Published data showed that the fatty acid profiles in amaranth oil from different species were as follows: palmitic (12-25%), oleic (19-35%), linoleic (25-62%), stearic (2-8.6%), and linolenic (0.3-2.2%) acid. The squalene levels range from 5% to 8% in

A. cruentus oil. Lower amounts of squalene (2–5%) are tested in some Amaranthus species of West Africa. Other lipids in amaranth – such as triglycerides, sterols, methylsterols, terpenic and aliphatic alcohols, tocopherols, and hydrocarbons – have also been identified. Recent results indicated that the major fatty acids in amaranth oil from 11 Amaranthus genotypes included palmitic acid (19.1–23.4%), oleic acid (18.7–38.9%), and linoleic acid (36.7–55.9%), with the S/U ratios of 0.26–0.32. The squalene content in amaranth oils from 11 genotypes of four species ranges from 3.6% to 6.1% (Table 7). These reports reveal the significant variation in oil composition content among various species and genotypes.

Squalene is a biosynthetic precursor to all steroids. It is an important functional ingredient in skin cosmetics and also as an excellent computer-disk lubricant. It has been reported that the decreased risk for some cancers might be associated with squalene consumption, and the use of squalene alone is effective in decreasing serum cholesterol levels. The traditional source of squalene is from shark and whale liver oil. Estimates in 1994 place the world market at 1400 t year<sup>-1</sup>. It is very expensive (\$53 000 per ton). Amaranth oil will attract more attention as an alternative plant source of squalene because of its abundant squalene content, although efforts to further increase the level are necessary.

Squalene can be easily extracted from amaranth grain by simple vacuum distillation. Recently, work to screen genotypes containing a higher content of squalene in a wide range of *Amaranthus* species and to develop efficient large-scale separation technique has been conducted; a method was established to separate and purify squalene from amaranth oils. After saponification, squalene content increased from 4.2% to 43.3% in the unsaponifiables by the removal of the saponifiables. The unsaponifiables were fractionated by a chromatographic method to

<sup>&</sup>lt;sup>b</sup>Least significant difference (p < 0.05) for comparison of means in the same column.

obtain highly purified squalene. The final purity of squalene reached 94-98%.

However, amaranth oil occurs in very low levels and at higher cost than oils of peanut, olive, rapeseed, etc. Unless a specialty starch or protein could also be produced from the defatted meal, a substantial decrease in raw product cost will probably be required before amaranth oil goes commercial.

# **Amaranth Pigments**

There has been an increasing trend toward replacement of synthetic colorants with natural pigments since the 1980s because of safety and health concerns. Red-colored vegetative tissues of Amaranthus plants contain a high concentration of betalain pigments and are often produced in high biomass (Table 8). They have attracted considerable interest as potential substitutes for the well-known betalains from beetroot (Beta vulgaris) which are extensively used in the food industry worldwide. In recent years, a comprehensive systematic study on amaranth pigments has covered field evaluation and selection of betalain-producing genotypes/species, extraction, identification and quantification of betalain components, determination and evaluation of properties and antioxidant activity, and pigment production and utilization in certain food systems.

Betalains are divided into two major structural groups, i.e., red-violet betacyanins and yellow betaxanthins. Amaranth pigments belong to betacyanins. They could be easily extracted with water or methanol. Sixteen kinds of betacyanins were isolated and characterized from plants in the Amaranthaceae. Figure 3 shows HPLC profile of amaranthine-type betacyanins and their molecular structures, which are predominant pigments in all Amaranthus species. Quantitative analysis data showed that the screened amaranth species/varieties yield higher betacyanin pigments than feasible in beetroot production.

**Table 8** Pigment content and fresh weight of 12 genotypes from seven *Amaranthus* species<sup>a</sup>

Species	Genotypes	Fresh weight of colored parts (kg ha <sup>-1</sup> ) <sup>b</sup>	Total betalain content (mg per 100 g FW) <sup>c</sup>
A. cruentus	12	56 390 ± 7225 (Lv,In)	42–199
A. caudatus	2	42 520 (In)	82-144
A. tricolor	3	20 520 <i>(Lv)</i>	51-143
A. hypochondriacus	1	29 160 ( <i>ln</i> )	47
A. hybridus	1	39 285 <i>(In)</i>	82
A. lividus	1	17 370 <i>(SI)</i>	46
A. paniculatus	1	44 550 ( <i>ln</i> )	127

<sup>&</sup>lt;sup>a</sup> Data from Cai YZ, Sun M, Wu HX, Huang RH, and Corke H (1998) Characterization and quantification of betacyanin pigments from diverse Amaranthus species. Journal of Agricultural and Food Chemistry 46(6): 2063-2070.

<sup>&</sup>lt;sup>c</sup>FW, fresh weight.

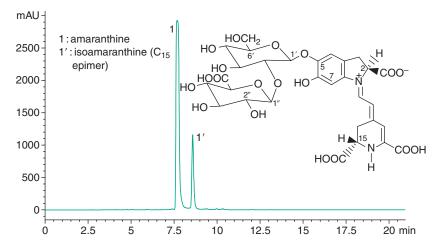


Figure 3 Structure and HPLC elution profile of amaranthine-type betacyanins from A. tricolor. The isomeric form (1') is the C-15 epimer of corresponding betacyanin (not drawn).

<sup>&</sup>lt;sup>b</sup>SI, seedlings; Lv, leaves; In, inflorescences. Estimated as SI, Lv, or In weight per plant × planting density.

Colorant properties and stability of the betacyanins are the key factors of pigment quality that directly influence their application in food systems. Assessment of color characteristics and stability of betacyanins from *Amaranthus* species showed that *Amaranthus* betacyanins exhibited bright red-violet color and favorable stability under selected conditions (pH 5–7 and temperature <14°C), as compared to red radish anthocyanins. Dried *Amaranthus* pigments had good storage stability ( $t_{1/2} = 23.3$  months) at room temperature. The presented data support the potential development and utilization of *Amaranthus* betacyanins as new natural colorants for use in the food industry, particularly for low-temperature uses.

Aqueous extracts of natural pigments are commonly unstable, and sensitive to storage environmental factors. Spray drying can significantly improve storage stability. *Amaranthus* betacyanin extracts were spray dried using a range of 10–25 DE maltodextrins as carrier and coating agents at different inlet/outlet air temperatures and various feed solid contents. The best-dried pigment powder made was superior to commercial red beet powder in terms of physical properties (Table 9). The results demonstrated the feasibility of production of spray-dried *Amaranthus* betacyanin extracts as a food-grade colorant.

Application of *Amaranthus* betacyanins in jelly, ice cream, and a model beverage was evaluated by comparison to commercial colorants (red radish anthocyanins and synthetic colorant FD&C Red#3). The results revealed the feasibility and potential of *Amaranthus* betacyanins for use in the model food systems at low temperature. *Amaranthus* betacyanins in the foods examined exhibited better color characteristics than red radish anthocyanins at the same levels, but were not as bright as FD&C Red#3. The betacyanins had color stability comparable to red radish and FD&C Red#3 at lower temperature (<14°C), and also similar color stability at 25°C, but not as stable as the two commercial colorants at higher temperature (37°C).

Additionally, all betalains isolated from plants in the Amaranthaceae exhibited strong antioxidant

activity. To date there has been evidence suggesting that certain natural colorants as nutritional antioxidants in the diet may reduce the risk of some diseases. This gives the possibility of these betalains being used both as natural colorants and as potential natural antioxidants.

# **Food and Feed Utilization of Amaranth**

Traditional foods are made from amaranth grains in many countries, e.g., "alegria" and "atole" in Mexico, "alboroto" in Guatemala, "bollos" in Peru, "Chapati" in Himalayas, "laddoos" in India, and "sattoo" in Nepal. These amaranth-based foods are still consumed today in various areas of the world. Modern food applications of amaranth grains include breakfast foods, infant/weaning food formulations, breads, pastas, flakes, drinks and beverages, etc. Currently available consumer food products include popped foods, baking mixes, malted flours, breakfast cereals, snacks, baked goods, and whole amaranth products. In China, a wide range of amaranth food products has been developed for retail sale. These include instant flour for beverages or porridge, packaged dried noodles, cakes, biscuits, and soy sauce made from brownblack amaranth and other grains (wheat, soybean). China has a wide diversity of traditional food products made from specialty grains, such as buckwheat, millets, sorghum, and legumes. By adapting products made from these, additional amaranth-based foods have been developed, such as composite flour instant noodles, distilled spirits, and vinegar. Furthermore, vegetable amaranth has been used as a traditional vegetable for several centuries in China. In sub-Saharan Africa and Central and Southern America, vegetable amaranth is also an important leafy vegetable, consumed as a cooked green vegetable, or used

Amaranth is a fast-growing, high-nitrogen, and high-biomass plant that can be used fresh or as silage for animal feed. Amaranth grain flour or powdered leaf meal can be used to make commercial pelletted or other compound feeds. Currently, feed application of

**Table 9** Comparison of amaranth pigment powder and commercial beet powder (No. 3600 or E162)<sup>a</sup>

Spray-dried powder	Betacyanin Hygroscopic moisture		Color parameters			Pigment retention <sup>c</sup> (%)	
	content <sup>b</sup> (%)	(g /100 g)	L*	a*	b*	H <sup>o</sup>	
Amaranth powder Beet powder	0.74 0.31	48.5 58.8	49.35 51.54	32.60 19.64	-2.47 0.71	355.7(-4.3) 2.0	87.9 ± 1.8 84.3 ± 1.4

<sup>&</sup>lt;sup>a</sup> Data from Cai YZ and Corke H (2000) Production and properties of spray-dried *Amaranthus* betacyanin pigments. *Journal of Food Science* 65: 1248–1252; amaranth pigment powder made by spray drying at 180°C inlet temperature using 19.8% solids in feed mixture (15 DE maltodextrin).

<sup>b</sup> Calculated as % amaranthine and % betanin.

<sup>&</sup>lt;sup>c</sup>Determined at 25°C and 32% RH after 16 week storage

amaranth has been successful and popular in China and has played a role in developing Chinese animal husbandry. Amaranth feeds made from leaves, stems, panicles, and grains include green fodder, silage, leaf and seed-meal feed, pellet feed, etc. The evaluation of feed use has shown that amaranth feeds efficiently raise pigs, chickens, cattle, sheep, and fish to improve the quality and productivity of animal production. Chinese peasant households are accustomed to raise livestock and poultry, and nowadays in many areas amaranth is the main source of silage for household pig production. However, it is not widely used as feed in other countries.

In summary, amaranth possesses high yield potential, and high stress tolerance to drought, salinity, alkalinity, or acidic soil conditions. Its grains have attractive chemical composition, superior nutritive value, and some special functional properties. Its vegetative parts with high biomass can be used for animal feed. Its red-colored vegetative tissue with high level of betacyanins is a potential alternative source of well-known beetroot pigments (betalains). Amaranth has been attracting worldwide attention and will be a high-potential new crop with multiple uses. However, intense and continuous efforts are necessary in several aspects, such as amaranth breeding and field cultivation, relevant food/feed processing and development, product industrialization, marketing, etc.

See also: Pseudocereals, Overview.

# **Further Reading**

- Becker R (1994) Amaranth oil: composition, processing, and nutritional qualities. In: Paredes-López O (ed.) *Amaranth: Biology, Chemistry, and Technology*, pp. 133–141. Boca Raton, FL: CRC Press.
- Bejosano FP and Corke H (1999) Protein quality evaluation of *Amaranthus* wholemeal flours and protein concentrates. *Industrial Crops and Products* 76: 100–106.
- Berghofer E and Schoenlechner R (2002) Grain amaranth. In: Belton PS and Taylor JRN (eds.) *Pseudocereals and Less Common Cereals – Grain Properties and Utilization Potential*, pp. 219–260. Berlin: Springer.
- Cai YZ and Corke H (2000) Production and properties of spray-dried *Amaranthus* betacyanin pigments. *Journal of Food Science* 65: 1248–1252.
- Cai YZ, Sun M, Wu HX, Huang RH, and Corke H (1998) Characterization and quantification of betacyanin pigments from diverse *Amaranthus* species. *Journal of Agricultural and Food Chemistry* 46(6): 2063–2070.
- Corke H, Wu HX, Yue SX, and Sun HL (1997) Developing specialty starches from new crops: a case study using grain amaranth. In: Campbell GM, Webb C, and

- McKee SL (eds.) Cereals: Novel Uses and Processes, pp. 91–102. New York: Plenum.
- He HP, Cai YZ, Sun M, and Corke H (2002) Extraction and purification of squalene from *Amaranthus* grain. *Journal of Agricultural and Food Chemistry* 50: 368–372.
- Irving DW, Betschart AA, and Saunders RM (1981) Morphological studies on *Amaranthus cruentus*. *Journal of Food Science* 46(4): 1170–1174.
- Leon-Camacho M, Garcia-Gonzalez DL, and Aparicio R (2001) A detailed and comprehensive study of amaranth (*Amaranthus cruentus* L.) oil fatty profile. *European Food Research and Technology* 213: 349–355.
- Lyon CK and Becker R (1987) Extraction and refining of oil from amaranth seed. *Journal of the American Oil Chemists Society* 64: 233–236.
- Paredes-López O (1994) Amaranth: Biology, Chemistry, and Technology. Boca Raton, FL: CRC Press.
- Saunders RM and Becker R (1984) *Amaranthus*: a potential food and feed resource. In: Pomeranz Y (ed.) *Advances in Cereal Science and Technology*, vol. 6, pp. 357–396. St. Paul, MN: American Association of Cereal Chemists.
- Segura-Nieto M, Barba de la Rosa AP, and Paredes-López O (1994) Biochemistry of amaranth proteins. In: Paredes-López O (ed.) *Amaranth: Biology, Chemistry, and Technology*, pp. 75–106. Boca Raton, FL: CRC Press.
- Singhal RS and Kulkarni PR (1988) Composition of the seeds of some *Amaranthus* species. *Journal of the Science of Food and Agriculture* 42(4): 325–331.
- Sleugh BB, Moore KJ, Brummer EC, et al. (2001) Forage nutritive value of various amaranth species at different harvest dates. Crop Science 41: 466–472.
- Teutonico RA and Knorr D (1985) Amaranth: composition, properties, and applications of a rediscovered food crop. *Food Technology* 39(4): 49–54.
- Williams JT and Brenner D (1995) Grain amaranth (*Amaranthus* species). In: Williams JT (ed.) *Cereals and Pseudocereals*, pp. 129–186. London: Chapman and Hall.
- Wu HX and Corke H (1999) Genetic diversity in physical properties of starch from a world collection of *Amaranthus*. *Cereal Chemistry* 76: 877–883.
- Wu HX, Yue SX, Sun HL, and Corke H (1995) Physical properties of starch from two genotypes of *Amaranthus cruentus* of agricultural significance in China. *Starch/Stärke* 47: 295–297.
- Yue SX, Sun HL, and Tang DF (1993) The Research and Development of Grain Amaranth in China (in Chinese). Beijing: Chinese Agricultural Science and Technology Press.

## **Relevant Website**

http://www.hort.purdue.edu/newcrop — Website of the New Crop Online Program of the Center for New Crops and Plant Products at Purdue University. NewCROP provides windows to new and specialty crop profiles, including *Amaranthus*.

# **ANIMAL FEED**

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# Introduction

Cereal grains are used widely as animal feed. Some plant species such as maize, sorghum, millet, triticale, and oats are grown predominantly for animal consumption, whereas others including wheat, barley, and rice are grown mainly for human consumption with grains not meeting market specifications frequently being fed to livestock. These latter grains are often small grains from screenings, broken grains, or damaged by weather. Cereal grains have a high content of starch relative to other ingredients used as animal feed such as forages or pulses and are offered to animals primarily as a source of readily available energy. Consequently, cereal grains are fed predominantly to intensively reared livestock such as dairy cows, feedlot cattle and sheep, pigs and poultry and may represent 60-80% of the ration, although they are also used for subsistence in times of poor pasture growth.

There is a large variation across cereal species, cultivars, individual grain samples, and animal types in the amount of energy available to animals from grains following ingestion. The extent of digestion of a grain by an animal depends on the adequacy of enzymes within the digestive tract capable of breaking specific chemical bonds in each grain component, the accessibility of the enzymes to the chemical components, and the time the enzymes and component are associated. Much of the variation between grains in energy availability can be explained by differences in gross chemical composition. However, other factors, particularly those that affect the accessibility of enzymes to specific grain components, can affect markedly the amount of energy in a grain that is available to different types of animals. Variation between animal types in the anatomy of the digestive tract and the digestive process has a substantial impact on the availability of energy and whether metabolic disorders are likely to result following grain consumption.

The variation between grains in energy availability is due in part to heritable characteristics, but much is due to the particular environmental conditions in which the grain was grown. From an understanding of the factors determining the availability of energy

for each animal type, it is possible to identify grain characteristics that could be changed by plant breeding or specific processing methods to increase their energy value to animals. Although cereal grains provide amino acids, minerals, and vitamins to livestock, these nutrients are of minor importance in grains relative to other dietary ingredients and are not discussed.

# Variation in the Energy Value of Cereal Grains

# **Definition of Available Energy and the Digestive Process**

The energy available from digestion of a dietary ingredient is defined for most livestock as the amount of energy that is in chemical components digested and absorbed along the entire digestive tract from mouth to anus in mammals or cloaca in poultry. Available energy is determined by subtracting the energy in collected faeces from the energy consumed and is termed digestible energy (DE, MJ kg<sup>-1</sup>). However, in poultry available energy is defined as apparent metabolizable energy (AME, MJ kg<sup>-1</sup>), because the material excreted from the cloaca includes uric acid from the kidneys as well as faecal material.

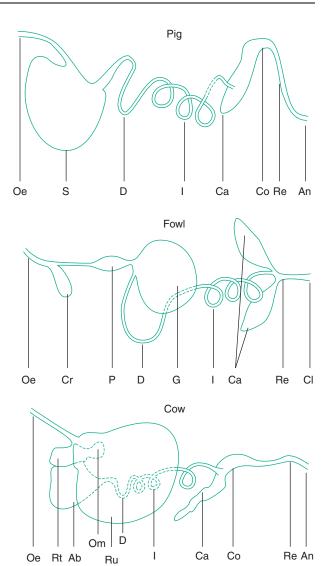
The value of digested energy to an animal for metabolic processes varies widely depending on whether the digestive process is a result of animal secreted enzymes or enzymes of microbial origin. In the latter case, the digestive process is frequently termed microbial fermentation, which results in the conversion of dietary carbohydrates and proteins into growing microbes, volatile fatty acids, and other compounds with the release of methane, ammonia, and heat of fermentation. The fermentation process within the digestive tract can result in the loss of 10–20% of the energy in the digested dietary material, depending on the composition of the diet, conditions of fermentation, and microbes present.

Nevertheless, the fermentation process is important for animals, because microbial enzymes can cleave chemical bonds in plant material that cannot be broken by enzymes secreted by mammals and birds. Glucose units in grain compounds are commonly linked by  $\alpha$ -(1-4),  $\alpha$ -(1-6), or  $\beta$ -(1-4) glycosidic linkages. The  $\alpha$ -(1-4) bonds, found predominantly in starch, can be hydrolyzed by animal digestive enzymes, whereas the  $\beta$ -(1-4) linkages, found in cellulose,

require microbial enzymes for cleavage. The  $\alpha$ -(1–6) glycosidic linkages also restrict the action of animal amylases. Starch digested by animal enzymes is used more efficiently than that digested by microbial enzymes. However, the nonstarch polysaccharides in grains including cellulose and endosperm cell wall constituents – arabinoxylans, xylans, and (1–3, 1–4)- $\beta$ -glucans – can be digested only by microbial enzymes. Although some energy is lost from the breakdown of these compounds during the fermentation process, 80–90% of the energy released is available to metabolic processes in the animal. The lignin content of grains, which consists of a range of phenolic polymers, is indigestible by animal enzymes and most intestinal microbes.

The anatomy of the digestive tract (Figure 1) and the digestive process varies widely between domestic livestock species. Feed consumed by ruminants is subjected to microbial fermentation within the rumen reticulum before it passes to the small intestines where animal enzymes are secreted. The starch in cereal grains is therefore first subjected to microbial digestion and, if degraded rapidly, can result in a significant increase in acid production and fall in rumen pH. Lactic acidosis caused by a rapid increase in the concentration of lactic acid and low pH disrupts the balance of microbes in the rumen, reduces digestion of other dietary constituents, particularly plant fiber, and can severely affect the health and productivity of the animals. The rate of starch fermentation in the rumen is influenced by characteristics of the grain and particle size. The latter depends on the extent of breakdown of grain during mastication and the effectiveness and type of grain processing. Sheep masticate grains more thoroughly than cattle and often grains are not processed before feeding to sheep, whereas cereal grains are frequently processed before being fed to cattle.

Although horses and pigs do not a compartment like the rumen reticulum within the stomach for the fermentation of feed, the hind-gut contains a large microbial population which digests much of the nonstarch polysaccharide component of their diet. The starch component of cereal grains is first subjected to degradation by the animal secreted enzymes. However, if a large quantity of starch passes through the small intestines undigested, it is fermented in the hind-gut and can cause laminitis in horses through a lowering of pH and an increase in the susceptibility of pigs to enteric diseases such as swine dysentery and Escherichia coli scours. Pigs masticate their feed poorly and unless the grain is processed before ingestion, large quantities of starch can be fermented in the hind-gut. Poultry do not have a significant microbial population within the digestive



**Figure 1** Diagrammatic representation of the digestive tracts of pigs, poultry, and ruminants (An, anus; Ab, abomasums; Ca, caecum; Cl, cloaca; Co, colon; Cr, crop; D, duodenum; G, gizzard; I, ileum; Oe, oesophagus; Om, omasum; P, proventriculus; Re, rectum; Rt, reticulum; Ru, rumen; S, stomach). (Reproduced with permission from McDonald P, Edwards RA, and Greenhalgh JFD (1988) *Animal Nutrition*, 4th edn. Essex, UK: Longman Scientific Technical.)

tract and there is insignificant digestion of the nonstarch polysaccharide components in grain. However, poultry have a gizzard within their digestive tract, and the intense muscular contractions of this organ are extremely effective for physically breaking the grain into small particles and disrupting the integrity of the endosperm cell walls.

# Variation in Available Energy between Grains and Animal Types

Examples of published variation in the available energy content of cereal grain species for poultry

**Table 1** Variation in published values for the available energy content of cereal grains for poultry and pigs<sup>a</sup>

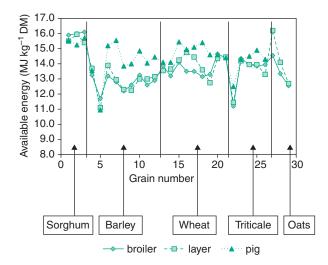
Cereal grain	Poultry AME <sup>b</sup> (MJ kg <sup>-1</sup> DM <sup>c</sup> )	Pigs DE <sup>d</sup> (MJ kg <sup>-1</sup> DM <sup>c</sup> )
Wheat Barley	10.3-15.9 10.4-13.5	13.3–17.0 11.7–16.0
Triticale	8.6–15.2	14.8–16.0
Sorghum Maize Oats	13.5-17.7 15.5-17.0 10.5-12.4	15.8–17.4

<sup>&</sup>lt;sup>a</sup>Constructed from (1) Hughes and Choct (1999) Premium grains for livestock. *Australian Journal of Agricultural Research* 50(5) and (2) van Barneveld (1999) Chemical and physical characteristics of grain related to variability in energy and amino acid availability in pigs: a review. *Australian Journal of Agricultural Research* 50: 667–688.

and pigs are shown in Table 1. The difference between values in AME for poultry of 3-6 MJ kg<sup>-1</sup> DM for wheat, barley, triticale, and sorghum would have a large impact on productivity, whereas the difference of  $1.5-2 \,\mathrm{MJ\,kg^{-1}\,DM}$  for maize and oats is less, but still important for commercial animal production. A similar difference between values in DE was observed for wheat and barley fed to pigs, but the difference for triticale and sorghum was less than reported for poultry. The results do not cover the same grains fed to both pigs and poultry, and the range in available energy values reported would include differences in experimental techniques between laboratories. Nevertheless, a difference of only 0.5 MJ kg<sup>-1</sup> DM in the energy content of cereal grains can have a significant effect on animal productivity, efficiency of feed use, and profitability of intensive animal enterprises.

A comparison of the available energy content of the same grain samples when fed to pigs, broiler chickens, and laying hens is shown in Figure 2. Other examples of the available energy content of the same grain sample offered to sheep, cattle, pigs, broiler chickens, and laying hens are shown in Table 2. The table employs the same grain species, cultivar, or type as used in the experiments, but the available energy values observed often are more closely related to the actual sample and the growing conditions rather than to the species and cultivar description.

There were relatively small differences in the available energy content of some individual grain samples when compared across the animal types, but for other grains the differences were marked. Figure 2 shows that the available energy content of cereal grains for pigs and poultry generally followed a close relationship. More energy tended to be available to pigs than poultry for barley and wheat samples. For most grains there were only



**Figure 2** Available energy content of grains (MJ kg<sup>-1</sup> DM) as apparent metabolizable energy for poultry and as digestible energy for pigs offered the same grains. (Updated from Black JL (2000) *Proceedings of the Australian Poultry Science Symposium* 13: 22–29.)

**Table 2** Available energy content of grains<sup>a</sup> (MJ kg<sup>-1</sup> DM) fed across animal species as digestible energy for sheep, cattle, and pigs and as apparent metabolizable energy for poultry<sup>b</sup>

Grain	Sheep	Cattle	Pigs	Broilers	Layers				
Individual samples	Individual samples from cereal grain species								
Barley	14.5	14.5	15.2	13.2	13.9				
Wheat	14.8	14.7	15.5	14.2	14.3				
Triticale	15.8	15.9	14.3	14.4	14.2				
Oats - normal	13.2	13.2		12.6	12.7				
Oats - naked	16.5			14.6	16.2				
Individual barley s	amples								
Arapilies frosted	12.3	12.7	11.0	11.7	11.1				
Reinette	13.1	13.5	14.1	12.6	13.0				
Galleon	14.1	14.6	15.2	13.2	13.9				
Merlin	15.5		15.3	12.6	13.9				
Individual sorghun	n samples	;							
Nonwaxy isoline	15.1	10.1	15.6	15.9	15.5				
Waxy isoline	14.7	13.7	15.2	16.0	16.0				
Buster	14.5	10.5	15.7	16.1	15.3				

<sup>&</sup>lt;sup>a</sup>All grains were dry-rolled prior to feeding to animals.

small differences in the available energy content of grains between broiler chickens and laying hens. The most striking difference was for a naked oat sample where the value was 16.2 MJ kg<sup>-1</sup> DM for laying hens and only 14.6 MJ kg<sup>-1</sup> DM for broiler chickens. The barley and wheat samples in the first section of Table 2 had a higher energy content for pigs than the other animal species. However, these differences were not apparent for the Arapilies and Reinette barley samples in the second section of Table 2. The

<sup>&</sup>lt;sup>b</sup>Apparent metabolizable energy.

<sup>&</sup>lt;sup>c</sup>Dry matter.

<sup>&</sup>lt;sup>d</sup>Digestible energy.

<sup>&</sup>lt;sup>b</sup> Revised from Black JL (2000) *Proceedings of the Australian Poultry Science Symposium* 13: 22–29.

available energy content of the barley samples examined ranged from 11.5 to 15.5 MJ kg<sup>-1</sup> DM for sheep, but only from 11.7 to 13.2 MJ kg<sup>-1</sup> DM for broiler chickens. The available energy value for the naked, waxy barley sample, Merlin, was high for both sheep and pigs, lower for layers, and substantially lower for broilers. Merlin was not fed to cattle.

The most striking differences between the animal types were for sorghum where the energy content for cattle of nonwaxy sorghum was only 65% of that for broiler chickens. The digestible energy content for cattle of a waxy isoline was substantially greater than that of the normal isoline (13.7 MJ kg<sup>-1</sup> DM compared with 10.1 MJ kg<sup>-1</sup> DM), but was only 86% of the value for broiler chickens. This difference in energy content between waxy and nonwaxy isolines of sorghum was not apparent for any other animal species examined. In addition, for all animal types except cattle, the available energy content of sorghum was higher than that of the other cereal grains. However, there are important differences between pigs and poultry in the site of sorghum digestion. The mean ratio of energy digestion to the end of the small intestines (ileal DE) to digestion along the entire digestive tract was 0.87 for pigs and 0.99 for broiler chickens. This result shows that  $\sim 13\%$  of the energy digestion from sorghum in pigs is through microbial fermentation and indicates that broiler chickens obtain significantly more energy for metabolism from sorghum than pigs.

Large variation has been observed in the digestibility of oat grains when fed to sheep and cattle. The variation in digestibility of whole oat grains suspended in a nylon bag in the rumen of cattle for 48 h (*in sacco* digestibility) or fed to growing cattle is shown in Table 3. The table shows also an indication of the lignin content of the hulls (lignin score — higher values represent higher lignin content) and growth rate of cattle. There was an extremely wide variation between the samples in digestibility and the performance of animals consuming the different oat grain samples. The oat samples containing high lignin contents had lower digestibility and resulted in slower growth rates than those with low lignin contents.

Storage of grain, particularly wheat, has been shown to significantly improve the AME content of some grain samples, but not others, for broiler chickens. Table 4 shows the AME values for six wheat samples at 1 and 4 months after harvest and the viscosity of water-soluble extract from the grains. Similar studies with wheat fed to pigs show that there is little effect of storage on the energy value of wheat for these animals.

The examples of available energy content of grains for different animal types provided above indicate

**Table 3** Lignin score and digestibility of selected oat samples, and cattle growth rate when grains are fed to the cattle

Oat sample	Lignin score	Whole grain in sacco dry matter digestibility (%)	Dry matter digestibility in cattle (%)	Live weight gain of cattle (kg day <sup>-1</sup> )
Echidna	5	5	60	0.41
Mortlock	4	13	67	0.88
Quoll	3	14	77	0.95
Eurabbie	2	47	72	1.00
MA 5237	2	37	81	1.10
Cooba (low)	1	24	75	1.03
Yiddah	1	38	79	1.27
Cooba (high)	1	55	81	1.27

Unpublished results from Kaiser AG.

**Table 4** Effect of storage of wheat samples for 1 month or 4 months on the AME content (MJ kg<sup>-1</sup>) and viscosity of the grain extract

Wheat sample	A (MJ	Grain extract viscosity (mPa s)	
Storage time	1 month	4 months	
1	10.14	12.73	4.8
2	10.31	13.30	4.1
3	12.35	13.95	4.8
4	11.18	11.10	12.5
5	12.02	13.94	3.8
6	11.80	11.98	8.2

Modified from Rowe JB, Choct M, and Pethick DW (1999) Australian Journal of Agricultural Research 50: 721–736.

that most grains with low-energy availability for one animal type will also have low-energy availability for other animal types. Similarly, most grains with high-energy availability for one animal type will have high availability for other animal types. However, some specific grain species or grain samples differ widely in their energy value between and within animal types. These observed differences between individual grains and animal types in available energy content of cereal grains illustrate the importance of being able to understand the reasons for the differences and to identify rapidly, the nutritional value of an individual grain sample.

# Reasons for Differences in Available Energy between Grains

To identify the reasons for the differences in the available energy content of grains between animal types and between grains within an animal type, it is necessary to understand the critical steps in the digestion

process. The extent of grain digestion by animals depends on the availability of enzymes capable of breaking the specific chemical bonds of each grain component, the ability of the enzymes to come in contact with the bonds, and the length of time the enzymes are in association with the substrates. The differences between animal species in terms of the availability of enzymes and the role of microbial fermentation in the digestion of nonstarch polysaccharides have been discussed, which also form the reason why wheat and barley samples have a higher energy availability in ruminants and pigs than in poultry. The inability of animal secreted enzymes to degrade endosperm cell wall components is of major importance for the nonruminant species. In addition, there appear to be some differences between animal species in the concentration or effectiveness of secreted enzymes for digesting specific components of cereal grains.

The accessibility of an enzyme to a grain component can be affected by particle size and surface area, physical barriers like cell walls or chemical barriers such as the tight helical structure of amylose chains, hydrophobic properties of lipid molecules, or the sequence of amino acids within proteins. The latter affects protein digestibility and may influence the accessibility of enzymes to other substrates within the grain. The rate of passage of digesta through the digestive tract can affect the time enzymes are in association with the grain components and thereby alter the extent of digestion. The main factors that are expected to contribute to differences in the energy value of cereal grains for livestock are discussed.

## **Gross Chemical Composition of the Grain**

The amount of energy available to an animal from a grain depends largely on the relative proportions of each chemical constituent because of differences in the extent of digestion or in the energy content of the constituent. There is a general positive relationship with starch content and negative relationship with fiber content of cereal grains and energy availability for all animal types. Relationships for sheep and broiler chickens are shown in Figure 3. Although the negative influence of fiber, cellulose, and nonstarch polysaccharides is less for ruminants than pigs and less for pigs than poultry, increasing fiber and lignin content of grains reduces the availability of energy from grains in ruminants. A clear negative relationship is presented in Table 3 between the lignin content of oat grains and digestibility in cattle. Lignin binds covalently to plant cell wall polysaccharides and proteins rendering them less accessible to digestive enzymes and reducing their digestibility.

The high energy availability for the naked oat sample for laying hens shown in **Table 2** is due to the high proportion of lipid and its higher energy content than other grain components. However, the energy available to broiler chickens from the same oat sample was 1.6 MJ kg<sup>-1</sup> DM lower than for layers, because of a lower concentration of lipase enzymes and lower digestion of the lipid in the younger birds.

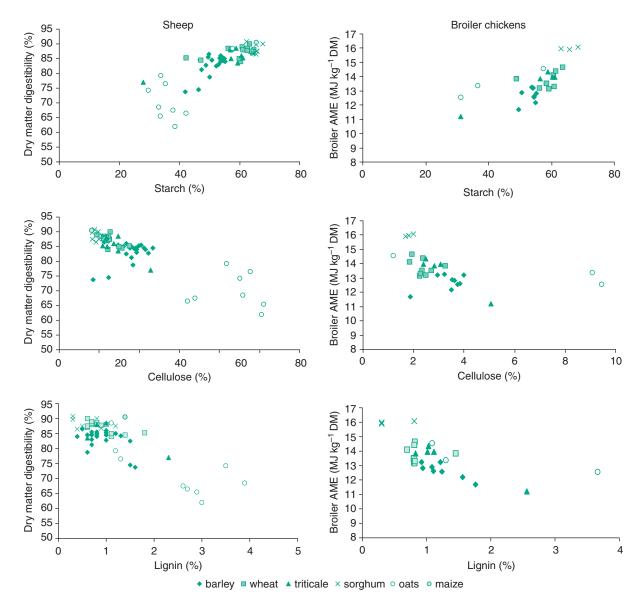
Although gross chemical composition of a grain and the digestive system are major determinants of available energy content of cereal grains for animals, other factors contribute to the variation observed between grain samples and animal types. Much of the variation that cannot be explained by chemical composition and digestive system relates to physical barriers limiting the enzyme contact with chemical components of the grain.

# Endosperm Cell-Wall Composition, Thickness, and Integrity

Endosperm cell walls are composed of a cellulose skeleton impregnated with soluble and insoluble arabinoxylans and  $\beta$ -glucans. Although these cell walls have little effect on the accessibility of starch from cereal grains for ruminants, they can reduce the contact of amylolytic enzymes with starch granules and lower-energy availability for nonruminant animals by acting either as a physical barrier or by increasing the viscosity of the digesta.

Endosperm cell walls act more as a physical barrier for pigs than for poultry. Grains eaten by birds are subjected to intense grinding in the gizzard, and most endosperm cell walls are ruptured. However, pigs appear to rupture few cells during mastication, and the availability of energy from cereal grains is increased substantially by fine grinding. Fine grinding breaks the integrity of the cell walls and exposes the starch to amyolytic enzymes. However, fine grinding does not increase the availability of energy from cereal grains for poultry, because the cells are broken during normal movement through the digestive tract. Although there is little scientific proof, it is logical to presume that cereal grains with large endosperm cells will require less processing for pigs than grains with small cells, because more starch would be available through the disruption of the same number of cell

There is strong evidence that the availability of energy from cereal grains in poultry is inversely related to the content of soluble nonstarch polysaccharides comprising largely arabinoxylans, xylans, and  $\beta$ -glucans. A linear decline has been observed in broiler AME values from 17.5 MJ kg<sup>-1</sup> DM for rice to 11 MJ kg<sup>-1</sup> DM for rye with increasing nonstarch



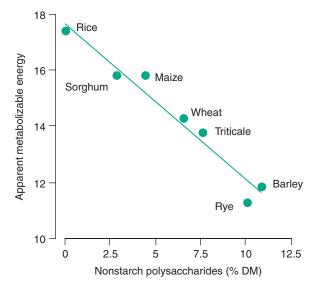
**Figure 3** Relationships between starch, cellulose, and lignin content of cereal grains and dry matter digestibility (%) in sheep and apparent metabolizable energy content (MJ kg<sup>-1</sup> DM) in broiler chickens. (Unpublished results from Flinn PC, van Barneveld RJ, and Hughes RJ.)

polysaccharide content of the grain (Figure 4). Soluble nonstarch polysaccharide compounds are believed to increase the viscosity of digesta, reduce the diffusion of digestive enzymes through the digesta, and reduce the rate of substrate digestion.

Chain length of soluble nonstarch polysaccharide polymers appears to be more important for reducing AME of wheat for broilers than is the total soluble nonstarch polysaccharide content, because of the greater increase in digesta viscosity, which reduces the digestion of starch, amino acids, and fatty acids. The addition of long chain pentosans to a sorghum-based diet fed to broiler chickens significantly reduced the availability of energy. However, if

the pentosans were first hydrolyzed to pentoses using arabinoxylanases and glucanases, viscosity of the digesta declines and the availability of energy was restored (Table 5).

Enzymes that degrade soluble nonstarch polysaccharide compounds are now regularly added to diets for poultry to reduce the viscosity of digesta and increase the access of enzymes to dietary substrates within the small intestines. The addition of nonstarch polysaccharide degrading enzymes to the Merlin barley sample listed in Table 2 increased the AME value for broiler chickens from 12.6 to 14.6 MJ kg<sup>-1</sup> DM. The observed increase in the AME content of cereal grains following several months storage after harvest



**Figure 4** Relationship between apparent metabolizable energy (MJ kg<sup>-1</sup> DM) and nonstarch polysaccharide content (% DM) of grains for broiler chickens. (Reproduced with permission from Choct M and Annison G (1990) *British Poultry Science* 31: 811–822.)

**Table 5** Effect of nonstarch polysaccharide chain length on the AME content, digestibility, and digesta viscosity for a diet fed to broiler chickens

Diet	AME (MJ kg <sup>-1</sup> DM)	Digestibility to end of small intestines (%)			Relative digesta <sup>a</sup>
		Starch	Protein	C18:0 fatty acid	viscosity
Control <sup>b</sup> Pentosan <sup>c</sup> Pentoses <sup>d</sup>	16.13 14.53 16.23	98 92 98	69 63 72	71 41 71	1.2 3.0 1.3

<sup>&</sup>lt;sup>a</sup> Relative digesta viscosity determined by time taken for an aliquot of digesta supernatant to flow through a viscometer relative to distilled water. <sup>b</sup> Control diet: 0.68 sorghum, 0.17 soybean meal, 0.076 meat and bone meal, 0.04 soybean oil plus amino acids, minerals, and vitamina.

Data from Choct M and Annison G (1992) British Poultry Science 33: 821-834.

is believed to be due to a reduction in the chain length of soluble nonstarch polysaccharides within the grain through the activity of endogenous grain enzymes.

Soluble nonstarch polysaccharides have a greater impact on energy availability for poultry than for pigs because of inherent differences between the species in both the normal viscosity of digesta and the transit time through the small intestines. The dry matter content of digesta is 16-20% in poultry compared with 7-10% in pigs and corresponding rates of passage of digesta through the small intestines are

2–4h for poultry and 12–24h for pigs. The fast transit time of digesta in poultry decreases the time digestive enzymes and grain components are in contact compared with pigs.

# **Protein Matrix Surrounding Starch Granules**

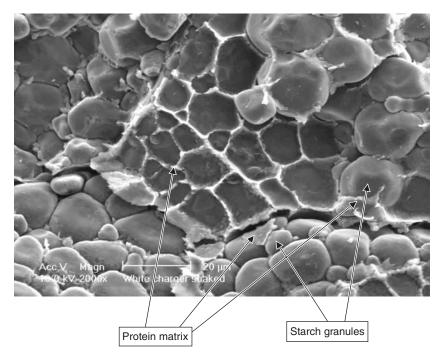
Starch granules in the endosperm of cereal grains are inserted to varying degrees in a protein matrix. In some grains such as sorghum, the protein matrix and embedded protein bodies can form a contiguous layer around individual starch granules. Figure 5 shows the protein matrix surrounding each of the starch granules of a sorghum grain when some starch granules were dislodged following soaking prior to preparation for microscopic examination.

The proteins surrounding the starch granules must be degraded to fully expose the starch to amylases. The protein matrix surrounding the starch granules in sorghum grain contains a high concentration of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -kafirins. These proteins contain increasing amounts of cysteine and methionine as they progress from the  $\alpha$ - to  $\gamma$ -types and are rich in disulfide bonds that are resistant to cleavage by some enzymes. There is strong evidence that the low availability of energy from sorghum grain for cattle is due to the inaccessibility of amylolytic enzymes to the starch granules encapsulated by the protein matrix. Waxy sorghum grains appear to have a lower proportion of γ-kafirins than normal cultivars. The marked difference in digestion of sorghum starch by cattle compared with pigs and poultry is most probably due to differences in the capacity of proteases from the different animal species to degrade the high disulfide bond proteins in the matrix surrounding the starch granules. There is also likely to be differences between animal species in the concentration of these enzymes within the small intestines, which favors the degradation of the matrix proteins by poultry and pigs.

The degree of starch granule encapsulation, amino acid composition of the protein matrix, nature of proteases, and the presence of anti-nutritional factors such as tannins and trypsin inhibitors will affect starch digestion. Many earlier cultivars of sorghum were high in condensed tannins, which bound to digestive enzymes. There is evidence that the presence of the protein matrix may affect the extent of starch digestion in maize and barley grains when incubated with mixed microorganisms from the rumen of cattle. Incubation of ground barley with proteases has been shown to increase significantly the digestion of starch. It is probable that the susceptibility of the protein matrix to proteases within the digestive tract of animals varies between barley grain cultivars as has been shown for sorghum cultivars.

 $<sup>^</sup>c$ Pentosan diet: control diet in which 0.035 (0.854 pure arabinoxylan) replaced sorghum.  $^d$ Pentose diet: control diet in which 0.015 arabinose and 0.015 xylose

<sup>&</sup>quot;Pentose diet: control diet in which 0.015 arabinose and 0.015 xylosi replaced sorghum.



**Figure 5** An electron micrograph of sorghum endosperm showing the protein matrix which encapsulates starch granules. (Unpublished results from Blakeney AB.)

# **Composition of Starch**

Cereal starch is composed primarily of amylose and amylopectin. The tight helical structure of the long chains of glucose in the amylose molecule makes it less accessible to amylases than amylopectin with its branched  $\alpha$ -(1–6) linkages. The  $\alpha$ -(1–6) glucose branches provide a more open structure to the starch molecule, which increases the accessibility to amylolytic enzymes. Grains that contain starches with low proportions of amylose are translucent in appearance and are termed waxy grains. Starches with high proportions of amylopectin have lower gelatinization temperatures than high-amylose starches, and waxy grains produce harder, more durable stock pellets due to the greater ease of gelatinization.

The rate of digestion of isolated starch from waxy sorghum is faster than that for a nonwaxy isoline and is compared with the digestion of starch from wheat and maize in Table 6. These results confirm that the rate of digestion of isolated starch is increased as the amylose content declines. Experiments with pigs have produced a significantly higher digestibility in the small intestines of starch when amylopectin-rich barley (9:91, amylose:amylopectin) was compared with normal barley (30:70, amylose:amylopectin). Similarly, the digestibility of low-amylase maize in the small intestines of rats has been observed at 96% compared with 68% for high-amylose maize. The higher intrinsic rate of digestion of starch from

**Table 6** Gelatinization temperature and susceptibility to  $\alpha$ -amylase digestion of starch isolated from several sorghum isolines, wheat, and maize

Grain	Gelatinization temperature <sup>a</sup> (°C)	α-Amylase susceptibility <sup>b</sup> (mg digested)	α-Amylase susceptibility relative to maize (100)
Sorghum			
Nonwaxy isoline	74.5	23.2	96
Waxy isoline	70.8	30.0	124
Sprouted	71.9	25.2	104
Conventional	72.6	23.5	97
Wheat	69.4	27.1	112
Maize	72.4	24.2	100

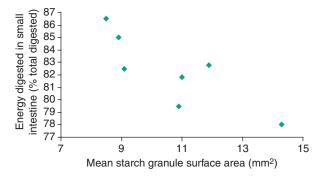
<sup>&</sup>lt;sup>a</sup>RVA 5 g/22 ml, model 4 standard 1 program.

waxy sorghum contributes to its higher rate of digestion, but the lower  $\gamma$ -kafirin content of the protein matrix is also an important factor for increasing the availability of energy in waxy sorghum for cattle.

#### Starch Granule Size

The size of starch granules in cereal grains varies widely from large A granules to small B granules. The surface area of the granules increases per unit of starch as the granule size decreases and exposes

 $<sup>^</sup>b$ 3 h digest with  $\alpha$ -amylase of 1 g starch. Unpublished results Blakeney AB.



**Figure 6** Relationship between the proportion of total energy digestion occurring in the small intestines of pigs and the average surface area of individual starch granules in wheat grain. (Unpublished results from Zarrinkalam M-R.)

a greater surface area for attachment of enzymes. The rate of digestion of cereal grains with small granules should be greater than for grains with a higher proportion of large granules, provided the small granules are not encapsulated within a protein matrix. There is evidence in pigs that increasing average granule size in wheat grain samples, as measured by average surface area of the granules, leads to a decrease in the proportion of energy digested in the small intestines (Figure 6). This result would be expected if the rate of digestion of grains with small starch granules was greater than of grains with larger granules.

#### Conclusions

The information presented suggests that the availability of energy from cereal grain varies widely between grain species, cultivars, and specific samples, and across animal types. An understanding of the reasons for these differences indicates that the energy value of grains could be improved by plant breeding and processing techniques. Clearly, an increase in the starch and decrease in the fiber content of cereal grains will increase energy availability for all animal species provided the grain compounds are accessible to digestive enzymes. A decrease in the lignin content of grains, particularly oat grain, would improve the availability of energy for ruminants. The availability of energy from cereal grains for pigs and poultry could be increased by breeding grains with thin endosperm cell walls and low-amylose starch. The digestibility of sorghum for cattle could be improved by breeding for a highly digestible protein matrix. Alternatively, the nutritional value of cereal grains could be increased by various processing techniques such as fine grinding for pigs and the addition of xylanase and glucanase enzymes to reduce the effect of cell wall constituents on digesta viscosity for poultry. Steam flaking, which gelatinizes the starch in sorghum and breaks the

integrity of the protein matrix surrounding the starch granules, has proved to be an effective way of restoring the energy value of sorghum grain in cattle to a level similar to that for pigs.

# **Acknowledgments**

The author acknowledges the contribution made by research within The Premiun Grains for Livestock Program, which is funded in part by the Grains Research and Development Corporation, Meat and Livestock Australia, Australian Pork Limited, Australian Egg Corporation Limited, the Rural Industries Research, and Development Corporation's Chicken Meat and Ridley Agribusiness. Much of the research reported was conducted by the collaborating scientists within the Program and their work is greatfully acknowledged.

See also: Cereals: Grain Diseases; Grain-Quality Attributes. Contaminants of Grain. Nutrition: Effects of Food Processing. Starch: Chemistry.

# **Further Reading**

Ammerman CB, Baker DH, and Lewis AJ (1995) Bioavailability of Nutrients for Animals. San Diego, CA: Academic Press.

Black JL (2000) Variation in nutritional value of cereal grains across livestock species. *Proceedings of the Australian Poultry Science Symposium* 13: 22–29.

Choct M and Annison G (1990) Anti-nutritive activity of wheat pentosans in broiler diets. *British Poultry Science* 31: 811–822.

Choct M and Annison G (1992) Antinutritional effect of wheat pentosans in broiler chickens; roles of viscosity and gut microflora. *British Poultry Science* 33: 821–834.

Fuller MJ (1992) In Vitro *Digestion for Pigs and Poultry*. Wallingford: CAB International.

Hughes RJ and Choct M (1999) Chemical and physical characteristics of grains related to variability in energy and amino acid availability in poultry. *Australian Journal of Agricultural Research* 50: 689–702.

Laplace JP, Fevrier C, and Barbeau A (1997) Digestive Physiology in Pigs. *Proceedings of the VIIth International Symposium*, Saint Malo, France. European Association of Animal Production Publication No. 88.

McDonald P, Edwards RA, and Greenhalgh JFD (1988) Animal Nutrition, 4th edn. Essex, UK: Longman Scientific Technical.

Moughan PJ, Verstegen MWA, and Visser-Reyneveld MI (2000) Feed Evaluation Principles and Practice. Wageningen, The Netherlands: Wageningen Pers.

Rowe JB, Choct M, and Pethick DW (1999) Processing cereals grains for animal feeding. *Australian Journal of Agricultural Research* 50: 721–736.

van Barneveld RJ (1999) Chemical and physical characteristics of grain related to variability in energy and amino acid availability in pigs: a review. *Australian Journal of Agricultural Research* 50: 667–688.

van der Poel AFB, Vahl JL, and Kwakkel RP (2001) Advances in Nutritional Technology 2001. *Proceedings* of the 1st World Feed Conference, Utrech, November 7–8. Wageningen, The Netherlands: Wageningen Pers. Wiseman J and Cole DJA (1990) Feed Evaluation. London: Butterworths.

# **Relevant Websites**

http://www.ksu.edu. http://www.ers.usd.gov. http://www.grains.gc.ca. http://www.grdc.com.au.



# **BAKERIES**

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# Introduction

Bread, in its many forms, is a staple food for mankind. It may be made in the home, one loaf at a time, or in a fully automated bakery, at a rate of tens of thousands of loaves per hour. Bread is baked and eaten in many different forms — thin or thick, soft or hard, sweet or plain, fresh or kept for many days (Figure 1). In some languages, the word for bread is the same as the word for food. Bread is practically the only food known almost universally around the world, from the most developed to the most primitive cultures, and it appears on the tables of the wealthiest and the poorest alike.

Bread is a generous gift of nature, a food that can be replaced by no other. When we fall sick, our appetite for bread deserts us last of all; and the moment we recover the appetite, we have shown a symptom of recovery. Bread is suitable to every time of the day, every age of life, and every temperament. It improves other foods, is the father of good and bad digestion. Eaten with meat or other foods, it loses none of its delight. It is so perfectly adapted to men that we turn our hearts to it almost as soon as we are born and never tire of it to the hour of our death.

Antoine Auguste Parmentier, Eighteenth Century French Nutritionist.

# **Historical Perspective**

The origins of bread making are lost in prehistory when primitive man discovered that the seeds of some grasses could be crushed, mixed with water, and heated on the fire to make an appetizing food. Advances came from extending his ability to recognize the more appropriate seed species (primitive wheats), and later to cultivate them, thus avoiding the tedium of searching and gathering them. Later, advances involved the development of better recipes and procedures for mixing and baking. Some of these arts became the trade secrets of specialists — bakers — who produced bread in quantity, for sale to their

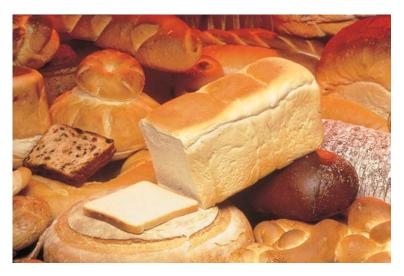


Figure 1 The square "sandwich" loaf, basic to the western diet.



Figure 2 The beehive oven was an essential part of the early bakery.

neighbors and beyond. Thus began the concept of the bakery – a place specifically designed for the efficient production of bread in quantity.

An early type of oven is illustrated in Figure 2, the so-called beehive oven. Shaped like an igloo as much as a beehive, this oven is timber-fueled, with a vent in the top for smoke to exit and to provide a draft. The stick at the right of the oven is a peel for placing dough pieces into the oven and for removing the baked bread.

# The Baking Process

Today, all bread bakeries, small or large, follow the same basic procedures. The principal differences are in the scale (batch size) and the degree of mechanization and automation. Total processing time from the mixer to the consumer for commercial pan breads can be as little as 1 h or up to more than 8 h, depending upon the process. These basic procedures carried out in the bakery are set out in Figure 3. The steps involved (1-12) are described in detail below.

- The most basic bread contains only flour, water, yeast (or a natural sour-dough starter), and salt. Its demand for use in bread and meat preservation caused salt to be one of the first items traded in antiquity.
- Ingredients must be stored carefully and measured accurately if the product is to be consistent. Home and small retail bakers may measure by volume, but large bakeries measure by weight, often with scales that automatically weigh and add ingredients to the mixer.
- 3. The dough is mixed until it is homogeneous and "developed," meaning that it is smooth and elastic

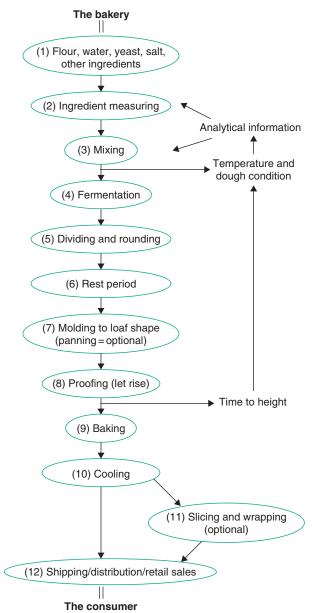


Figure 3 The basic procedures carried out in the bakery.

and able to hold the gas formed during fermentation and baking. Large batch mixers may hold 1 t or more of dough (Figure 4), but the home baker may mix just enough for one loaf by hand.

Chemical and physical test information on the dough is provided to the baker, which helps in adjusting the process for the particular formula and for the natural variation in ingredients. The dough temperature is monitored, and information on its characteristics is fed back to adjust the next batch.

4. Fermentation typically takes  $\sim 1-4$  h, though some "short time" processes take much less. The



Figure 4 A horizontal dough mixer used in a commercial bakery.

Sponge-and-Dough process involves mixing and fermentation, followed by the addition of more flour and ingredients; thereafter mixing resumes.

- 5. The dough is divided into smaller pieces, taking into account the amount of weight loss (~10%) that will occur during baking, to get the final loaf weight. Dividing typically occurs at ~100 loaves per minute in a commercial bakery, with many modern production lines now approaching 200 loaves per minute. A boutique bakery, on the other hand, may make fewer than 100 loaves per day.
- 6–7. After the dough relaxes for a few minutes, it is "molded," or shaped into loaves, usually by flattening out into a disk, then rolling into a cylinder before being placed into a pan for common round-top or square sandwich bread.
- 8. The dough is allowed to rise, or "proof" to the desired height for ~1 h under moist and humid conditions. The yeast generates additional gas by fermenting the natural and added sugars into carbon dioxide and alcohol.
- 9. Baking can take less than 2 min at very high temperatures for thin "flat breads," or up to half an



**Figure 5** Baked bread emerging from a traveling oven. (Reproduced with permission of BRI-Australia Ltd.)

hour at moderate temperatures for pan breads (Figure 5). A few very large, dense, hearth bread loaves may take several hours and bake very slowly. The loaf's interior temperature typically reaches  $\sim 95-98$ °C.

- 10–11. After the bread is baked, it is usually cooled as quickly as possible to below 40°C inside before it is sliced and packaged. If it is too warm, it will "gum" the slicer bands and tear the bread. If it is bagged too warm, condensation forms in the package and mold spots form. The slicing and bagging operations are most prone to mechanical difficulties and to product losses.
- 12. Distribution can be as simple as placing the bread on a table or rack in front of the bakery where the walk-by customer purchases it unwrapped and still warm. Alternatively, it can involve overnight truckload shipments to distant warehouses and distribution facilities before reaching the retail store where shoppers buy it after it is anywhere from 1 day to 1 week old.

## **Home Baking**

The concept of the commercial bakery has never replaced the practice of bread making at home. Irrespective of time in history or of social status, there is an irresistible attraction to the aroma of yeasted bread baking in the home kitchen and the taste of freshly baked bread, straight from the oven. In today's westernized society, the traditional art of baking yeasted bread in the home may be lost to some extent, being replaced by the baking of chemically leavened cakes and cookies, with yeasted bread being bought at the

supermarket. These changes are presumably due to the more complex procedures involved in the production of yeasted breads.

However, today's obsession with consumer goods has partly solved these problems with the provision of the baking machine, a "high-tech" computerized appliance that performs the three major steps of baking in the one unit, namely, mixing, proofing, and baking. These appliances were developed initially in Japan in the 1980s, in response to its attraction to the relatively new food, leavened pan bread, and the liking for hot, fresh bread in the home, especially at breakfast time.

The use of these baking machines has since spread worldwide. The baked product is appetizing while hot, but in general it is technically inferior to the quality of bread from a commercial bakery or even that produced by a skilled home baker. It has a relatively coarse crumb texture (due to inadequate development of the dough), less flavor and aroma (due to a short fermentation stage), and stales relatively quickly (due to the general use of a "lean" formula, lacking sugar and emulsifier, and a lower protein flour). It is thus unlikely that home baking will replace the manufacture of yeasted bread from a commercial bakery; nevertheless, the kitchen still has a significant place in the bakery story.

# **Bakeries in Western Societies**

# The Large Plant Bakery

The plant bakery is at the opposite extreme, compared to the home kitchen. Technological advances in baking have led to the development of very large bakeries, in which bread of uniform type and quality is mass produced using automatic control and uniform ingredients by expert staff with a minimum of staff numbers. There would normally be no sales at the bakery, but all baked product would be transported over a wide area to retail outlets, mainly supermarkets (see Oven Technologies).

Production in these large bakeries mainly involves sandwich loaves ("Pullman" loaves of square cross-section baked in lidded pans), with the potential to put out more than 10 000 loaves per hour. "Round-top" loaves might also be produced in plant bakeries, but there is a very limited variety of bread types. A second class of bread type from plant bakeries is the bun or dinner roll, and the rate of their production might be as high as 100 000 units per hour. Due to the need for extensive transport of the bread, a long shelf life is essential, and sophisticated packaging is needed.

Features of the plant bakery include the following:

• Computerized control of all stages of the baking process, including the prediction of consumer

- needs and scheduling the production volume for the day. The bakeries may operate 5 or 7 days per week.
- Very large bulk-storage bins for flour delivered from mills in bulk by road or rail (*see* Wheat: Dry Milling).
- Uniform quality properties for flour specifications (*see* Wheat: Dough Rheology).
- Pneumatic movement of flour and other ingredients around the bakery, thus avoiding manual handling and avoiding dust hazards.
- Filtration and temperature regulation of water used in dough mixing.
- Large mixing equipment, each mixer frequently making more than 1000 kg of dough (Figure 4).
- High-speed dough dividing and forming equipment, operating totally "hands free," with very narrow weight control.
- Automatic proofing cabinets that control the temperature and humidity; so the dough rises at the correct rate and is ready for the oven at the right time.
- Large traveling ovens to accommodate many loaves in a continuous process of baking (Figure 5) (see Oven Technologies).
- Regulation of oven temperatures, humidity, heat transfer rate, and speed to ensure uniform baking of all loaves.
- Automated facilities for the cooling, slicing, and packaging of loaves.
- Automated palletizing and loading of the delivery trucks.

This type of large bakery is most common in major centers of population in westernized countries — particularly North America, Europe, Australia, and New Zealand — and is characterized by stiff competition and a very low profit margin. France may be an exception, with a continuing preference for the smaller trade bakery, providing freshly baked bread to a local neighborhood.

# The Independent "Scratch" Bakery

Like the French model of a local-neighborhood bakery, this is the traditional type of bakery, with one or a few skilled bakers making a wide range of bread types for sale on-site on a scale much smaller than that of the plant bakery. Bread is made "from scratch," i.e., all basic ingredients are incorporated in the bakery, as distinct from recent developments such as the "in-store" bakery (see below) where only the final stages of baking are performed. This type of bakery is often a family business catering for local needs and parochial preferences for bread types. Some "ethnic" bread types are still made in those

bakeries located in neighborhoods with many people from a common ancestry or country. It thus provides a valuable local resource, even a local meeting place, but it may be much less efficient than the plant bakery in terms of the production cost per unit. In recent decades, there has been a loss of this type of bakery, in some cases because the operators of plant bakeries have bought them out in order to expand their distribution network. In North America, the few remaining small local bakeries often produce principally cakes and pastries rather than the low-margin white pan bread. The majority of the 1200 British in-store bakeries (in supermarkets) are based on scratch production - not frozen dough or bake-off. Some other European countries have similar high numbers of scratch in-store bakeries (e.g., France, Hungary, and Portugal).

# **Hot-Bread Shops**

This type of modest-sized operation has reappeared in recent decades in the form of the franchised "hotbread shop," often located in shopping centers. In this type of bakery, all goods are baked within the bread shop for direct sale at the shop-front. In general, recipes and equipment are standardized. Some products might be produced "from scratch," but in many cases the earlier stages of production might be short cut, for example, by the use of bakery premixes containing all ingredients so that the main addition is water before mixing. In such bakeries, only a modest level of skill may be needed on the part of the bakery staff, thereby reducing costs. Nevertheless, a wide range of goods may be produced, including many "fancy" types of bread, sweet pastries, and filled doughs such as pies.

#### **In-Store Bakeries**

A further innovation of recent decades involves "taking the bakery to the people," namely, into the supermarket. This concept involves performing the last stage of the process, the baking step, within the supermarket so that the baked products are presented freshly baked for immediate sale. In this case, relatively unskilled staff can be used to load parbaked or frozen dough pieces into the oven for baking (rack- or reel-type oven) under prespecified conditions (see Oven Technologies). Although skill requirements may be modest, a range of bread types can be offered by the in-store bakery, thereby satisfying local demands.

The critical contribution to the success of the instore bakery is the supply of frozen doughs or parbaked foods. These are produced at a centrally located "commissary bakery" and may be distributed relatively long distances. The commissary bakery may be a large plant bakery with automated facilities to take the baking process through to either the proof stage (before freezing) or the partly baked stage as parbaked (before refrigeration or freezing and transport). Recent trends favor the parbaked product for this concept. It can be stored and transported at room temperature for up to  $\sim$ 7 days, though some bakers claim shelf life of up to 2 weeks. Longer storage requires cooling or freezing to extend the shelf life of frozen dough to  $\sim$ 3 weeks and of parbaked dough up to several months if properly packaged and deep-frozen.

Frozen dough pieces are sometimes stored for much longer but the freezing process damages both the yeast and the gluten quality, so that higher levels of gluten must be used, together with special yeast strains (*see* Gluten and Modified Gluten). There have been some attempts to sell the frozen dough pieces directly to the homemaker, but a substantial amount of time is still required to properly thaw, proof (raise), and bake the dough. As a result, the use of frozen dough has never reached predicted levels in direct home sales. However, certain institutional applications, such as frozen pizza crust disks, have been very popular in some niche markets where, even when some skilled staff are available, they can save on the mixing and forming stages with their long lead times.

# **Old-World Bakeries**

Bakery operations in the Middle East, Asia, and Latin America are generally on a small scale, catering for the local community, and producing baked goods that may once have been regarded as "exotic" by westerners. Labor and skill input is considerable, sales are on-site, and there is little need for packaging or for extensive transportation of the product.

The range of products is unlikely to include much of the western-style leavened loaf, but its popularity is increasing in these regions. More likely, these bakeries produce traditional breads suited to the local demand. In the Middle East, this includes various types of flat breads (Figure 6). Some of these are pocket breads that open in two layers for the insertion of pulses or other fillings. Thicker flat breads are popular in Turkey, North Africa, and India. Baked at very high temperatures, they have a very coarse, open, texture with a thick crust and are often "decorated" with the baker's finger marks on the top.

Tortillas were once considered to be "ethnic" breads (*see* Tortillas). With the growth of the Hispanic population in North America, however, they



Figure 6 A bakery in which Arabic flat breads are produced.

are now widely available and their production is switching from small artisan bakeries to large mechanized high-speed production. Hispanic types are speckled with dark brown spots and an irregular surface. Chinese preferences are for tortillas to be very white and smooth. Both types are used as wraps for filling in the center, as for a "Taco" or "Peking Duck."

A number of small "boutique" bakeries have begun to appear in some areas. They tend to use very limited equipment to produce a small (and expensive) product, baked by residual heat in preheated heavy masonry ovens derived from the ancient "beehive" designs (Figure 2).

# **Specialized Baked Goods**

Beyond the scope of what we would normally class as "bread" in its widest sense, there is a wide range of specialized bakery products. Many of them are sweet but some are savory, some are yeast leavened, but others are chemically leavened. The list includes foods such as cinnamon rolls, pretzels, doughnuts, snack foods, bagels, many types of cookies and crackers, cakes, and pastries. Bakeries differ considerably depending on the procedures and equipment needed for this diverse array of foods.

Some of these such as crackers and cookies are produced in vast quantities on very large, highspeed, automated ovens, whereas most of them individually involve small product runs. An outstanding example in the high-speed class is given by the many types of cookies and crackers made by Nabisco, making it the largest baking company in the USA, yet the company produces no bread.

# **Future Perspective**

The future is likely to see the spread of larger planttype bakeries into the centers of population in countries where traditional bakeries have always been the norm. This transition will involve the adaptation of automation and mechanization to bread types that have not previously been produced in such quantities and uniformity. Nevertheless, history has shown that the "bottom line" that determines the success or otherwise of any such innovation is the reaction of the consumer. It is the common person who buys the bread, selecting the source and type of their choosing, that ultimately determines the fate of any bakery.

See also: Cakes, Chemistry of Manufacture. Milling and Baking, History.

# **Further Reading**

Faridi H and Faubion J (1995) Wheat End Uses around the World. St. Paul, MN: American Association of Cereal Chemists.

Kulp K, Lorenz K, and Brummer J (eds.) (1995) Frozen and Refrigerated Doughs and Batters. St. Paul, MN: American Association of Cereal Chemists.

Pyler EJ (1988) Baking Science and Technology, 3rd edn. Kansas City, KS: Sosland.

Qarooni J (1996) Flat Bread Technology. New York: Chapman and Hall.

Quail K (1996) Arabic Bread Products. St. Paul, MN: American Association of Cereal Chemists.

# **Relevant Websites**

http://www.aaccnet.org - American Association of Cereal Chemists.

http://www.aibonline.org – American Institute of Baking.

http://www.asbe.org - American Society of Baking.

http://www.asb.org.au – Australian Society of Baking.

http://www.bbga.org - Bread Baker's Guild of America.

http://www.bri.com.au - BRI Australia Ltd.

http://bsb.org.au - British Society of Baking.

http://www.campden.co.uk - Campden and Chorleywood Food Research Association.

http://www.oznet.ksu.edu/dp\_grsi - Kansas State University.

# **BARLEY**

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Genetics and Breeding
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# **Genetics and Breeding**

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# Introduction

Knowledge about barley genetics is expanding at a rapid rate, as molecular and information technologies provide increasingly detailed information about gene structure and function. The purpose of this article is to give an overview of the subject area, with references to recent reviews and online databanks that provide in-depth coverage of specific topics. The discussion will focus on traits and methodologies that have the greatest significance for applied breeding programs.

Barley (Hordeum vulgare L.) is one of the oldest domesticated crops, and is expected to have originated in the Fertile Crescent region of the Near East around 8000 BC. It has been used both as food and as a principal ingredient in fermented beverages since ancient times. Today, about two-thirds of the annual world production of 136 million metric tons (Mt) is used for animal feed (FAOSTAT). Among the cereal crops, barley ranks fourth in production after wheat, rice, and corn.

Cultivated barley is one of 31 *Hordeum* species, belonging to the tribe Triticeae, in the grass family (Poaceae). It is an annual, self-pollinating, diploid species with 2n = 14 chromosomes. The relative simplicity of the genetic system and diversity of the species make it an ideal organism for genetic studies and breeding efforts. Currently the wild ancestor of barley (*H. vulgare* subsp. *spontaneum*) is expected to be a subspecies of cultivated barley, and cultivated barley is classified in the subspecies *vulgare*. Wild barley has a brittle rachis and occurs only in the two-row

form. Cultivated barley has a nonbrittle rachis and may be the two-row or the six-row form. *H. vulgare* subsp. *spontaneum* may be a transitional form between the true progenitor of barley and the cultivated species.

# **Morphological Diversity**

Full descriptions of morphological mutants and the genes that control them were published in the "Barley Genetics Newsletter" in 1997 (http://wheat.pw.usda.gov).

# **Two-Row versus Six-Row Barley**

Barley has a single floret in each spikelet. There are three spikelets at each rachis node, alternating on opposite sides of the barley head or spike. In two-row barley, the central floret is fertile and the two lateral florets are sterile, resulting in a single seed at each node, giving the head a flat appearance (Figure 1a). In the six-row barley, all of the florets are fertile (Figure 1b). The central seeds are round and plump, but the laterals tend to be slightly asymmetric. A single head of barley can produce up to 80 seeds.

The development of lateral spikelets and lateral awns is controlled by alleles at the *vrs1* locus on the long arm of chromosome 2 (2H). Most six-row cultivars possess the recessive *vrs1.a* allele. *Vrs1.b* occurs in many two-row cultivars and is dominant to *vrs1.a*. Rather complex interactions occur with other alleles at this locus, and with alleles at the *int-c* locus on chromosome 4 (4H) to create

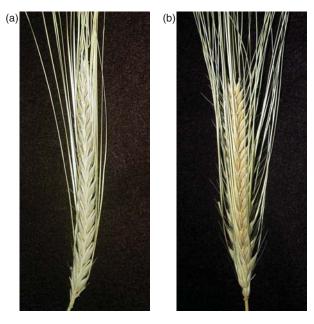


Figure 1 Heads of the: (a) two-row barley and (b) six-row barley.

intermediate phenotypes. Commercial two-row varieties are usually recessive for *int-c* while six-row cultivars have the dominant *int-c* allele.

Two-row varieties usually have a higher number of tillers per plant and plumper, heavier kernels than sixrow varieties. Six-row varieties, on the other hand, usually have more seeds per inflorescence. Thus, the compensatory effects of yield components lead to similar levels of yield potential. Two-row barleys are favored for malting throughout most of the world, but in the USA and Mexico, six-row barleys are used extensively for this purpose. Given that two-row and six-row germplasm have historically been maintained in separate breeding pools, one might expect that crosses between the two groups would produce some useful segregants with superior agronomic and malting characteristics. The experience of plant breeders, however, has been that two-row × six-row crosses do not generally provide useful progeny for variety development.

# **Specialty Types**

Most barley varieties are hulled types, with the lemma and palea remaining attached to the seed at maturity. Threshing involves removal of the kernel from the rachis and removal of the awns or hoods from the tip of the lemma. Varieties that are difficult to thresh retain part of the awns or rachis, and may have lower test weight and quality for feed or malting. In hullless or naked types, the seed separates from the lemma and palea during threshing. The hull-less characteristic is controlled by a simple recessive allelle at the nud locus on the long arm of chromosome 1 (7H). Hull-less types are produced for various food and beverage uses in East Asia, primarily, China, Japan, and Korea. Hull-less barley is an important subsistence crop in the Andes and Himalayan regions and in Ethiopia. In Canada, hull-less varieties are commonly grown as feed for swine.

Variation in the morphology of barley awns may also influence the suitability of cultivars for particular end uses. Most cultivars have awns, which may be rough or smooth, but awnless types also exist. Hooded types have modified awns that consist of a deformed, inverted floret with leaf-like appendages. This trait is controlled by a single dominant gene at the *Kap* locus located on the short arm of chromosome 4 (4H). Hooded varieties are preferred for hay, silage, and green chop.

Blue aleurone is another common variant of barley, which is controlled by alleles at five *Blx* loci with complementary effects. In general, white aleurone types are preferred because the kernels have a brighter appearance.

# **Adaptive Characteristics**

Different gene pools have evolved in the major barley production areas of the world, with the necessary physiological characteristics for adaptation to each environment. Appropriate maturity, vernalization, and photoperiod response are all major features required for adaptation. The distinct attributes of gene pools may also reflect requirements for important end uses of barley, or personal preferences established over time as the result of the history of barley in the region.

Yield is a primary objective of any breeding program, and steady increases have been attained over the years. Modern cultivars have a higher harvest index than older cultivars, indicating that more of the aboveground biomass has been partitioned to the grain. Improvements in yield are achieved through manipulation of yield components, including number of spikes per unit area, number of kernels per head, and kernel weight. Modern cultivars have several productive tillers per plant and are resistant to shattering. Shattering may be due to seeds separating from the head or from spikes breaking off before harvest. Varieties that are highly resistant to shattering may be difficult to thresh, so breeders must consider several traits simultaneously when making selections.

Good lodging resistance requires short, strong stems and a sturdy root system to anchor the plant in the soil. Lodging has been reduced in modern varieties through increased straw strength and reduced plant height. Semi-dwarf stature may be controlled by one or several genes. Selection for reduced height can have undesirable effects on yield and other characteristics, so again it is important to consider multiple traits when attempting to improve lodging resistance.

Barley varieties should be bred to have the optimum heading date for their target environments. Early maturity is a very important adaptive trait at higher latitudes. For areas with a longer growing season, it is best to grow varieties that take full advantage of the available moisture and favorable temperatures. Barley generally requires long days to initiate flowering, but cultivars vary in their photoperiod sensitivity. Barley has a slightly shorter life cycle than other cereal crops, which may enable it to escape drought in some environments. It is also relatively tolerant to drought, and has the capacity to send up new tillers when conditions become favorable. Plants are most adversely affected by drought during floral initiation and anthesis.

Barley is more salt tolerant than many other crops and is often used to reclaim saline soils.

Breeding programs have been successfully carried out for increased salt tolerance. Varieties have also been developed with tolerance to aluminum and soil acidity.

# Winter Barley

Winter barley varieties usually require a period of exposure to cool temperatures (vernalization) to initiate flowering, and must also be cold hardy. Facultative varieties do not require vernalization, but they are tolerant to cold temperatures, so they can be planted in the fall or in the spring. Winter barley is potentially higher yielding than spring barley, in areas where it is adapted. However, winter barley varieties are generally not as tolerant to cold as other winter cereals. Cold tolerance in barley has been a difficult trait to improve with conventional breeding methods. It is best to screen breeding material under uniform cold stress in the laboratory, because screening for winterhardiness in the field is seldom reliable. Good lodging resistance and disease resistance are also important characteristics for winter varieties.

## **Disease and Insect Resistance**

Commercial varieties must possess adequate levels of resistance to the disease and insect pests prevalent in their target production zones. It is often stated that qualitative resistance determined by single genes can be overcome by new strains of a pathogen, whereas quantitative resistance, although not absolute, tends to be more durable over time. This is a useful guiding principle for development of resistant varieties, but there are exceptions to this rule. Barley is affected by many diseases, most of which are typical of small grain cereals, e.g., rusts (*Puccinia* spp.), smuts (*Ustilago* spp.), root rots (*Rhizoctonia*, *Pythium*, *Fusarium*), bacterial blights, and viruses (*see* Cereals: Grain Diseases).

Breeding for insect resistance is a difficult task, because it is usually necessary to rear the insects to obtain uniform, reliable screening environments. Green bug (*Toxoptera graminum*), Russian wheat aphid (*Diuraphis noxia*), jointworm (*Tetramesa hordei*), and the cereal leaf beetle (*Oulema melanopus*) have all received some attention in barley breeding programs.

# **Breeding Strategies**

## **Breeding Objectives**

Breeding programs have traditionally emphasized yield and adaptation. The primary goal has been to

develop malting types, because they receive a premium price in the market.

Furthermore, malting and brewing industries have provided much-needed support for breeding programs. In contrast, relatively little effort has been made to improve barley for feed or food uses.

A common strategy in breeding programs is to breed for good malting quality, with the expectation that resulting varieties will also be good for feed. This approach is based on the assumption that the nutritional requirements of yeast in the brewing process are not too different from those of animals, particularly monogastrics. This theory has been researched and debated for many years. Among existing varieties, there does appear to be some association between quality for feed and quality for malting and brewing. Others argue that existing varieties may not truly represent the potential for high-quality feed barleys, because feed quality has not been a specific objective of breeding programs.

There has been considerable debate about the best ideotype to select to increase yield in barley, because there are often compensatory effects among yield components. Studies of yield components have led to a better understanding of changes brought about by selection, but have provided few useful tools with which to improve selection efficiency.

#### **Quantitative and Qualitative Traits**

Many of the important traits determining yield, malting, and feed quality are quantitatively inherited, which means that they are controlled by many genes and are measured on a continuous scale. Quantitative traits may be influenced considerably by environmental conditions during the growing season. Genotype—environment interaction is also common for quantitative traits, which complicates selection in a breeding program, because genotypes that perform the best in one environment may not perform as well in another. Genotypes must be evaluated over multiple sites, and advanced lines must be tested in several seasons to assess quantitative characters.

Qualitative traits that are controlled by one or just a few genes are fairly easy to manipulate in a breeding program, because progeny from crosses between selected parents usually exhibit predictable Mendelian segregation patterns. The situation with quantitative traits is far more complex, because one is never sure whether the parents selected have all of the favorable genes controlling the trait. Furthermore, it is necessary to screen large numbers of progeny to have a good chance of obtaining the favorable genes from both parents in one of the offsprings.

# **Breeding for Quantitative Characters**

Barley breeders and geneticists have approached the challenge of understanding the inheritance of quantitative traits using biometrical tools. Certain traits such as plant height, disease resistance, vernalization requirement, and kernel composition tend to have high heritabilities, which means that the phenotype is determined to a large extent by genetics. Traits with high heritabilities are fairly responsive to selection. Others, such as yield and many malting quality parameters, have lower heritabilities and are more subject to environmental influences. Information about genetic variances and heritabilities have helped to refine selection methods, but still leave many questions unanswered regarding the relationship between genes and adaptation.

The advent of molecular markers and quantitative trait locus (QTL) analysis in the late 1980s provided a means to determine how many genes are controlling a trait, their relative importance in determining the phenotype, and their location on the chromosomes. QTL markers have made it possible to track the transmission of favorable genes from one generation to the next, assisting breeders to choose the best parents for a cross and to select the best offspring.

# **Barley Cultivation in a Breeding Program**

The management practices needed to grow barley in a breeding program are similar to those used in commercial production (*see* Barley: Agronomy). Cool temperatures are needed during early growth and establishment and spike development, particularly if crosses are to be made. Anderson and Reinbergs reviewed the techniques available for emasculating and pollinating barley. The anthers must be removed or destroyed when they are still plump and green or greenish-yellow, to prevent self-pollination. Stigmas of the female flowers become feathery and receptive to pollen 1–3 days later. Barley pollinations are most successful in the morning, when the anthers naturally dehisce.

#### **Breeding Methods**

The most common approach utilized for barley improvement has been pure line variety development through conventional pedigree and backcross breeding techniques. In other programs, bulks of segregating families are evaluated in parallel with pure line development, to permit assessment of quantitative characters in earlier generations. Single seed descent (SSD) has also been used effectively for spring or facultative germplasm, to rapidly advance material from the  $F_2$  generation to the  $F_4$  or  $F_5$  generation in the greenhouse, where it is possible to grow three

generations in a year. For germplasm with a winter growth habit, one can rely on the natural cold temperature exposure in the field, or use a growth chamber to advance generations more quickly in the greenhouse. Another possibility is to use doubled haploid techniques to advance directly from the F<sub>1</sub> generation to homozygous pure lines. One possible advantage of SSD compared to doubled haploid production is that the additional generations of selfing provide additional opportunities for genetic recombination. Another consideration is the genetic stability of completely homozygous lines derived from haploid methods. With a few exceptions, the general conclusion from experiments comparing conventional, SSD, and haploid breeding methods is that the mean and variance of yield performance are similar for lines derived from the three methods. The choice of methods will depend on breeding objectives, the relative time and expense involved, and the facilities available.

Once the desired level of homozygosity has been attained, single head rows may be evaluated in a breeding nursery for highly heritable traits such as disease resistance and agronomic type. A bulk from a single head row can be evaluated in preliminary yield trials at two locations. In the next generation, adequate seed should be available to initiate advanced yield trials and tests for malting quality.

#### **Mutation Breeding**

Chemical mutagens and ionizing radiation have been used extensively to induce new variation in barley. Most mutations are recessive and most are deleterious. Nonetheless, mutation-induced variation has been used in conjunction with conventional breeding methods to generate barley varieties with semi-dwarf stature, early maturity, and disease resistance. A number of useful mutations in kernel composition have been induced, including high protein, high lysine, low  $\beta$ -glucan, and proanthocyanidin-free mutants.

#### **Doubled Haploids**

In barley, several techniques have been used to create doubled haploids from female or male gametes; among the most effective are anther or microspore culture and the "bulbosum" method. To apply the "bulbosum" technique, female F<sub>1</sub> lines of *H. vulgare* are crossed to male lines of *H. bulbosum*. After fertilization, the *H. bulbosum* chromosomes are spontaneously eliminated from the hybrid embryos. The haploid embryos are excised and grown in tissue culture in the presence of colchicine to double the

chromosome number. The resulting plants are comparable to those that would be obtained after many generations of selfing from an  $F_1$  plant.

# **Barley Hybrids**

Many sources of genetic male sterility have been identified in barley, and cytoplasmic male sterility and restorer systems are known in *H. vulgare* subsp. *spontaneum*. Gametocide treatments can also be used to induce male sterility.

Although hybrid vigor may be advantageous for hybrid production, it may have undesirable consequences for malting quality. Uniform germination is essential for the malting process, but the  $F_2$  grain harvested from an  $F_1$  hybrid may be highly variable. The use of parents with similar malting quality may reduce this problem, but may also reduce the heterosis in the cross if the parents are genetically related.

Another barrier for hybrid production is the fact that barley normally sheds pollen while it is still in the boot stage, which limits the extent of cross-pollination to a male-sterile female line. Increased cross-pollination and hybrid seed set can be achieved through selection, however.

# **The Barley Genome**

The haploid barley genome size is  $(4.9-5.3) \times 10^9$  bp, which is considerably larger than the rice genome. However, only ~12% of the DNA codes for functional genes, and at least 75% of the genome consists of highly repetitive DNA sequences. The genome is organized into regions that have many genes and regions that contain few functional genes.

Barley chromosomes were originally numbered from 1–7 based on relative length during mitotic metaphase. The first five chromosomes have no satellites, and are ordered from longest (chromosome 1) to the shortest (chromosome 5). Chromosome 6 is longer than chromosome 7, and both have satellites (terminal sections of the chromosomes separated by a narrow constriction). Each of the chromosomes of barley can be distinguished with the use of Giemsa Cand N-stains which reveal distinct banding patterns during mitotic metaphase.

#### **Molecular Mapping in Barley**

Comparisons of genetic maps among the members of the grass family have shown that different species have many of the same genes and genetic markers, which often occur in a similar order along the chromosomes. Within the tribe Triticeae, the barley genome is highly collinear with the A, B, and D genomes of wheat, with only a few minor inversions and translocations between the two species. Chromosomes 1–7 in barley are expected to be homeologous to chromosomes 7H, 2H, 3H, 4H, 1H, 6H, and 5H, respectively, in wheat. Some regions of the barley genome are highly collinear with rice, but there are others that show some rearrangements. Nonetheless, genetic probes for rice have proven to be very useful for mapping in barley.

Molecular mapping in barley was facilitated by the existence of many morphological mutants that could be readily scored and assigned to linkage groups. Cytogenetic stocks, such as the barley—wheat substitution lines, were invaluable tools that enabled researchers to assign markers and linkage groups to specific arms of the barley chromosomes, and to determine the location of centromeres. Based on the location of telomeric markers, the combined length of all of the barley chromosomes has been estimated to be ~1500 cM.

Initial genetic maps of barley relied on morphological markers, isozymes, and known genes for disease resistance and storage proteins. Since the late 1980s, many genetic maps of barley have been published using various types of genetic markers including RFLPs, RAPDs, SSRs, and AFLPs. Mapping populations consist of randomly derived, segregating progeny from crosses between diverse parents. The distance between markers in a linkage group is estimated based on the frequency of recombinant (nonparental) genotypes in the mapping population. A database of barley maps is maintained on the GrainGenes website (http:// wheat.pw.usda.gov/index.shtml). Collectively, over 2000 molecular markers have been mapped in the barley genome. However, many markers are unique to particular mapping populations, or map to slightly different locations in different genetic backgrounds. A bin map has been developed which attempts to integrate information from many different sources (http://barleygenomics.wsu.edu/). Markers are assigned to ~10 cM segments or bins along each chromosome, which can be identified by specific markers that are common to many of the published genetic maps. This map is a good starting point for researchers who wish to obtain a fine resolution map of a particular chromosome region.

Although the occurrence of genetic markers and their locations on chromosomes are unique to each mapping population, in general, there have been few, if any large-scale rearrangements of chromosomes observed among the mapping populations studied.

The doubled haploid technique has proven to be very useful in developing mapping populations that can be maintained over time. The genetic maps can be refined using increasing numbers of markers, and the lines can be evaluated phenotypically in different experiments for many traits of interest (*see* Genome Mapping).

Physical Maps Maps of physical distances along the chromosomes present quite a different picture of the barley genome than genetic maps based on recombination frequencies. Recombination is uncommon in the region of the centromeres, so genetic distances tend to be relatively longer than the physical map in those regions. Recombination appears to be greatest near the end of chromosome arms, but defined regions of high recombination are also found in interstitial areas of each arm. The barley genome is organized into gene-rich and gene-poor regions. There is evidence that the gene-rich areas correspond to areas of high recombination, where marker densities are greatest.

# **Analysis of QTLs**

Individuals in a mapping population are evaluated both for their marker genotypes and for phenotypic traits of interest. Statistical techniques are used to determine if there is a relationship between particular phenotypes and the occurrence of specific marker alleles. If the relationship is significant, then it is assumed that a QTL for the trait is closely linked to that marker. Haves et al. provide summaries of all of the published results from QTL studies in barley (http://barleyworld.org/NABGMP/qtlsum.htm), cluding traits associated with abiotic stress resistance, agronomic traits, biotic stress resistance, and malting quality. More than 750 QTLs have been identified, and for each category, QTLs have been distributed across all seven chromosomes. A high number of QTLs mapped to the vicinity of the vrs1 locus on chromosome 2 (2H), which controls the two-row versus the six-row character. It is not known whether this is due to close linkage of QTLs with vrs1 or due to pleiotropic effects at that locus.

#### **Marker-Assisted Selection**

Molecular markers have been utilized successfully to select for closely linked genes and QTLs. Laboratory screening can be carried out during the off-season, to attain more rapid progress in a breeding program. Commonly used markers such as SSRs can discriminate homozygous dominant from heterozygous genotypes, which would have identical phenotypes in a conventional selection program. Marker-assisted selection (MAS) is particularly useful for introducing genes from exotic germplasm, because selection can be carried out both for the genes of interest from the donor parent and the background genotype of the

recurrent parent, reducing the deleterious effects of linkage drag. In the USA and Canada, MAS is being used to incorporate mutations for low phytic acid into elite germplasm. MAS has been used successfully in barley to pyramid multiple genes for stripe rust resistance, providing a higher and more durable level of resistance against diverse strains of the pathogen. MAS was used to develop the variety "Valier" in the USA with superior agronomic and feed quality characteristics. Despite these benefits and successes, MAS has not been applied widely in breeding programs. Its greatest advantage appears to be for traits such as yield and malting quality that have low heritabilities and require extensive screening using conventional approaches. Thomas provides further discussion of the potential and limitations of MAS in barley.

# **Feed Barley**

Barley is fed to livestock both as grain and as forage (see Animal Feed). Research has shown that there is considerable genetic diversity among barley varieties in terms of nutritional quality and suitability for feed and food uses, but these objectives have not received much emphasis in breeding programs. The price of feed barley is usually determined by simple physical characteristics such as percentage of plump kernels and high test or volume weight. The genetics of feed quality attributes have been reviewed recently.

High test weight is a positive attribute for feed barley, as higher kernel density tends to indicate higher nutritive value, due to higher content of starch and protein, lower fiber, and reduced air space. Kernel plumpness and uniformity are desirable, because plump kernels tend to have higher starch (energy) content. Uniformity is important during feed processing, which may involve dry or steam rolling. Particle size of cracked barley is a characteristic that has recently been shown to influence feed quality for ruminants. Studies to date indicate that larger particle size may confer a slight benefit to animals, but some compromise in particle size may be necessary to optimize all of the parameters used to determine feed quality.

Starch content varies considerably among barley varieties, but typically constitutes  $\sim 55\%$  of the kernel. Thus, the main value of feed barley is as an energy source. Typically, starch in barley is composed of 75% amylopectin (a branched chain molecule) and 25% amylose (an unbranched, straight-chain molecule). Genetic variants range from little or no amylose (waxy types) to a high of  $\sim 45\%$  amylose. Both mutations are controlled by single genes and tend to be associated with reduced starch and increased  $\beta$ -glucan levels. Waxy types are homozygous

recessive at the *wax* locus on the short arm of chromosome 1 (7H). High amylose mutants have recessive alleles at the *amo1* locus on chromosome 3 (3H).

Levels of protein and amino acids vary among barley varieties and are also influenced by environmental conditions. QTLs for grain protein content have been identified on all seven chromosomes of barley, with concentrations of QTLs on chromosomes 2 (2H), 4 (4H), and 7 (5H).

Like all cereals, barley is relatively low in lysine. High lysine mutants were first reported in 1970, and have been the subject of much research and breeding effort. Most of the high lysine mutants are determined by single recessive genes, but many have pleiotropic effects on other components of the kernel, such as the composition of starch, lipid, and protein fractions. Shrunken endosperm is another common attribute of high lysine mutants. The nutritional benefits of high lysine barley have been clearly demonstrated in nonruminant diets, but undesirable pleiotropic effects present a challenge to breeding programs. The agronomic characteristics of high lysine varieties have never met commercial standards, despite intensive breeding efforts.

Compared to other cereal crops, lipid levels are relatively low in barley. Approximately 25% of the kernel lipids occur in the embryo, with the remaining 75% in the endosperm. Attempts to screen germplasm collections for high fat barley mutants have had only limited success; the maximum lipid content identified was ~7%. Nonetheless, barley lipids may be important from a human nutrition standpoint because they contain antioxidant tocotrienols, which inhibit cholesterol synthesis.

The hull constitutes 10-15% of the dry weight of the kernel and is the major contributor of crude fiber, which for the most part is nutritionally unavailable to nonruminants and poultry. The fiber content of hullless barley is lower, comparable to that in maize and wheat. The source of fiber is primarily the structural components of cell walls (nonstarch polysaccharides and lignin). Acid detergent fiber (ADF) measures insoluble fiber (primarily cellulose and lignin) and is a commonly used indicator of fiber content. Values for ADF range from 2% to 12%. Other components of fiber are soluble to some extent, including the  $\beta$ -glucans, which predominate in the starchy endosperm cell walls, and arabinoxylans (pentosans), which are concentrated in the cell walls of the hull and aleurone. QTLs have been identified for  $\beta$ -glucan content and ADF on chromosomes 2 (2H), 4 (4H), and 5 (1H), indicating that the inheritance of these traits is complex.

The testa of barley contains proanthocyanidins, which are polyphenolic or flavonoid compounds similar to those found in sorghum. There is some evidence

that these compounds may form insoluble phenolprotein complexes, which create haze in beer and reduce the digestibility of protein in animal feed. Other studies have shown that the effects on digestion are minimal because the phenolic compounds occur in very low concentration. Numerous mutants that are deficient in proanthocyanidins have been identified. All that have been studied to date show single-gene recessive inheritance. The most useful mutants from a breeding standpoint appear to be those that alter anthocyanin production in the last step of the flavanoid pathway. Other mutations that affect anthocyanin or proanthocyanin production also reduce vigor and yield.

Minerals occur in barley kernels in low concentrations but are important for animal nutrition. Phosphorus, potassium, and calcium occur in greatest quantities with lesser amounts of chlorine, magnesium, sulfur, and sodium. The hull has the highest concentration of minerals, followed by the embryo, with lowest concentrations in the embryo. Phosphorus is an important mineral in animal nutrition, but 80% of the phosphorus in barley is unavailable because it is bound to phytic acid. Phytic acid also binds to other important minerals, including calcium, iron, magnesium, and zinc. Animals cannot digest these phytate compounds, and the minerals that are not absorbed are excreted in feces. Excess phosphorus in animal wastes can lead to contamination of groundwater, creating environmental problems. Recently, low phytic acid mutants of barley with normal phosphorus levels have shown promise as a means for increasing the availability of phosphorus in animal diets while reducing levels of phosphorus in animal waste.

#### **Animal Performance Tests**

Animal performance is the ultimate test for feed quality, but measurement of animal response requires large quantities of barley feed, and considerable time and expense. To effectively breed for feed quality, one would need to assess many genotypes. Several approaches have been developed to measure digestibility, using much smaller quantities of feed. The nylon bag method involves introducing feed into an animal's digestive system in a small mesh bag, and then monitoring the disappearance of the feed after the bag is recovered from the rumen or the feces. The rate of disappearance of feed *in vitro* when incubated with an animal's digestive enzymes is another commonly used measure of digestibility. At some point these results must be verified with full-scale animal feeding trials. Rats, chicks, or weanling pigs are also used for digestion or metabolism trials, and provide good indications of feed quality using limited sample sizes. Results from animal feeding trials have shown that feed quality characteristics in barley are heritable, i.e., they can be manipulated in a breeding program because they are determined to some extent by genetics.

# **Malting Barley**

There are no absolute definitions of malting and brewing quality, due to differences in malting and brewing practices and consumer preferences. In the USA, the American Malting Barley Association (http://www.ambainc.org/index.htm) provides breeders with recommended target levels for traits associated with malting and brewing quality.

#### **The Malting and Brewing Process**

During malting, barley kernels are germinated under controlled conditions. Good malting varieties will break dormancy rapidly and germinate uniformly. These characteristics are controlled to some extent by endogenous levels of the plant growth regulators gibberellic acid (GA) and abscisic acid (ABA). During germination, hydrolytic enzymes are synthesized or activated to partially degrade large starch, protein, and nucleic acid molecules into sugars, amino acids, and nucleic acids that can support the growth of yeast and fermentation processes during brewing. The "diastatic power" of a malt indicates its enzymatic potential to convert starch into sugar. After  $\sim$ 4 days, the green malt is kilned to arrest modification. Modification refers to all of the polymer-degrading processes that occur during malting. During the next step (mashing), the malt is treated with hot water to obtain an extract (wort). Varieties with high extract levels are desired for malting. The extract must provide adequate nourishment to the yeast to permit fermentation, and must contain sufficient sugar to attain the desired alcohol level. During brewing, the malt sugar solution is boiled with hops for seasoning. The solution is cooled and yeast is added to begin fermentation. Finally, the yeast ferments the sugars, releasing CO<sub>2</sub> and ethyl alcohol (see Barley: Malting).

# **Genetics of Malting Quality**

Genetic control of malting quality has recently been reviewed.

The endosperm is composed of large and small starch granules that are packed in a protein matrix. The amount and types of storage protein in barley affect the rate of modification and malting quality. During malting and mashing, the barley starch should be almost completely degraded into sugars that can be utilized by the brewing yeasts, whereas only  $\sim$ 45% of the barley protein should be solubilized. Too much protein solubilization is expected to result in beers with poor foaming characteristics. When insufficient protein hydrolysis occurs, the remaining proteins may interact with polyphenols to form beer haze precipitates. Grain protein in the range of 11.5-13.5% is desired for malting. The hordeins are the major storage proteins, and these can be divided into three groups: the sulfur-rich B hordeins, the sulfur-poor C hordeins, and the high molecular weight D hordeins. These proteins are encoded by the *Hor2*, *Hor1*, and *Hor3* loci, respectively, all of which are located on chromosome 5 (1H).

Four amylolytic enzymes are thought to participate in converting the starch in malted barley into fermentable sugars: these are  $\alpha$ -amylase,  $\beta$ -amylase, α-glucosidase, and limit dextrinase. A fifth carbohydrase, isoamylase, has recently been discovered, but the precise role of this enzyme is not yet known. The α-amylases are particularly important because they have sufficient thermostability to remain active throughout the mashing process. The Amy1 and Amy2 loci, on chromosomes 6 (6H), and 1 (7H), respectively, encode two  $\alpha$ -amylase isozymes that differ somewhat in their biochemical properties. Alpha-amylase 2 is expected to make the greatest contribution to α-amylase activity in grain. QTLs for important malting quality traits have been detected in the region near the Amy2 locus on chromosome 1 in several mapping populations (Figure 2).

Two genes, Bmy1 and Bmy2, on chromosomes 4 (4H) and 2 (2H), respectively, encode the  $\beta$ -amylases. Genetic differences in thermostability of  $\beta$ -amylase could potentially be exploited in breeding programs to improve malting quality.

The enzyme  $\alpha$ -glucosidase is encoded by the Ag1 locus on chromosome 1 (7H), and appears to be important in the mobilization of starch during seed germination.

The enzyme  $\beta$ -glucanase plays an important role in degrading  $\beta$ -glucans in endosperm cell walls during malting. High  $\beta$ -glucans cause problems during modification and filtration and lead to excess haze in the beer. A similar problem may result from an excess of arabinoxylans in the mash. Two genes known to encode  $\beta$ -glucanase isozymes are located on chromosomes 5 (1H) and 1 (7H).

The enzyme systems that reduce the barley storage proteins to "soluble protein" for fermentation are very complex. The rate-limiting step for protein degradation is the hydrolysis of the original proteins into soluble protein by endoproteinases, so it is the activities of these enzymes in malt that will usually determine whether a barley genotype is acceptable for malting. The small peptides and amino acids that

are released by the exopeptidases comprise the majority of the free amino nitrogen (FAN) fraction. FAN concentration is measured to indicate how well the original protein material can be utilized by yeasts during brewing.

Several other proteins have been identified that may indirectly affect malting quality by inhibiting the activities of some of the enzymes that are involved in degradation of starch, arabinoxylans, and proteins.  $\alpha$ -Amylase 2 and limit dextrinase are examples of enzymes rendered less active by inhibitors. It might be possible to improve malting quality by reducing the effects of inhibitors as well as through selection for increased enzyme levels.

# **Breeding for Malting Quality**

There are a few characteristics that breeders can select routinely to improve malting quality, such as kernel plumpness and protein content, but other assays are expensive or require large sample sizes, limiting the number of genotypes that can be evaluated. Tests for malting quality are usually applied at the prerelease stage. Newly released varieties will be designated as either a malting variety, or by default, a feed barley. To be designated as a malting variety, rigorous test criteria established by the malting and brewing industry must be met. A variety must pass through a series of micromalting and malt analysis tests, followed by pilot-scale malting tests, and finally, plant-scale malting and brewing tests including taste tests. The processes of varietal development and quality testing may take 15 years.

Many of the traits determining malting quality can be improved through breeding, but developing a variety that has good agronomic characteristics and falls in the accepted range for all quality traits is a challenge. Figure 2 shows an SSR map of the 88Ab536/Strider population. "88Ab536" is a variety developed by the USDA at the University of Idaho that combines winterhardiness from a Nebraska line "NE76129" with the malting quality of "Morex." "Strider" is a highly productive winter feed barley adapted to the Pacific Northwest of the USA. The graph reveals results from OTL analysis of malting quality in four different populations, showing QTLs consistently in the region of the Amy2 locus on chromosome 1 (7H), and on the short arms of chromosome 4 (4H) and 7 (5H). Molecular markers can be used to simultaneously select for this quality profile and important QTLs determining cold tolerance. The ability to make selections for malting quality at early stages in the breeding program should greatly increase the likelihood that good malting varieties will reach the prelease stage for pilot-scale malt testing.

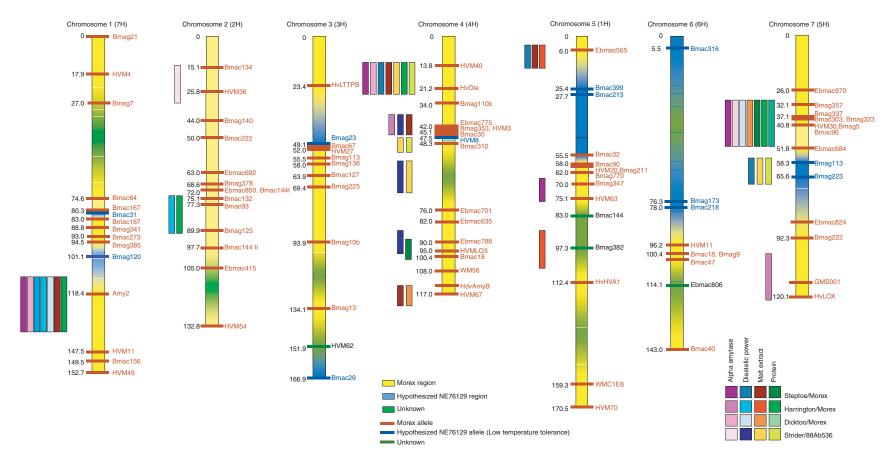


Figure 2 Genetic map of the 88Ab536 × Strider mapping population showing polymorphic SSR markers. Colored bars indicate location of malting quality QTL in four populations. (Courtesy of Tanya Filichkina and Luis Marquez-Cedillo, Oregon State University – previously unpublished.)

#### **Future Directions**

Experience with QTL analyses has shown that QTL results may be as variable and subject to environmental influences as the traits they were meant to define. Efforts are underway to develop expressed sequence tag (EST) libraries, which are cloned sequences of cDNA derived from actively transcribed mRNA. This will lead to identification of genes that have a direct functional relationship to quantitative trait expression, which will serve as more precise targets for MAS.

Discrepancies between the genetic and physical maps of barley present problems for fine structure analysis of gene function. QTLs that map to regions corresponding to large physical distances may be suitable for MAS, but are not sufficient for map-based cloning. Several approaches are being used to obtain higher resolution maps. Near isogenic lines are proving to be useful tools for genetic dissection of particular traits and chromosome regions. SNP and STS markers are being used to saturate chromosome regions with low recombination frequencies and low marker densities. Two barley yeast artificial chromosome (YAC) libraries and a barley artificial chromosome (BAC) library have been developed. The BAC library will be a useful tool for physical mapping of the barley chromosomes and map-based cloning.

Despite the potential of MAS to improve quantitative traits, there are only a few examples of the application of this technique in applied breeding programs. One of the reasons for this is the high cost/benefit ratio for application of molecular approaches, compared to conventional breeding methods. The development of microarray chip technology may overcome this barrier by greatly increasing the rate of throughput and information content of laboratory samples.

Another limitation of QTL analysis to date is that it may be specific for the mapping populations that are utilized. Germplasm from important barley production areas of the world, including Africa, Asia, the former USSR, and the Middle East, is under-represented in these studies. New statistical techniques for association mapping may permit rapid identification of potentially useful QTLs in a broader array of germplasm.

The likelihood of obtaining acceptable varieties is greatest when crosses are made between elite varieties. Breeders typically cross elite varieties and attempt to improve specific traits such as disease resistance, while maintaining the desired agronomic and quality characteristics. This approach is inherently conservative because it relies on a limited gene pool. There is a wealth of genetic diversity with potential utility in cultivated barley, and perhaps

in H. vulgare ssp. spontaneum as well. Effective use of these resources requires long-term efforts to develop useful source populations. The availability of better selection techniques for malting quality and yield using molecular markers will greatly facilitate the use of more diverse genetic resources.

See also: Animal Feed. Barley: Agronomy; Harvesting, Storage, and Transport; Grading and Marketing; Milling and Processing; Malting. Cereals: Grain Diseases; Grain-Quality Attributes. Genome Mapping. Genomics. Taxonomic Classification of Grain Species, Variety Registration and Breeders' Rights.

# **Further Reading**

Anderson MK and Reinbergs E (1985) Barley breeding. In: Rasmusson DC (ed.) Barley, Agronomy Monograph No. 26. Madison. WI: American Society of Agronomy.

Hayes PM, Castro A, Marquez-Cedillo L, et al. (2003) Genetic diversity for quantitatively inherited agronomic and malting quality traits. In: von Bothmer R, van Hintum T, Knüpffer H, and Sato K (eds.) Diversity in Barley (Hordeum vulgare L.). Amsterdam: Elsevier.

Kleinhofs A and Graner A (2001) An integrated map of the barley genome. In: Phillips RL and Vasil IK (eds.) DNA-Based Markers in Plants. Advances in Cellular and Molecular Biology of Plants, vol. 6. Dordrecht, The Netherlands: Kluwer Academic.

Künzel G, Korzun L, and Meister A (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics 154: 397-412.

Logue S (ed.) (2000) Barley Genetics VIII. Proceedings of 8th International Barley Genetics Symposium, Adelaide University, Glen Osmund, South Australia.

MacGregor AW and Bhatty RS (eds.) (1993) Barley Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Mathre DE (ed.) (1997) Compendium of Barley Diseases, 2nd edn. St. Paul, MN: APS Press.

Raboy V, Cook A, Wesenberg D, and Rossnagel B (2001) Genetics and breeding of seed phosphorus and phytic acid. Journal of Plant Physiology 158: 489-497.

Shewry PR (ed.) (1992) Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. Wallingford: CAB International.

Swanston JS and Ellis RP (2002) Genetics and breeding of malt quality attributes. In: Slafer GA, Molina-Cano JL, Savin R, Araus JL, and Romagosa I (eds.) Barley Science: Recent Advances from Molecular Biology to Agronomy of Yield and Quality. Binghamton, NY: The Haworth Press.

Thomas WTB (2002) Molecular marker-assisted versus conventional selection in barley breeding. In: Slafer GA, Molina-Cano JL, Savin R, Araus JL, and Romagosa I (eds.) Barley Science: Recent Advances from Molecular Biology to Agronomy of Yield and Quality. Binghamton, NY: The Haworth Press.

Ullrich SE (2002) Genetics and breeding of barley feed quality attributes. In: Slafer GA, Molina-Cano JL, Savin R, Araus JL, and Romagosa I (eds.) Barley Science: Recent Advances from Molecular Biology to Agronomy of Yield and Quality. Binghamton, NY: The Haworth Press.

#### **Relevant Websites**

http://barleygenomics.wsu.edu — Comprehensive barley molecular mapping information and data compiled by A. Kleinhofs at Washington State University as part of NABGP.

http://barleyworld.org — The North American Barley Genome Project (NABGP) is a multi-institutional, multi-disciplinary project that applies the latest genetic technology to barley improvement. The long-term goal of the NABGP is to locate and characterize genes of economic importance and to use these genes in applied plant breeding.

http://barleyworld.org — A summary of published barley QTL reports by Hayes *et al.*, 2001.

http://apps.fao.org — FAOSTAT is an on-line database containing international statistics on agricultural production, utilization and trade.

http://wheat.pw.usda.gov — The Barley Genetics Newsletter consists of informal reports which are presented to further the exchange of ideas and information between research workers. The newsletter is maintained by GrainGenes courtesy of the American Malting Barley Association, Inc.

http://wheat.pw.usda.gov — Barley Genetics Stocks is a special edition of the Barley Genetics Newsletter (Vol. 26, 1997) that provides comprehensive descriptions of known barley mutants and their sources. http://wheat.pw.usda.gov — GrainGenes is a compilation of molecular and phenotypic information on wheat, barley, rye, triticale, and oats. The project is supported by the USDA-ARS Plant Genome Research Program, and by the community of scientists who provide the information.

# **Agronomy**

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#### Introduction

Barley is the cereal crop with the widest range of production areas in the world. It is often the last cereal crop

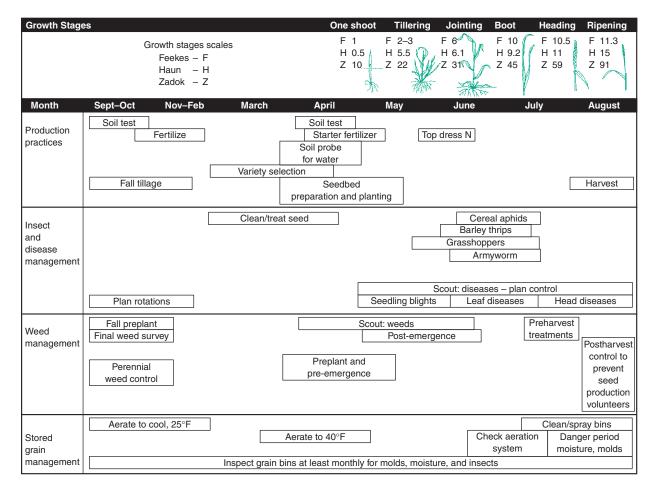
grown at the highest altitudes in the Andes and Himalayan mountains; adjacent to the deserts of Africa, the Middle East, and China; and near the Arctic Circle in the northern reaches of Asia, Europe, and North America. Barley has many uses, including livestock feed, human food, and for production of malt. Because of the diverse growing areas for barley and its many uses, production practices vary greatly. Production practices for malting barley are more stringent than those for barley produced for other uses because of the specifications put on the crop by the malting and brewing industries. Barley to be used for malting must meet specifications for germination, kernel size and weight, grain protein, and many other traits. Barley for livestock and human food uses has much fewer restrictions.

# **Growth Stages**

Before the effects of different production practices and environmental stresses on the performance of barley can be understood, knowledge of the different growth stages and yield components of barley are needed. Different scales are available for describing the growth of barley, including the Feekes, Haun, and Zadoks growth stage scales. Figure 1 compares these three different growth stage scales and lists suggested production practices for spring barley grown in the Mid-west United States.

Components that determine yield in barley are the number of spikes per area, the number of kernels per spike, and kernel weight. Stresses during a certain period of the growing season will reduce one or more of the yield components. Factors affecting the number of spikes per area include those that directly affect the number of plants per area and the number of tillers per plant. The number of plants and tillers can be impacted most severely from sowing to shortly before the jointing stage. Factors that can reduce the number of plants per area include sowing rate, insufficient moisture for germination, waterlogged soils following sowing that may kill the seed or cause soil crusting, winter-kill, weed competition, insects, and diseases. The number of tillers per plant can be negatively impacted by excessive temperatures, insufficient moisture, insects, and weed competition.

Before jointing, the developing barley plant is tolerant to freezing temperatures because the growing point is protected below the ground. At the jointing stage, the growing point of the barley plant comes aboveground and is vulnerable to environmental stresses that will cause fewer spikelets per spike. The number of kernels per spike also can be impacted by stresses during pollination, such as freezing or excessive temperatures that can kill the pollen. Pollination in barley generally occurs during the late-boot stage.



**Figure 1** Barley growth stages and suggested production practices for barley produced in the Midwest United States. (Reproduced with permission from Peel MD (2000) *Barley Production Guide*. Fargo, ND: North Dakota State University Extension Service.)

Stresses following fertilization will affect kernel weight and plumpness, possibly reducing grain quality. These stresses include excessive temperatures, insufficient moisture, weed competition, diseases, and insects. Damage to the flag leaf and the leaf below it by insects or diseases more severely impacts kernel weight than damage to the lower leaves. Thus, growers are encouraged to scout their crop to make sure that the top two leaves of the plant are free from damage caused by disease and insects. Thin lightweight kernels are undesirable for malting because they typically are high in grain protein and are difficult to malt uniformly. Also, the resultant malt generally has overall poor-quality malt, especially low malt extract.

# **Plant Nutrition**

Barley responds well to fertilizers containing nutrients limiting in the soil, especially nitrogen. When nitrogen is limiting, additional amounts applied through different forms of fertilizer will increase yield and grain

protein. The amount of fertilizer to apply is dependent on the end use of the harvested grain, soil test results, and expected yield. In most areas of the world except North America, barley used for malting must have grain protein below 11.5%. Many North American maltsters and brewers will accept grain protein up to 13.5%. One reason why brewers in this region accept higher barley grain protein levels is that they often use higher levels of brewing adjuncts (e.g., corn or rice) that have a diluting effect on the total barley protein in the beer. There are no upper limits on grain protein content on barley for livestock feed or human food uses.

Nutrients and amounts of them to add are dependent on the results of a soil test that measures residual levels of nutrients. **Table 1** is an example of a soil recommendation chart used in the Midwest United States based on soil test results and yield goal. Higher levels of nitrogen fertilizer are recommended for barley produced for nonmalting uses; however, excessive nitrogen can result in a lush crop that is prone to lodging and foliar diseases. Also, excessive fertilizers can be environmentally problematic in well-drained

Table 1 Nutrient recommendations for malt and feed barley in the Mid west United States

Yield		Soil N plus fertilizer		Sol	il test ph	osphorus ( <sub>k</sub>	(mda			Soil te	Soil test potassium (ppm)	(mdd) m	
$goal$ $(tha^{-1})$	N required for malt barley (kg ha $^{-1}$ (0.6 m) $^{-1}$ )	N required for feed barley (kg ha $^{-1}$ (0.6 m) $^{-1}$ )	Bray Olsen	Very low Low Medium High 0-5 6-10 11-15 16-20 0-3 4-7 8-11 12-15	Low 6-10 4-7	Medium 11–15 8–11	High 16–20 12–15	Very high 21+ 16+	Very low 0–40	Low 41–80	Medium 81–120	Very low Low Medium High 0-40 41-80 81-120 121-160	Very high 161+
						kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	T				kg K <sub>2</sub> O ha <sup>-1</sup>	-	
2.2	65	80		35	20	15	0	0	20	32	20	0	0
3.2	100	110		45	35	20	10	0	80	22	35	10	0
4.3	135	150		09	45	30	10	0	100	2	45	15	0
5.4	170	190		80	22	35	10	0	130	06	55	15	0

Nitrogen recommendation = 1.5 VG - STN + SDA - PCC. Bray-IP recommendation = (0.785-039 STP)YG. Olsen P recommendation = (0.785-039 STP)YG.

Olse'n P recommendation = [0.785-0.050 STP)VG.
Potassium recommendation = (1.286 0-0.008 5 STK)VG.
The abbreviations used in the equations are as follows: YG = yield goal, STN = soil test phosphorus, STK = soil test potassium, SDA = sampling date adjustment, PCC = previous crop credit. Reproduced with permission from Peel MD (2000) Barley Production Guide. Fargo, ND: North Dakota State University Extension Service

soils or areas with a high water table if they leach into the groundwater.

Accurate estimates of a yield goal are extremely important in choosing the proper amount of nitrogen fertilizer to apply. Application of too much nitrogen fertilizer can result in the barley having excessive protein and being rejected for malting. In environments and/or years where moisture is limiting, the recommended rates of nitrogen to apply to malting barley may be excessive and result in unacceptable barley for malting. As mentioned earlier, high-protein barley is difficult to malt uniformly and results in poor-quality malt. Thus, it is not uncommon for growers to apply less than the recommended rate of nitrogen to increase their likelihood of producing acceptable malting barley.

Fertilizers can be applied throughout the growing season; however, most nitrogen is applied prior to sowing to ensure sufficient amounts are available for seedling establishment and tillering. Growers sowing barley during spring often apply their nitrogen during fall to reduce their spring workload; however, fall application of fertilizers is not recommended for well-drained sandy soils because excessive leeching may occur over winter. The method of nitrogen fertilizer application is dependent on the source of nitrogen. Anhydrous ammonia is knifed into the soil  $\sim$ 10–15 cm below the soil surface. Nitrogen in the form of urea, other dry formulations, or liquid can be broadcast over the soil and then incorporated into the soil using shallow tillage. In soils deficient in phosphorus and potassium, these nutrients are often applied during sowing to supply a "quick" start for the crop. Top dressing of fertilizers during the growing season is an option that is available if a nutrient deficiency is detected.

Knowledge on the effects of micronutrient deficiencies on barley is limited. Under some conditions, barley has been found to respond favorably to addition of chloride, copper, iron, and sulfur. More is known about the toxic effect of some micronutrients. In low pH soils, in areas of South America and Australia, aluminum toxicity can be a problem. Also, in areas of Australia, boron toxicity can severely interfere with plant growth. To overcome problems associated with these problematic soils, barley cultivars have been developed that are tolerant to higher levels of boron and aluminum.

# **Cultivar Selection**

Choice of cultivar for production is especially important for growers producing barley for malting and as a method to combat potential problems such as diseases, insects, and micronutrient toxicity. In all major malting-barley-growing regions, different

organizations evaluate and determine which barley cultivars meet their specifications. To aid barley breeders, the organizations responsible for evaluating potential new malting barley cultivars often provide specifications that cultivars must possess before they will be recommended for malting and brewing (see Barley: Malting). Some of these groups also provide lists of cultivars that fit their specifications and are recommended for malting and brewing. In choosing malting barley cultivars for production, growers need to be aware of what cultivars are preferred by their local buyer. Even though there may be up to a dozen cultivars recommended for malting in a grower's area, local buyers may be purchasing only one or two specific cultivars. Growers should not only consider yield potential when choosing a malting barley cultivar to produce, but also its grain protein and kernel plumpness when grown in their area. For example, if going into the growing season, the grower knows that stored soil moisture is limiting and residual nitrogen is high, they should choose a cultivar that has inherently lower grain protein even if it has a lower yield potential. Because of the premium paid for malting barley versus feed barley, it is often better to sacrifice some yield to ensure the crop has acceptable grain protein and kernel plumpness.

Recommendations for barley cultivars to be used for livestock feed or human food are limited. "Valier" barley was developed by the Montana Agricultural Experiment Station in the United States specifically for feeding to cattle. However, much of the barley for the livestock market is grain that has been denied for purchase for malting due to one or more deficiencies (e.g., excessive grain protein, diseased kernels, low kernel weight, etc.). Barley cultivars for human food often are bred to have greater levels of soluble fiber, especially beta-glucan, and to have the hull-less character. Cultivars with these characteristics have been found to have hypocholesterolemic effects and a low glycemic index that may be beneficial in regulating blood glucose and reducing the risk of diabetes.

Most barley recommended for malting and brewing has the two-rowed spike type (Figure 2). However, in North America over 50% of the barley used for brewing is six-rowed. Historically, six-rowed barley has had higher enzyme levels that are required in beers brewed with adjuncts. Beers brewed with adjuncts tend to be lighter than traditional European-style beers made with 100% malt.

# Sowing Date, Depth, and Rate

The timing of sowing varies greatly across the globe. Barley with a spring growth-habit is generally sown in



**Figure 2** Comparison of the spike morphology of two-rowed barley (left) and six-rowed barley (right).

spring; however, it can be sown during fall in environments where winters are not severe and more than one cropping season per year is available. For example, spring barley often is sown in fall in east-central China. In this situation, barley is sown in October, harvested in early May, and rice is sown shortly thereafter. Another example of fall-sown spring barley occurs in the southwest United States. In this desert area, barley is sown in October, harvested in April, and a forage crop such as sudan grass is then sown. Areas where spring barley is predominantly spring-sown include Australia, northern Europe and Asia, North America, and South America. Barley with the winter-growth habit is generally less winterhardy than winter wheat; therefore, winter barley is grown in areas with less severe winters. In Germany, the ratio of winter to spring barley is  $\sim 60:40\%$ .

The desired sowing depth of barley is between 20–30 mm; however, the sowing depth may need to be deeper, as the seed is placed in moist soil. Because of the emergence mechanism of barley, it should not be sown deeper than 55 mm. The type of seedling emergence in barley is referred to as the elongating coleoptile. In this type of emergence, the maximum

length of the coleoptile will be only 35-55 mm. During seedling emergence, the coleoptile ceases to elongate once it breaks through the soil surface and is exposed to sunlight. Emergence of the remaining leaves occurs through the tip of the coleoptile. During germination, the stem internodes do not elongate, so the first node, coleoptilar node, and terminal bud (i.e., growing point) remain at the sowing depth. About 2-3 weeks following emergence for spring-sown barley and after vernalization and temperatures warm up in the spring for winter barley, the second internode of the plant elongates until the terminal bud and other stem nodes are  $\sim \! 10$  mm from the soil surface. The first and second nodes remain at the sowing depth, and the tillers and crown roots develop from the remaining nodes.

The seeding rate for barley is dependent on many items, including the type of barley being grown (two-rowed versus six-rowed) and whether the barley is being grown under dryland or irrigated conditions. The desired plant population can range between 215 and 375 plants m<sup>-2</sup> (i.e., between 2.15 and 3.75 million plants ha<sup>-1</sup>). The lower plant population is recommended for barley grown under dry conditions while the higher population is recommended for barley grown under irrigation. Also, since two-rowed barley generally produces more tillers than six-rowed barley, lower plant populations for two-rowed barley can be used.

To determine the correct number of seeds to sow per hectare, the grower must know the desired final plant population, the estimated seedling mortality, and the percent germination of the seed. The estimated seedling mortality can be based on prior experience and the percent germination of the seed is usually supplied with the purchased seed. If the seed is old or if the germination is not known, the grower needs to determine the percent germination themselves. This can be done using a simple test where 100 seeds are placed between two pieces of damp paper towel and counting the percent germinated seeds after three days. It is important that the paper towel is kept moist during the 3-day period. The number of seeds to sow per hectare can be calculated using the following formula:

```
# of seeds to sow ha<sup>-1</sup> = # of desired plants ha<sup>-1</sup>
\times (1/(1-\% \text{ seedling mortality}))
\times (1/\% \text{ germination})
```

For example, if the grower desires a population of 3.0 million plants  $ha^{-1}$ , the seedling mortality is 3%, and the percent germination is 95%, the grower would need to sow  $\sim 3.25$  million seeds  $ha^{-1}$  (i.e., (3.0 million plants  $ha^{-1}$ ) × (1/0.97) × (1/0.95)).

The seeding rate to achieve the desired plant population is dependent on seed weight. To determine the correct seeding rate the grower can use the following formula:

```
kg of seed ha^{-1} = # of desired seeds ha^{-1}
× (1000-kernel weight/1000)/1000
```

For example, if the grower wants to sow 3.25 million seeds ha<sup>-1</sup> and the 1000-kernel weight is 42 g, the grower would need to sow 136.5 kg ha<sup>-1</sup> (i.e., 3.25 million seeds  $\times$  (42/1000)/1000).

# **Irrigation**

Barley has a high water-use efficiency and can extract moisture from a  $0.8-1.0\,\mathrm{m}$  depth. Moisture needs throughout the growing season is  $\sim\!400\,\mathrm{mm}$ . Because of its high water-use efficiency, barley is most often grown without irrigation. However if irrigation is used, it is done more for malting barley than feed barley because of its higher potential value and the requirement for plump kernels and low grain protein.

The goal of any irrigation schedule is to maintain available soil moisture levels above 50%. Management of any irrigation schedule is important because insufficient water will result in reduced yields, thin kernels, and excessive grain protein for malting. Excessive moisture will result in grain that lodges, making it prone to disease and difficult to harvest. Also, lodged grain often has thin kernels and high grain protein that may make it unsuitable for malting.

#### **Disease Control**

The list of barley diseases caused by bacteria, fungi, and viruses is extensive. Table 2 provides a partial list of important bacterial, fungal, and viral diseases of barley. Barley diseases exist that can infect all parts of the plants. A series of different root rots severely limit root development. The effects of this group of diseases are especially noticed during dry years or in dry areas of the field such as hilltops because roots are unable to uptake sufficient water. Diseases that affect the foliage of the plants such as the rusts, blotches, and leaf blights can severely reduce the photosynthetic capacity of the plants. These diseases often result in yield losses and excessive grain protein for malting due to reduced kernel weight. Finally, diseases of the spike such as the different kernel blights and smuts can reduce yield, but more importantly can limit the marketability of the grain. Grain that is contaminated with smut or kernel blight can be severely discounted or rejected at the point of sale. Also, some pathogens such as the kernel blighting Fusarium spp. produce

Table 2 A partial list of common diseases of barley, causal organism, and most common growth stage of infection

Disease	Causal organism or vector <sup>a</sup>	Plant parts infected
Bacterial		
Bacterial leaf blight	Pseudomonas syringae	Leaves
Bacterial kernel blight	P. syringae	Kernels
Bacterial blight	Xanthomonas translucens	Leaves
Fungal		
Common root rot	Bipolaris sorokiniana	Roots
Pythium root rot	Pythium ssp.	Roots
Rhizoctonia root rot	Rhizoctonia solani	Roots
Spot blotch	B. sorokiniana	Leaves
Net blotch	Pyrenophora teres	Leaves
Scald	Rynchosporium secalis	Leaves
Powdery mildew	Erysiphe graminis	All aerial plant parts
Leaf rust	Puccinia hordei	Leaves
Stripe rust	P. striiformis f. sp. hordei	Leaves
Stem rust	P. graminis f. sp. tritici	Leaves
Covered smut	Ustilago hordei	Kernels
Loose smut	U. nuda	Kernels
Head blight (scab)	Fusarium ssp.	Kernels
Viral		
Barley stripe mosaic	Seed-borne	Leaves
Barley yellow dwarf	Aphids	Leaves
Barley yellow mosaic	Soil-borne fungus Polymyxa graminis	Leaves

<sup>&</sup>lt;sup>a</sup>Causal organism for bacteria and fungi, and vector for viruses.

mycotoxins. In the Midwest United States in 2000, barley with greater than 0.5 ppm of the mycotoxin deoxynivalenol (DON) was discounted nearly \$20 per ton. Grain with DON greater than 3.0 ppm was rejected entirely for malting.

Disease control can be accomplished by use of resistant cultivars, cultural methods, and chemicals. The easiest and most cost-effective method of disease control is growing resistant cultivars. Cultivars that are resistant or tolerant to most economically important diseases exist; however, like all cultivars they have limited areas of adaptation. When a disease is new to an area or resistance breaks down in currently grown cultivars, it may take breeders a short time to develop improved cultivars if they have been working on the problem. However, when a disease is new to an area and no resistance is found in any of the adapted cultivars or advanced breeding germplasm, it may take more than 10 years to develop new resistant cultivars. Development of disease-resistant malting barley cultivars may take even longer, since the breeder must incorporate disease resistance with the stringent characteristics specified by the malting and brewing industry.

Crop rotation and tillage are cultural methods that are commonly used for disease control. Many of the diseases that infect barley also infect other crops. For example, *F. graminearum* and *Bipolaris sorokiniana* are the causal organisms of common root rot in barley

and wheat, and *F. graminearum* is the causal organism of head blight in barley and wheat, and stalk rot in maize. Thus, when choosing a crop rotation, growers must be aware of their potential disease problems and alternative host crops of those diseases. A minimum of 1 year between susceptible crops is needed; however, to ensure reduction of potential disease problems, 2–3 years between susceptible crops is preferred.

Tillage can reduce potential disease problems by burying crop residue that harbors pathogens. However, as more growers use minimum tillage or no-till methods, the potential for residue-borne disease is likely to increase. Chemical control for disease management can include application of fungicides applied to seed and foliage, and insecticides to control virus vectors. Laws controlling chemicals that can be applied to crops differ for each country; thus, the mention of a particular chemical does not indicate that it is a permissible treatment everywhere. Fungicides applied to seeds are done to protect the seedlings from root rot, control loose smut (incited by Ustilago nuda), covered smut (incited by U. hordei), and other seed-borne diseases. Chemicals often used for seed treatment include carboxin; carboxin with captan, maneb, or thiram; imazalil; metalaxyl; and tebuconazole.

Application of fungicides for foliar-disease protection is generally done to protect the top two leaves of the plant. However, control of polycyclic diseases

such as leaf rust (incited by *Puccinia hordei*) and stripe rust (incited by *P. striiformis* Westend f. sp. *hordei*) may require fungicide applications to seedlings and additional applications later in the season to protect the plants' top two leaves. Adequate coverage of the spikes by fungicides to control the kernel-blighting diseases is difficult because the lemma awns intercept the chemical before it reaches the kernel surface and the fact that most crop sprayers are designed to apply chemicals to weed and crop foliage. A sprayer configuration comprised of front and back-facing nozzles has been found to provide the best coverage of spikes with fungicide.

#### **Insect and Nematode Control**

Damage due to insects can be grouped into two categories: (1) damage due to consumption of plant parts during feeding and (2) damage due to virus or toxins transferred during feeding. Insects that can cause substantial losses due to plant consumption include grasshoppers, crickets, thrips, the cereal leaf beetle, wireworms, and cutworms. Wireworms tend to cause their damage by feeding on the roots and underground portions of the plant while the remainder of insects listed above cause their damage by feeding on the aboveground portions. Control of these insects is recommended after their population is above an economic threshold. Wireworms and cutworms need to be controlled early during the growing season so the plant population is not significantly reduced. Similar to controlling diseases, growers need to ensure that the top two leaves of the plant are not damaged by grasshopper, crickets, and other foliage-eating insects since they are the most important leaves in contributing photosynthates to the developing kernels.

Four aphids are known to transfer the barley yellow dwarf virus during feeding. These aphids are: the bird cherry-oat aphid (*Rhopalosiphum padi*), corn leaf aphid (*R. maidis*), English grain aphid (*Sitobion avenae*), and green bug (*Schizaphis graminum*). Barley yellow dwarf is the most important viral disease of barley and is found worldwide. Use of resistant cultivars is the best control for this disease. However, if resistant cultivars are not available, systemic insecticides can be used to control aphids if their population gets above the economic threshold.

Hessian fly (*Mayetiala destructor*) is a pest of wheat throughout the world and can cause damage to barley as well by injecting a toxin during feeding. However, damage to barley is rare and this insect is not often considered a serious pest of barley.

Two nematodes are known to cause economic damage to barley. Cereal cyst nematodes are a complex of several species of nematode that belong to the genus

Heterodera. In seriously damaged plants, the roots are stunted and produce many lateral roots. Early aboveground symptoms of the plants infected by cereal cyst nematode mimic those caused by nutrient deficiencies. When older infected plants are pulled from the ground and the roots are examined, the white cysts attached to the roots can be identified. To confirm that damage is due to cereal cysts nematodes, a laboratory diagnosis is encouraged. Control of this pest has been accomplished using several different methods, including crop rotation, use of resistant cultivars, and use of fungal biocontrol agents.

Cereal root-knot nematode, belonging to the genus *Meloidogyne*, is the other nematode that can cause economic yield losses in barley. Damage caused by this complex of nematodes is similar to that of the cereal cyst nematodes. The root system of infected plants appears bushy due to a stunted root system with many lateral roots. When adult plants are pulled from the ground and the roots are examined, galls ranging from small to near pea-sized can be seen. To confirm infection by cereal root-knot nematodes, a laboratory diagnosis is recommended. Control of damage due to cereal root-knot nematodes is usually accomplished using crop rotation.

#### **Weed Control**

Weed control is an integral part of almost every commercial barley production scheme. Excessive weeds can reduce yield and negatively impact grain quality for malting by reducing kernel plumpness and increasing grain protein. Many growers use tillage, herbicides, crop rotation, or a combination of these practices to control weeds in their fields. In some areas of the world, these methods may not be feasible or available because of environmental or marketing circumstances.

Tillage is a viable option for most barley production systems including organic production, where herbicide use is not an option. Most weeds are easily controlled with tillage when they are small and can be covered by small amounts of soil. Barley can be harrowed or rotary-hoed either pre-emergence (when coleoptile is 10–15 mm below the ground) or postemergence (2-3 leaf stage). At these stages, the crop can more easily overcome damage and root pruning caused by tillage implements. Since tillage is a nonselective weed-control method and both crop and weed species can be injured, care must be taken when harrowing, rotary-hoeing, or using other types of tillage. Many times, the barley stand can be reduced 20% or more by using tillage. In addition, the level of weed controlled may not be an

acceptable excuse for subjecting the barley crop to this harsh, nonselective method of control.

Herbicides are the most popular method of weed control for growers worldwide. In a properly managed integrated pest management (IPM) program, herbicides can be a valuable asset. Herbicides can provide both nonselective and selective means of controlling weeds in a cropping situation. For most broadleaf weeds, many herbicide options are available. These broadleaf herbicides take advantage of obvious physiological differences between grass and broadleaf plants. Grass weeds can be the most important yield-limiting factor in a crop production situation. Since barley is in the same taxonomic family as other grasses, Poaceae, grass weed control in barley is difficult or impossible to achieve without some injury.

Since there are fewer chemical options when controlling grass weeds, resistance to specific herbicides and herbicide families is becoming a major problem. For this reason, it is important to use different herbicide families when controlling weeds to remove resistant biotypes of various weed species. Many weeds have developed resistance to acetolactate synthase (ALS) and acetyl CoA carboxylase (ACC-ase) inhibiting herbicides. Kochia (Kochia scoparia) is an example of an ALS inhibitor-resistant weed that has become a major problem. Resistant kochia plants evolved when herbicides such as chlorsulfuron and metsulfuron-methyl from the ALS inhibitor group of herbicides became popular in crop protection. Kochia plants developing resistance to one of these herbicides also become more tolerant of herbicides that affect the same exact site of enzymatic activity. As a result, kochia may be resistant to other herbicides that belong to the same group. Only one plant is needed to create a resistant population that can exponentially increase over time. More serious weedcontrol problems can occur when weed species develop resistance to more than one herbicide family or group; this is known as multiple resistance.

Crop rotation plays an important role in weed control. By growing a different crop on the same piece of land each growing season, different herbicides can be used; thus, reducing the chance of resistant weed biotypes becoming problematic. By growing a broadleaf crop before rotating into barley, the grower typically has a better opportunity to get some grass weeds under control. Likewise, volunteer barley from the previous crop can be easily controlled in a broadleaf crop by a number of different herbicides the following growing season. Another good crop rotation practice is rotating spring and winter crops. This allows herbicide applications at different times during the year and may control certain weed populations that may not be controlled during a spring-type cropping scenario.

Not all weed control options are available to every barley grower. Environmental conditions, such as lack of moisture, may cause tillage to be a prohibitive practice. For example, tillage may not be an option in a no-till situation, but may work in a minimum-till setting. Organic growers are restricted from using herbicides on weeds and genetically modified crops in their rotations, but may get weeds under control by tillage and use of crops like rye in their cropping rotation. By using any of these methods of weed control, growers can protect their yield potential and make their crop more profitable.

See also: Barley: Genetics and Breeding; Harvesting, Storage, and Transport; Grading and Marketing; Malting.

# **Further Reading**

Bauer A (1975) *Irrigation of Small Grains*. Fargo, ND: North Dakota State University Extension Service.

Dahnke WC, Fanning C, and Cattanach A (1992) Fertilizing Malting and Feed Barley. Fargo, ND: North Dakota State University Extension Service.

Mathre DE (ed.) (1997) Compendium of Barley Diseases, 2nd edn. St. Paul, MN: The American Phytopathological Society.

Peel MD (2000) Barley Production Guide. Fargo, ND: North Dakota State University Extension Service.

Rasmusson DC (ed.) (1985) Barley. Madison, WI: American Society of Agronomy.

US Department of Agriculture (1979) Barley: Origin, Botany, Culture, Winter Hardiness, Genetics, Utilization, Pests. Washington, DC: Superintendent of Documents, US Government Printing Office.

# **Relevant Websites**

http://www.ambainc.org — This website provides information on recommended barley cultivars for the United States, and suggested harvest and storage practices.

http://www.albertabarley.com — This website provides a great deal of information on production of barley in western Canada. Topics discussed include land preparation, cultivar information, fertility, diseases and other pests, and weed control.

http://www.dpi.qld.gov.au — This website provides information on barley production practices specifically for the state of Queensland in Australia. However, the information provided is relevant for much of the barley production area in Australia.

http://www.ebc-nl.com — Information on cultivars being evaluated in the European Brewing Congress's EBC Barley and Malt Committee Field Trials can be obtained from this website. This information is useful in knowing what malting barley cultivars are

currently being used and which ones may be used in the near future.

http://www.weedscience.org - This website provides information on herbicide resistant weeds around the world. Information is contained in a database that can be gueried based on common or scientific name of the weed, herbicide mode of action, and by country.

# Harvesting, Storage, and **Transport**

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#### Introduction

Harvesting, storage, and transport of barley aims to deliver a year round supply of sound grain to a variety of barley end users. The barley must satisfy the demands of the food, feed, and malting industries. In turn, the malting industry must deliver a product suitable for food, distilling, and brewing industries. Barley for malting must retain good germination, and this is a key issue in the handling of barley. Harvesting aims to recover maximal yield of barley and avoid environmental damage and contamination. Storage must maintain varietal purity and avoid loss of germinability. Transport should preserve storage conditions and ensure that the grain is not contaminated.

#### **Unique Features of Barley**

The harvesting, storage, and transport of barley require consideration of the issues associated with other grains and the additional constraints imposed by the use of barley in malting. Malting requires clean grain that can be germinated uniformly. Barley is also unique in comparison to grains such as wheat in being a hulled grain (except in the case of naked barley). The lemna and palea make up a husk that adheres to the grain. The presence of the husk provides some protection to the grain against abrasion during handling. The husk is, however, a site for microbial infestations (fungal and bacterial) that may be more difficult to remove than those on the surface of naked grains. Hull-less barley varieties may have very different storage and handling properties.

# **Harvesting**

The timing of harvest aims to optimize yield and quality while minimizing environmental risks such as preharvest sprouting, associated with delayed harvesting. The grain must be mature before it is harvested. Early harvesting will result in higher moisture content of the grain, which will require careful storage and/or drying. The timing of harvesting may be more critical in some environments than others, so harvesting capacity must be sufficient to harvest the crop within a very short period of time in high-risk environments. Areas with a high rainfall, conducive to preharvest sprouting, require this approach. In some areas, high humidity can restrict the time available for harvest each day. Storage and transport capacity requirements may also be determined by these factors. Crops can be harvested at very high moisture contents (more than 50%) with combines but yield will be lost, because the crop would not have reached maximum grain dry-weight and costly grain drying would be needed before storage. The grain must be mature before it is harvested.

# **Grain Cleaning**

"Dockage" is a term used to describe the grading of grain such as barley, by removing nongrain parts of the plant and other debris.

#### **Grain Size**

The size of barley is a key measure of grain quality, and harvested grain is also subjected to screening to remove small and broken grains prior to storage or transport. The proportion of grain in 2.5 and 2.8 mm sieves is a further measure of grain plumpness used to assess barley quality. The uniformity of size is important because of the influence of grain size on the rate of modification of the grain during malting. Grain may be fractionated to manipulate the uniformity before use in malting.

#### **Skinning**

The skinning of barley during harvest or handling can result in loss of germination potential. Skinning may result in the loss of the embryo or damage to tissues such as the aleurone, important in malting. However, some abrasion of the grain may assist malting by accelerating water absorption during steeping or by contributing to the breaking of dormancy.

#### **Shattering**

The shattering of barley results in poor recoveries of grain in the field and therefore significant losses. This is more likely when harvesting dry grain or grain that

has matured under unfavorable environmental conditions.

#### **Grain Drving**

The drying of barley requires extra care to preserve germinability for malting. Heat and mechanical damage may result from some drying protocols. Aeration of stored grain with a fan can both reduce the grain temperature and dry the grain especially if dehumidified air is used or low ambient humidity prevails. Heated air needs to be used with caution to avoid damage to the viability of the barley, especially in the case of malting barley.

# Storage

Defined hazards associated with storage of grain include:

- excessive moisture;
- extremes of temperature;
- microbal infestations;
- insects and arachnids (e.g., weevils, beetles, and
- rodents (e.g., mice and rats);
- birds (both before harvest and in grain stores);
- biochemical deterioration; and
- mechanical damage in handling.

Other general concerns of particular significance in relation to barley are introduction of environmental contaminants, preservation of germinability, and avoidance of admixture.

#### **Environmental Contaminants**

Environmental contaminants in barley are of concern to brewers as these may contaminate the finished beer. These contaminants include soil pollutants and naturally occurring toxins (e.g., heavy metals), components of fertilizers, fungicides and pesticides, and air pollutants. Seeds are often coated with preparations designed to protect the seeds from pests and diseases when planted in the field. The composition of these seed protectants may be of concern to brewers. Fungi introduce anther type of hazard, the possible presence of mycotoxins. Treatments to prevent the growth of fungi in stored barley may be required. Beer consumers are considered very sensitive to concerns about the purity of the product, and brewers are keen to ensure that their product retains a reputation for purity relative to competing products. This issue has resulted in the introduction of detailed quality assurance protocols to control the production and storage of barley. The use of insecticides and fungicides, especially those applied during storage may result in residues on malt that could end up in beer or other cereal products.

#### Germinability

Storage of malting barley also aims to preserve the viability of the grain. This is only a constraint for seed uses in other grain species. A ready germination of at least 95% of the grain is the usual standard applied to the acceptance of barley for malting. A supply of barley with high viability is required throughout the year to allow malting facilities to be used to maximum capacity. Storage of malting barley for at least 12 months after harvest is required unless transport between northern and southern hemispheres is used to supply the maltings. However, because of transport costs, most malting is based on locally produced barley.

#### **Moisture Content**

The relationships between moisture content and temperature and longevity in storage can be used to determine the probable storage life of barley samples. Equations to predict the safe storage period for barley based upon temperature and moisture content have been developed (Figure 1). These equations can be adapted to allow for differences in preharvest sprouting damage.

Barley should have less than 130 g per kg of moisture for safe storage for 1 year, and less than 110 g per kg for safe storage for 5 years. Grains with more than 140 g per kg of moisture may heat and spoil rapidly. The moisture content of barley at 25°C will vary with relative humidity and at equilibrium will be 97 g per kg at 97% relative humidity, 135 g per kg at 70%, and 268 g per kg at 100%.

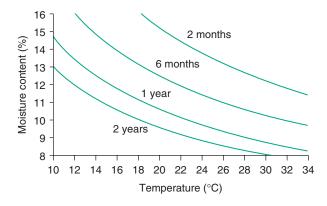


Figure 1 Safe storage of barley at different moisture contents and temperatures.

#### **Dormancy**

Some barley varieties have a brief period of dormancy immediately following harvest. This may be desirable in areas in which preharvest sprouting damage is prevalent. Malting of barley is usually delayed for some weeks or months after harvest to avoid dormancy. This may require barley to be stored for malting considerably more than 12 months after harvest, so that supplies are available for malting from the previous year's harvest until the new crop has emerged from dormancy. Dormancy of barley may be influenced by the variety (genetic factors) and growth conditions (environmental factors). Some varieties (when dried) with strong dormancy are treated (warm storage) to break dormancy to allow more immediate processing. Another strategy in some areas is to malt a minor variety with low dormancy immediately after harvest until preferred but more dormant varieties are ready. Some varieties are treated to break dormancy prior to processing. Dormancy of Triumph barley may be removed by warm storage immediately prior to malting.

#### **Admixture**

Barley varieties may germinate at different rates, complicating the malting of samples of mixed genotypes. This constraint means that malting barley storage requires more careful segregation of varieties than that of most other grain types. This adds to the cost of barley storage and transport. The same issues make the blending of barley parcels with widely differing specifications (e.g., protein content) to produce a target specification highly undesirable. Blending of malts after malting is the best way to ensure consistent malt specifications for the brewer. Planting of pure seedlots is a key approach to ensuring the harvesting of grain of a single variety. Testing of seed before planting and of plants in the field before harvest can be used to confirm the identity and purity of barley varieties. Testing of the harvested samples can be used to confirm these advance tests. Unfortunately, the analysis of the harvested samples cannot always be completed before the harvested grain is bulked or mixed in storage placing more importance on the preharvest testing. Varietal identity and purity have been analyzed based upon the analysis of seed proteins but methods based on deoxyribonucleic acid (DNA) analysis have increasingly been employed. The analysis of simple sequence repeats in barley DNA is widely used for this purpose. Analysis of identity and purity, and other characteristics influencing storage decisions may ultimately be determined by automated visual assessment using video and computer analysis.

# **Factors Influencing Storage Life**

Moisture content and temperature are the key determinants of storage life. Barley storage is adversely affected by factors such as preharvest sprouting. Insect or microbial infestations may occur in storage but often originate in the field prior to harvest, only becoming obvious in storage. Storage treatments may be applied to protect the grain in storage. Insects may be managed using chemical agents, controlled atmospheres, or the addition of products based upon diatomaceous earth. Aeration of barley during storage can be used to control temperature and moisture distribution within the bulk.

# **Malting Quality**

Malting quality requires that grains be of a suitable malting variety, have a high germinative capacity and energy, be clean and free from microbial or chemical contaminants, and not be an admixture. Brewers are increasingly concerned about possible contaminants at all of the steps in the production chain leading to beer. Beer consumers are often influenced by perceptions of the quality and purity of the product. Gushing is a specific problem of malting barley which has been linked to *Fusarium* infection. (Gushing is excessive frothing of the product.)

#### **Feed Quality**

Feed quality requires freedom from contaminants likely to detract from the feed value of the grain. Feed users also supply the food chain and aim to eliminate any undesirable chemical or microbiological contaminant.

#### **Controlled-Atmosphere Storage**

Storage of barley under controlled atmospheres can reduce the use of chemical pest control agents and eliminate residues. Protection of viability for malting requires special considerations when this approach is used with barley. High CO<sub>2</sub> concentrations may reduce oxygen levels to dangerously low levels for malting barley. Temperature and method of CO<sub>2</sub> introduction need to be managed for the success of this technology in barley.

# **Transport**

# **Barley**

Barley transport from field to maltings or feedlot may involve many steps. Transport from the harvester to primary storage or off-farm may be followed by repeated steps of transport by road, rail, and sea, and storage in a wide range of storage vessels. The control of environment during transport may not always be as optimal as in the best fixed storages.

#### Malt

Malt is usually stored at very low moisture contents of ~4%. Removing more moisture may preserve the malt and reduce transport costs for malt that is transported large distances internationally. However, the energy cost of drying malt to very low moisture content may be high. Transport of malt to breweries involves many of the same issues as these associated with transport of the barley prior to malting.

See also: Barley: Agronomy. Contaminants of Grain. Wheat: Harvesting, Transport, and Storage.

# **Further Reading**

- Bason ML, Wrigley CW, and McCleary BV (1993a) Modelling the effects of sprouting damage on malting barley, based on viscometric or amylase analyses. In: Walker-Simmons MK and Reid LJ (eds.) Pre-Harvest Sprouting in Cereals 1992, pp. 417-425. St. Paul, MN: American Association of Cereal Chemists.
- Bason ML, Ronalds JA, and Wrigley CW (1993b) Prediction of safe storage life for sound and weatherdamaged malting barley. Cereal Foods World 38: 361 - 363.
- Edeny MJ (1996) Barley. In: Henry RJ and Kettlewell PS (eds.) Cereal Grain Quality, pp. 113-151. London: Chapman and Hall.
- Ellis RH and Roberts EH (1980a) The influence of temperature and moisture on seed viability period in barley (Hordeum distichum L.). Annals of Botany 45: 31 - 37.
- Ellis RH and Roberts EH (1980b) Improved equations for the prediction of seed longevity. Annals of Botany 45: 13 - 30.
- Fleurat-Lessard F (2002) Qualitative reasoning and integrated management of the quality of stored grain: a promising new approach (Review). Journal of Stored *Products Research* 38(3): 191–218.
- Kent NL and Evers AD (1994) Storage and pre processing Technology of Cereals, 4th edn, pp. 103-128. UK: Elsevier.
- Kettlewell PS (1996) Agronomy and cereal quality. In: Henry RJ and Kettlewell PS (eds.) Cereal Grain Quality, pp. 407-437. London: Chapman and Hall.
- Korunic Z, Fields PG, Kovacs MIP, et al. (1996) The effect of diatomaceous earth on grain quality. Postharvest Biology and Technology 9(3): 373-387.
- Krysinska-Traczyk E, Kiecana I, Perkowski J, and Dutkiewicz J (2001) Levels of fungi and mycotoxins in samples of grain and grain dust collected on farms in eastern poland. Annals of Agricultural and Environmental Medicine 8(2): 269-274.

- Majumdar S and Jayas DS (2000) Classification of cereal grains using machine vision: IV. Combined morphology, color and texture models. Transactions of the ASAE 43(6): 1689-1694.
- Mills JT (1996) Quality of stored cereals. In: Henry RJ and Kettlewell PS (eds.) Cereal Grain Quality, pp. 441–478. London: Chapman and Hall.
- Miyake Y, Hashimoto K, and Matsuki H (2002) Fate of insecticide and fungicide residues on barley during storage and malting. Journal American Society Brewing *Chemists* 60(3): 110–115.
- Rameshbabu M, Jayas DS, Muir WE, White NDG, and Mills JT (1996) Bulk and handling properties of hulless barley. Canadian Agricultural Engineering 38(1): 31 - 35.
- Smilanick JL, Goates BJ, Denisarrue R, et al. (1994) Germinability of tilletia SPP teliospores after hydrogen peroxide treatment. Plant Disease 78(9): 861-865.
- Wright EJ, Banks HJ, and Darby J (1997) Integration of harvest and storage strategies for maximum return. Proceedings of the 8th Australian Barley Technical Symposium, pp. 213.1–213.14.

# Grading and Marketing

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# Introduction

Annual world barley production is expected to rise to more than 150 million tons (Mt) by 2011. It is anticipated that over 21 Mt of that production will be traded on the world market as either feed, malting, or food barley. The remaining production will be used in domestic markets for similar purposes. In either case, considerable effort in marketing and grading will be required to ensure that producers get paid appropriately while end users receive barley with the required quality and at a competitive price.

Malted barley, the major ingredient in beer, represents only a minor portion, ~25%, of total world barley trade, but it routinely offers the greatest return for producers. End users of malting barley, as well as barley for whiskey production and for food, are obliged to use at least some barley in their products. They also require the barley to have a unique set of quality characteristics. As a result, barley demand in these markets is more consistent and prices are higher.

The largest portion of barley production is used as feed on farms or sold in domestic markets, where there are limited demands on quality. Feed barley, especially in international markets, is often replaced by less expensive grains such as corn or wheat. Two specific markets, namely, Japan and Saudi Arabia, do not, for traditional reasons, substitute it with other grains, which makes them the two most consistent international markets for feed barley.

Marketing of barley consists of a series of activities that move grain from production to its end use. Marketing is a mechanism by which the conflicting demands of producers and end users can be reconciled. For barley with a given quality, producers look for the highest possible price, while end users want the lowest possible price. Barley marketing ranges from a simple exchange of commodities among neighboring farms to the complex use of handling companies and grain merchants on an international scale. It might require the use of intake points in the production area for collection of grain as well as export-handling facilities at ports for shipment. The silos and handling facilities at intake and export positions can be owned by private companies, large multinational grain companies, producer cooperatives, or by barley processors. Grain merchants are often involved in actual sales. They can be representatives of handling companies, independent marketing companies, or a state trading enterprise (STE).

Marketing is facilitated through the use of standards and grades that act as a marketing language through which buyers and sellers can communicate on the quality of barley to be delivered. Grades and standards sort barley into groups of similar quality. They indicate the expected performance of barley during processing and requirements for special storage, as well as any need for additional handling such as cleaning or drying. Sanitary factors such as the presence of contaminants can also be included while grading to indicate fitness of barley for human or animal consumption. Grades and standards are dynamic, changing with grain technology, weather conditions, and the changing demands of processors and consumers.

Marketing and grading mechanisms can vary considerably among the different exporting and importing countries. Local supply and demand as well as government involvement affect final arrangements.

# Marketing

Marketing of barley is a complex process involving all the activities required to move grain from the field to the end user. It can involve three major components. The primary component is the exchange of barley for money. Another important component is arranging physical activities such as transportation or storage that are required for moving the product from field to the end user. Finally, marketing will include many facilitating functions such as standardization, financing, risk bearing, gathering of market intelligence, promotion, customer technical support, and after-sales service.

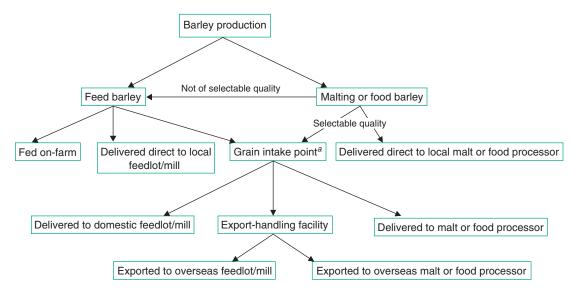
The marketing of barley begins with a producer's decision to grow grain for feed, alcoholic beverages, or food. First, there must be a local outlet available for delivery to the chosen market. Producers should discuss with these outlets, including local processors and handlers as to which markets are expected to be available at time of harvest, and any specific varieties that will be in demand. Only specific varieties can be effectively processed into alcoholic beverages or food and end-users will demand only those varieties. The feed industry is usually not concerned with specific varieties.

Environmental growing conditions will ultimately determine the appropriateness of a barley sample for malting or food processing compared with feed barley. Soil composition and fertility can influence barley quality, predominantly the protein content, while weather conditions during the growing season can have devastating effects on quality. Barley that has been downgraded due to poor growing or harvesting conditions can always be sold to the feed industry, given the right price. In contrast, it may be impossible to convert such barley into an end product that is acceptable to the malting or food industries.

Barley producers generally aim for the malting and food markets, when available, as they tend to be the most lucrative. However, occasionally, when price spreads are narrow, producers will sell top quality (malting or food) barley into feed markets in order to obtain quick cash and to avoid possible rejection of the barley later in the selection and/or delivery process. In order to be selected for malting or food, a barley must be deemed to have appropriate quality. Barley unsuitable for processing usually reverts back to the feed industry, but producers may store their barley and resubmit it for consideration if standards are relaxed due to changes in demand.

Barley that has been selected, be it for malting or food, can be destined for either domestic or overseas processors (Figure 1). In some countries, producers deliver their barley directly to domestic processors, but often barley is transferred to domestic processors through local grain intake points or to port facilities for export to overseas producers. In other countries, the barley is delivered directly to export facilities.

The decision on where to market feed barley depends on prices of the barley in local and international



**Figure 1** Flow diagram of delivery options of barley for feed and malting or food. <sup>a</sup> Deliveries of feed and selected barley can go directly to an export-handling facility at some locations.

markets. Prices depend on domestic supply and demand. The majority of feed barley is fed on-farm or sold to neighbors or local feedlots and/or mills. A second choice is delivery to a local grain intake point for transfer to more distant feedlots and/or mills or to export facilities. Once again delivery straight to the export facility is possible. In some countries, the final choice can be farm storage of the barley until a more appropriate time.

# **Grain Merchandizing**

Grain merchandizing is a complex process involving grain-intake points, export-handling facilities, and grain merchants, including STEs. At grain-intake points, decisions have to be made on whether to store, condition, or blend delivered grains. Conditioning can consist of cleaning, separating, or drying the barley. In most cases, where intake points and export facilities are jointly owned, the barley is simply transferred to export facilities, where most of the storage, conditioning, and blending takes place.

Marketing of barley is not just a matter of a seller finding a buyer and agreeing on a price, although that is the extent of it in some sales. More typically, arrangements between seller and buyer include much more detail. In some cases, quality terms are on the basis of known standards or grades of the seller. However, buyers often stipulate their own specifications, especially for malting barley. In either case, specifications are included in the sales contract along with the agreed method for determining and documenting the quality delivered. In most cases, an independent testing agency is agreed upon by

the parties. Other factors included in a sales contract can be adjustments and penalties for specifications outside of tolerances, shipping terms and demurrage clauses, and dispute-settlement clauses.

Contracts are often based on the templates of the Grain and Feed Trade Association (GAFTA) of the UK. GAFTA offers over more than one hundred different contracts to cater for the type of grain, the origin of grain, the units (imperial or metric), and many other variables. Much of the barley trade worldwide is carried out under GAFTA contracts. The quality specifications and tolerances can be specified in detail in the contract or they can be on the basis of fair average quality (FAQ) for the origin in question. The definition of FAQ must be agreed upon and will depend on the origin of grain plus the growing year.

Quality specifications are of less importance in the world market for feed barley, which is more supply-sensitive and competitive. Price is the compelling factor. World feed-barley trade is dominated by a small number of companies, and demand is concentrated in a limited number of countries.

Marketing of barley, in many cases, involves a long-term relationship between buyer and seller. Relationships involve market development activities, technical support, and customer service programs. Many grain-exporting countries have developed specific institutes to assist with these activities through educational and promotional programs. Examples of such institutes include the Canadian International Grains Institute and the Canadian Malting Barley Technical Centre in Canada, the US Feed Grains Council in the USA, the Home Grown Cereal Authority in the UK, and the France Export Cereals in France.

# **Grading and Standardization**

Grading of barley is used to facilitate grain merchandizing through the use of uniform tests and terms. Grades are measuring sticks of quality, where quality is defined as a subjective property of a barley sample that indicates its usefulness or value to an end user. In simple transactions, such as those between neighbors or with local feedlots and/or mills, inspection of the product at the time of delivery may suffice in agreeing on the grain's value. In contrast, international sales require a set of specifications in order for buyer and seller to agree on a value — grain unseen. The grades act as a market language allowing producers, marketers, and end users to communicate. As a result, the marketing process is simplified and marketing costs are reduced.

Grades separate barley into groups that meet certain standards. All the grains within a group have similar, but not necessarily identical, quality. Grades and standards can indicate potential processing value of the grain, its nutritional value, as well as other factors that may affect the overall value of the grain such as a need to dry or clean a sample, or even the need for special storage conditions. Grades and standards can be based on visual appearance or more objective machine testing.

Standardization, or the use of grades and standards, has benefits for all involved in the marketing of grains (Table 1). Grades ensure that grains of equal value receive equal monetary compensation and that a high-quality product receives a premium that allows end users to communicate their needs to producers. Grades foster a more efficient price-discovery process; they can reduce costs of handling and transportation through the use of bulk-handling systems. Grades lead to consistency in processing and satisfied end users. Grades can also reveal clear variations in quality,

**Table 1** List of advantages and disadvantages of using grades and standards

Advantages	Disadvantages
Maintain consistent pricing and ensure premiums for top quality product Allows end users to communicate their needs to producers Reduce handling and transportation costs through bulk handling Attract and keep customers through delivery of consistent product Reduce sanitary hazards	Grading contributes to costs and advantages must outweigh this cost Grading of grains can affect throughput at handling Quality is subjective — agreements on actual specifications can be difficult

indicating opportunities for improvement such as drying, cleaning, and special storage. Enforcement of grades can reduce sanitary hazards such as contaminated grain.

The number of grades cannot be exaggerated without increasing costs and thus negating advantages. The grades must be easily applied such that they do not significantly slow down the handling and transportation of barley. However, the methods must still be accurate and allow for uniform measurement. There cannot be absolute uniformity at any cost. A grade must identify enough of average production to be a meaningful category. The best test of any grade is the degree of its use by end users.

#### **Grading to Determine Processing Potential**

Barley must meet quality specifications in order to be acceptable for malting or, to a lesser extent, for processing as a food (i.e., tea, pearling, sochu (a barley liquor)). The factors considered in selecting a barley for malting (Table 2), the predominant use for selected barley, indicate how well the barley will process in the malt house. Factors identify barley that takes up water at a quick and consistent rate, germinates in a uniform manner, and is capable of producing a final product of acceptable quality. Of special interest is the need for varietal purity. Each variety malts slightly differently, and mixtures of varieties often do not malt to their full potential. Each malting barley variety has been bred specifically for malting, and acceptance of its potential is indicated by the variety's registration or listing on an official eligibility list, often maintained by domestic barley industries. Barley deemed unacceptable for processing as malt or food is reverted to the feed industry.

Barley selected for barley tea or pearling must also show specific characteristics in order to ensure

**Table 2** Examples<sup>a</sup> of grain characteristics that may be used in selecting barley for malting

Characteristic	Desired quality
Protein level	9-12% dry matter
Barley variety	Locally recognized malting barley variety
Varietal purity	>98%
Germination after 3 days	>95%
Falling number	>300 s
Rapid Visco Analyzer	>130 stirring number
Pesticide residues and mycotoxins	Minimal (according to national standards)

<sup>&</sup>lt;sup>a</sup>Characteristics will vary among countries.

straightforward processing. Barley for pearling must have an appropriate endosperm texture to prevent kernel breakage, and kernel size must be uniform in order to ensure a consistent pearled product. Barley for tea must be of a desired variety and also show a consistent kernel size in order to produce a uniformly roasted end product. Similarly to malting barley, these characteristics are often ensured by offering varieties that are known to be acceptable to end users. Feed-barley end users often demand a barley with special processing characteristics. This is especially true when barley is rolled prior to feeding. Uniform size is essential if rolled barley is to be sufficiently cracked without production of excess fines.

# **Grading to Determine Overall Value**

The overall value of barley, be it for feed, food, or malt, is determined by grading a barley sample's size, moisture, cleanliness, and appearance. Factors considered include kernel size and weight, presence of damaged kernels and appearance, as well as the presence of foreign matter such as other cereals, small seeds, and wild oats. The application of grades varies among countries, with some applying premiums and discounts for particular factors, while in others a series of parameters are used to determine a grade, and each grade is assigned an appropriate price.

Examples of grade specifications for a number of exporting countries are listed in Tables 3–7. The

Table 3 Some malt and feed grade specification used at barley receival in Australia

Characteristic	Malting barley 1	Malting barley 3	Feed barley 1	Feed barley 2
Kernel plumpness	< 30.0	<42.0	< 60.0	< 80.0
(% below $2.5 \times 12.5$ mm screen) Screenings	< 7.0	no	no	20
(% below 2.2 × 12.5 mm screen)	< 7.0	na	na	na
Test weight (kg hl <sup>-1</sup> )	65.0	65.0	62.5	60.0
Moisture (%)	13.5	13.5	13.5	13.5
Ergot	nil	nil	nil	nil
Sprouted grains (per 100 grains)	nil	nil	Free from rootlets	5 sprouted kernels or less
Skinned kernels (per 100 grains)	15	15	na	na
Broken kernels (% weight per 100 grains)	2	2	5	5
Other cereals (seeds per half liter)	85	85	500	1500
Wild oats (seeds per half liter)	25	25	50	100

na = not applicable.

Data from ABB Grain Ltd.

Table 4 Grade specification applied for some feed and malting grades used at grain-intake points in Canada

Characteristic	Special select (malting) <sup>a</sup>	Select (malting) <sup>a</sup>	No. 1 Canadian Western (feed) <sup>b</sup>	No. 2 Canadian Western (feed) <sup>b</sup>
Kernel plumpness (% over 2.38 × 19.05 mm screen)	>85.0	>80.0	na	na
Screenings (% through a 1.98 × 19.05 mm screen)	< 3.0	<3.0	na	na
Test weight (kg hl <sup>-1</sup> )	63.0	61.0	63.0	57.0
Moisture (%)	13.5	13.5	14.8	14.8
Ergot (% by weight)	nil	0.025	0.05	0.1
Sprouted grains (% by weight)	nil	0.5	10.0	20.0
Peeled and broken kernels (% by weight)	4.0	6.0	15.0 <sup>c</sup>	25.0 <sup>c</sup>
Other cereals (% by weight)	1.0	1.0	2.5	8.0
Wild oats (% by weight)	0.2	0.5	1.0	2.5
Stones (% by weight)	0.02	0.02	0.15	0.15
Total foreign matter (% by weight)	1.0	1.5	2.5	10.0

<sup>&</sup>lt;sup>a</sup>Specifications for two-rowed barley.

<sup>&</sup>lt;sup>b</sup>No distinction between two- and six-rowed barley.

<sup>&</sup>lt;sup>c</sup>Broken only.

 $na = not \ applicable.$ 

Data from Canadian Grain Commission.

parameters, as well as specifications for the parameters, vary among countries, reflecting differences in infrastructure, customers, and growing conditions.

# Establishing and Analyzing Grades and Standards

The establishment of specifications for grades and standards can be difficult. Scientists and the trade tend to develop standards that are easily measured but not necessarily informative or accurate. Conflicts may arise between buyers and sellers, and compromises may form part of marketing negotiations. In the end, specifications must be indicative of value to the end user, otherwise the end users will not use the grades.

**Table 5** Some of the EU intervention standards for feed barley

Characteristic	Feed barley
Test weight (kg hl <sup>-1</sup> )	62.0
Moisture (%)	14.5
Sprouted grains (% by weight)	6.0
Broken kernels (% by weight)	5.0
Overheated grain (% by weight)	3.0
Other cereals (% by weight)	5.0
Total foreign matter (% by weight)	12.0

Data from European Commission.

Changes in the preferences of consumers, the ultimate end users, have increased the pressures on the system. Marketing agreements have had to evolve and now often include more specific and tighter quality conditions. Marketers and end users have increased their reliance on grower contracts and identitypreserved systems in order to meet and document specifications. In some cases, documentation is required on the type of seed and variety planted and a history of pesticide use, as well as specific information on the nutrient content of the grain. The result is tighter control and a need to preserve the integrity of the product as it moves through the system. Such extra control leads to greater costs, with higher prices for end users but not necessarily better prices for producers.

Determination of a barley's grade is totally dependent on obtaining a representative sample for testing. At large grain-intake points and export-loading facilities, representative samples are obtained with automated equipment that takes samples from a moving grain stream. At smaller facilities, samples must often be obtained using manual probes of a lot at unload. Considerable documentation exists on proper sampling techniques.

Grades and standards are largely determined using objective, machine-based methods. Levels of protein

Table 6 Grade specifications (used by buyers and sellers) of barley in Russia and Ukraine

Characteristic	Barley for brewing (malting)	Barley 1 Class (fit for humans)	Barley 2 Class (feed)
Test weight (kg hl <sup>-1</sup> )	No limit	63.0	No limit
Moisture (%)	17.0	19.0	19.0
Other cereals (% by weight)	2.0	9.0	15.0
Small seeds (% by weight)	5.0	5.0	No limit
Total foreign matter (% by weight)	2.0	4.0	8.0

Data from GOST.

Table 7 Grade specifications used for some of the grades used in the USA

Characteristic	US No. 1 (malting) <sup>a</sup>	US No. 2 (malting) <sup>a</sup>	US No. 1 (feed) <sup>b</sup>	US No. 2 (feed) <sup>b</sup>	US No. 3 (feed) <sup>b</sup>
Screenings	<5.0°	<7.0°	< 10.0 <sup>d</sup>	<15.0 <sup>d</sup>	<25.0 <sup>d</sup>
Test weight (kg hl <sup>-1</sup> ) <sup>e</sup>	64.3	61.8	60.5	57.9	55.3
Damaged Kernels (% by weight)	na	na	2.0	4.0	6.0
Peeled and broken kernels (% by weight)	5.0	7.0	4.0 <sup>f</sup>	8.0 <sup>f</sup>	12.0 <sup>f</sup>
Wild oats (% by weight)	1.0	1.0	na	na	na
Total foreign matter (% by weight)	0.5	1.0	1.0	2.0	3.0

<sup>&</sup>lt;sup>a</sup> Specifications for two-rowed barlev.

Data from Federal Grain Inspection Service.

<sup>&</sup>lt;sup>b</sup>No distinction between two- and six-rowed barley.

 $<sup>^{\</sup>it c} Through \ a \ 2.18 \times 19.05 \ mm \ screen.$ 

 $<sup>^</sup>d$ Through a 1.98  $\times$  19.05 mm screen.

<sup>&</sup>lt;sup>e</sup>Based on Winchester bushel weight × 1.287.

<sup>&</sup>lt;sup>f</sup>Broken only.

na = not applicable.

and moisture are measured using near-infrared technology, which can also indicate the processing potential of a sample for malting or even the nutritive value of a sample for feeding. Automated agitators and screens are used to determine kernel size distribution, although imaging technology can provide more information on size homogeneity. Imaging can also quantify damaged kernels, but this largely remains a visual task. Varietal purity is based on visual inspection with confirmation, often on a random basis, through more objective methods such as gel separation of proteins or deoxyribonucleic acid (DNA). Machine tests such as falling number and rapid visco-analyzer indicate any preharvest sprouting in a sample and thus its storability. However, a three-day germination test remains the only reliable method for ensuring a sample's germination potential. The single-kernel characterization system is used to identify barley with appropriate endosperm texture for pearling and further processing. It can also determine the homogeneity of a sample with respect to size, weight, moisture, and protein. Measuring pesticide and mycotoxin residues in a sample requires specialized lab equipment and is usually done only on a random basis unless specified. General appearance of a sample remains largely a visual process, but some countries rely on machinebased methods for quantifying grain color.

# Specific Details for Some Major Barley-Exporting Countries

#### Australia

Australia is the leading malting-barley exporter and one of the major feed-barley exporters in the world (Table 8).

Traditionally, Australian barley has been marketed by an STE, but recent deregulation has allowed for increased private trading of barley. The boards, such as the GrainPool and the former Australian Barley Board (now ABB Grain Ltd.), have no monopolies on the marketing of barley domestically. Monopoly powers continue to exist, in some states, for the export of feed and malting barley, although additional changes could further reduce these powers.

Australian marketing agencies set the quality specifications (Table 3) of the various grades of feed and malting barley purchased from producers and apply price adjustments accordingly. These agencies work with the local storage and handling corporations (in some cases they are one and the same) to provide payments to growers, which reflect the quality of the barley delivered.

When exporting grain the marketing agencies trade according to the specifications agreed with the importer, and the quality standards may vary by contract. In most cases, the marketing agencies provide analysis of specifications, although independent private analyzers are used when required.

#### Canada

Canada is the second leading exporter of malting barley in the world and continues to export significant amounts of feed barley but not to the extent it did in the 1980s. A majority of barley production is used in the domestic feed industry.

Barley is marketed in Canada through the Canadian Wheat Board (CWB), an STE with marketing monopoly, which operates under government legislation. Domestic feed barley is exempt from the CWB monopoly and is, therefore, bought and sold on the open market. However, all other categories of barley must be marketed through the CWB. Thus, exports of feed, malting and food barley, and sales of malting and food barley to domestic processors must go

Table 8 World	l exports (millic	n tons) of f	eed and malting	g barley b	v country (	(1997–2011)
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	1	Feed barley		Λ	lalting barley	
	Averages	Proje	ctions	Averages	Proje	ctions
	1997–2001	2006	2011	1997–2001	2006	2011
Australia	2.2	1.7	1.3	1.4	1.9	2.1
Canada	0.5	0.4	0.4	1.1	1.8	2.0
European Union	5.4	4.0	4.7	1.1	1.5	1.5
Russia	0.9	2.2	2.5	0.0	0.1	0.1
Ukraine	1.2	2.5	2.9	0.0	0.1	0.1
USA	0.8	0.6	0.6	0.2	0.2	0.2
Others	1.5	2.0	2.6	0.3	0.3	0.4
World total	12.7	13.7	15.2	4.1	5.9	6.4

Data from Canadian Wheat Board.

through the CWB. Export sales to international customers can be direct from CWB or indirect via approved accredited exporters, including Canadian and international grain trading companies.

Growers receive payment for feed, malting, and food barley delivered to the CWB based on official grades (Table 4) of the Canadian Grain Commission (CGC). The CGC, a federal government agency, determines, with industry consultation, the grade standards and provides third-party determination of grades. Official standards are not always applied to feed barley sold on the open domestic market, which often trades on specifications of the domestic feed industry.

In general, CGC grades are applied in international sales of Canadian feed barley. However, malting-barley exports are normally sold according to individual contract specifications as agreed with international buyers. Sales of malting barley and food barley to domestic processors can be according to official CGC grades.

#### **European Union**

The European Union (EU) is the largest exporter of feed barley in the world and the third leading exporter of malting barley. The EU is treated, for this discussion, as a single entity even though a number of countries contribute to barley production and market their barley independently.

Feed and malting barley are traded on a private basis in the EU. Open-market barley trades freely and finds its own price level on the domestic market as in most other countries. However, if domestic prices are higher than world prices, the EU can grant export refunds to allow domestic barley to be competitive in world markets. Feed barley is sold periodically in the domestic market or to non-EU markets from intervention (the internal EU-guaranteed floor price system) stocks using a tendering system.

The EU has a common set of minimum standards for feed barley (**Table 5**) that are applied to domestic intervention purchases as well as for all exports, either from intervention stocks or from the free market. Exports of malting barley are based on specifications in GAFTA contracts.

#### Russia/Ukraine

In recent years, Russia and Ukraine have emerged as significant exporters of feed barley. Barley is marketed in an open-market-type system, with the export of barley being handled by the private trade.

For internal trade, the State Committee of the Russian Federation for Standardization and Metrology (GOST) establishes grades for barley. Sellers and

buyers conduct trade according to GOST standards (Table 6). Product specifications are analyzed and certified by the State Grain Inspectorate.

Exports are made on the basis of contract specifications negotiated between buyer and seller and agreed in the sales contract. GAFTA contract templates are often used to specify the agreed quality standard and method of analysis. Specifications are certified by the State Grain Inspectorate.

# USA

The USA is a major exporter of feed barley. It also exports small quantities of malting barley.

US barley is marketed under an open-market system. Various private traders compete for supplies of barley and for export business. Prices paid to producers are determined at the intake point and are based on a series of discounts determined from analysis of the delivered barley. The majority of malting barley is now production-contracted by maltsters and brewers who often specify their own barley varieties.

In the USA, the Federal Grain Inspection Service (FGIS), a division of the Grain Inspection, Packers and Stockyards Administration (GIPSA), is responsible for grade analysis and, in consultation with industry, the setting of standards (Table 7) for barley. Official inspection and weighing of US barley is mandatory for export but not for domestic commerce. Inspection services, or official grade analysis, can be performed by private agencies that have been licensed by the FGIS.

# Specific Details for Some Major Barley-Importing Countries

# **China (Malting-Barley Imports)**

China is the largest malting-barley importer in the world. Until the mid-1990s, all malting barley imports were done through an STE, the China National Cereals, Oils and Foodstuffs Import and Export Corporation (COFCO). Recently, imports have been liberalized, and now end users and local trading companies from China are free to import barley directly and to conclude their own contracts, in terms of both price and quality.

Sales contracts can be based on GAFTA terms or on individual contract terms, depending on origin, seller, or importer/end-user preferences. They specify the exact quality specifications to be delivered. The State General Administration of the People's Republic of China for Quality Supervision and Inspection and Quarantine (AQSIQI) verifies the quality of all imported barley. Only barley imports with an AQSIQ certificate are released for distribution.

# Japan (Feed-Barley Imports)

Japan is a major feed-barley importer in the world market. Imported feed barley has traditionally been the main ingredient used in the production of a special type of heavily marbled beef called Kobe beef. For this use, barley is steam-rolled before feeding. Feed barley is also milled into compound feeds for other livestock such as dairy.

Japan is one of the few countries in the world that still retain STE, the Japanese Food Agency (JFA), with monopoly powers for buying and importing barley. The JFA is responsible for purchase and distribution of barley, for both human and animal consumption. The JFA conducts regular calls for tenders from supplying countries. Tenders are usually based on the official standard quality grades used in the country of origin, which are familiar to the sellers and the JFA. The JFA does its own testing to verify the quality of all delivered barley. Imports from tenders go into the JFA's stock and are distributed to domestic end users at agreed prices. In this system, there are no negotiations, with respect to price or quality specifications, between individual end users from Japan and international traders.

However, in recent years, the JFA has allowed for simultaneous buying and selling (SBS). Although the JFA still administers them, SBS purchases allow for individual negotiations between Japanese end users and international traders. Each year the JFA approves the total tonnage of grain that can be imported under the SBS system.

#### Saudi Arabia (Feed-Barley Imports)

Saudi Arabia is the leading feed barley-importing country in the world. In recent years, imports have been fully liberalized and privatized, with local traders and feed mills working with international trading agencies to source their barley. The only government constraint is that all imported barley be dyed with a red colorant in order to distinguish it from the locally produced and heavily subsidized barley. Typically, the majority of the barley that is imported is fed as whole barley to sheep, goats, and camels by Bedouins. Feed mills are sophisticated and import bulk barley for use in their rations, given favorable prices. Contract terms have become quite flexible since market deregulation, but they normally conform to GAFTA contract terms. The major parameter for selling barley into Saudi Arabia is a competitive price.

See also: Animal Feed. Barley: Harvesting, Storage, and Transport; Milling and Processing; Malting. Variety Identification of Cereal Grains. Wheat: Grading and Segregation; Marketing.

# **Further Reading**

Kohls RL and Uhl JN (1996) Marketing of Agricultural Products. Upper Saddle River, NJ: Prentice-Hall.
MacGregor AW and Bhatty RS (eds.) (1993) Barley Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

#### **Relevant Websites**

http://www.usda.gov — This site gives barley grade determinants for the USA.

http://www.gost.ru.

http://www.ddic.dom.

http://www.fao.org.

http://grainscanada.gc.ca - This site gives grade specifications from Canada.

# **Milling and Processing**

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#### Introduction

Barley is one of the most widely cultivated cereal crops that can provide valuable nutrients required by humans and domestic animals. Its high adaptability to various climates and growing conditions has led to worldwide production averaging 147 million ton (Mt) per year since the early 1990s. Barley has had three distinct end uses – human foods, alcoholic beverages, and animal feed – but patterns of its utilization have changed throughout history. Barley was grown in the ancient world mostly to provide food staples for human nutrition. In ancient Rome, for example, barley was used in bread making, while later it became more common in soup and porridge dishes, usages that have survived into present times. Drastic changes in consumption patterns in the Western world took place at the beginning of the twentieth century, when grains such as rice, wheat, and maize gained greater preference. The utilization of barley for food dropped drastically, except for barley used in the production of alcoholic beverages, especially beer. The world's use of malting barley for beer production significantly increased after the Second World War, but this trend reversed itself at the end of the twentieth century, especially in the West. The use of malting barley in Asia, however, continues to increase. Cultivation of barley for feed is a more recent development, but it has significantly exceeded other uses, and the majority of barley production today is used for feeding animals.

Although barley has lost its earlier pre-eminent position in human nutrition, at least in industrialized countries, recent collaborative efforts in a variety of disciplines – such as medicine, nutrition, and agriculture – have elucidated the role of grains in prevention and treatment of many human diseases and revived interest in barley for food purposes. Current developments in processing barley and incorporating it in various food products stem from the many health benefits associated with certain components of this cereal. The food industry is now facing the challenge and opportunity to develop novel, palatable, attractive, and healthy barley-based products that will gain acceptance among consumers.

# **Composition of Barley Grain**

Barley grain consists mainly of carbohydrates, proteins, and lipids (Table 1). Carbohydrates are major components, with starch being the principal constituent. Barley is known for its high content of total dietary fiber ( $\sim 10-20\%$ ), and mixed linkage  $\beta$ -glucan has been shown to lower plasma cholesterol, reduce the glycemic index, and reduce risk of colon cancer. The nutritional value of other dietary fiber components – such as arabinoxylans, cellulose, fructans, galactomannans, and arabinogalactans – has

Table 1 Barley composition

Component	Content (%, dry weight
Carbohydrates	78-83
Starch	60-65
Arabinoxylans	4.4 - 7.8
$\beta$ -Glucans	3.6-6.1
Cellulose	1.4-5
Simple carbohydrates (glucose, fructose, sucrose, maltose)	0.41-2.9
Oligosaccharides (raffinose, fructosans)	0.16-1.8
Lipids	2–3
Proteins	8-15
Albumins and globulins	3.5 - 4.5
Hordeins	3-5
Glutelins	3–5
Minerals	2–3
Others <sup>a</sup>	5-6

 $<sup>^</sup>a\text{Barley}$  also contains small quantities of B-complex vitamins, including thiamine (B1), riboflavin (B2), nicotinic acid, pyridoxine (B6), and pantothenic acid, biotin, folic acid, and vitamin E.

not yet been thoroughly elucidated. Barley proteins are poor in certain amino acids, e.g., lysine and threonine, but considerable improvement of lysine content has been achieved with the development of high-lysine barley mutants. Barley grain contains some important lipids, such as tocols (tocopherols and tocotrienols), which posses some antioxidant properties. The concentration of  $\alpha$ -D-tocotrienol, known to inhibit cholesterol synthesis in livers of experimental animals, is higher in barley than in other grains. A second cholesterol inhibitor, α-linoleic acid, has also been found among the barley fatty acids. The recently recognized involvement of antioxidant compounds in preventing the formation of carcinogens from precursor compounds has directed attention to such barley components as phenolic acids, phytin, vitamin E, proanthocyanidins, and catechins. Barley is also an excellent source of B-complex vitamins, especially thiamine, pyridoxine, pantothenic acid, niacin, as well as biotin and folacin. Elemental minerals occur in barley grain with concentration between  $\sim 2\%$  and 4%. The major components are phosphorus, potassium, and calcium, with lesser amounts of chlorine, magnesium, sulfur, and sodium. Phosphorus is one of the most important mineral nutrients in barley, but up to 80% of barley phosphorus may be nutritionally unavailable because it is bound to phytic acid. This problem is being addressed by the development of low-phytic acid barley genotypes.

Chemical composition of barley is strongly influenced by both genetic and environmental factors. The presence or absence of the hull after harvesting, and the inherent starch characteristics, are the two fundamental genetic factors influencing barley composition. The hull or hull-less characteristic is established during development and maturation of the grain. In hull-less barley, the unattached, loose husk is visibly separated from the kernel during threshing; in hulled barley, the husk remains attached. Generally, hulled barley is chosen for malting, whereas hull-less barley has been used in animal feeds and shows better potential for incorporation into human foods.

The distribution of various chemical constituents is not uniform throughout the component tissues of barley grain, i.e., husk, pericarp, testa, aleurone layer, endosperm, and embryo (Figure 1). The husk and pericarp, the two outermost and protective tissues of the barley grain, consist primarily of cellulose, hemicellulose, lignin, and lignans — the major components of insoluble dietary fiber. The husk contains the highest proportion of minerals, followed by the embryo and the endosperm. Proanthocyanidins and catechins occur between the cuticularized layers of

Adapted from MacGregor AW and Fincher GB (1993) Carbohydrates of the barley grain. In: MacGregor AW and Bhatty RS (eds.) *Barley Chemistry and Technology*, pp. 73–130. St. Paul, MN: American Association of Cereal Chemists

testa. The embryo, which constitutes  $\sim 2.5\%$  by weight of the barley grain, is rich in protein (34%), lipids (14-17%), and ash (5-10%), and is very rich in sugars (sucrose, 15%; raffinose, 5-10%; and fructosans). The cell walls of the scutellum are composed mainly of hemicelluloses and contain some phenolics, mainly ferulic acid. The thick walls of the aleurone layer  $(2-5 \mu m)$  are composed mainly of

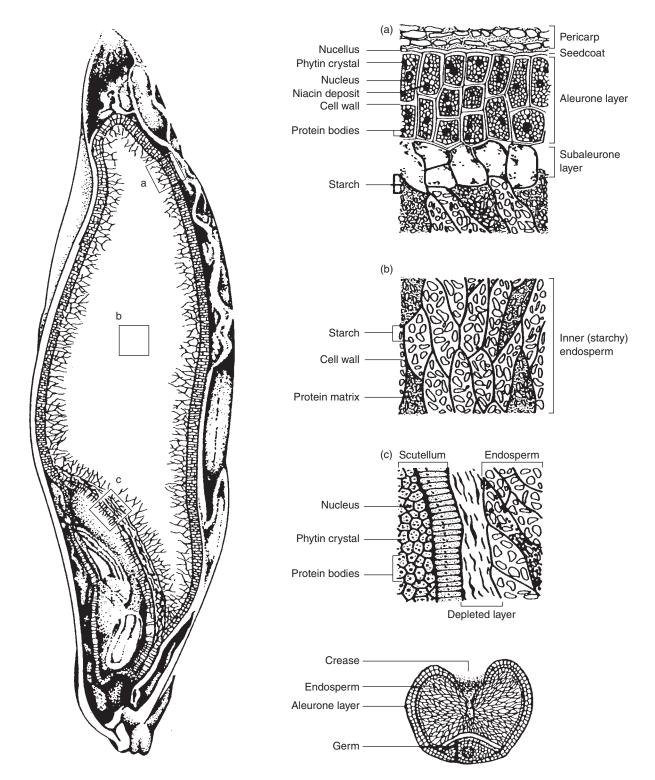


Figure 1 Structure of the barley kernel sectioned longitudinally (left) and transversely (right), with detailed bran layer (a), starchy endosperm (b), and embryo (c). (Courtesy of Fulcher RG and Wong SI.)

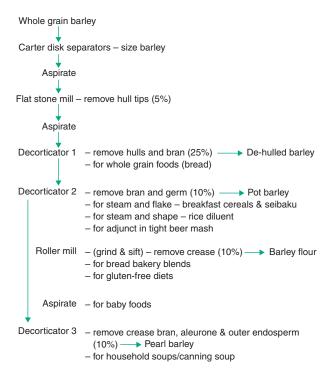
arabinoxylans (67–71%) and  $\beta$ -glucans (26%), and also contain phenolic acids. This tissue also abounds in proteins (17–20%), triacyl glycerol (20%), minerals, phytin, and sugars (including sucrose, raffinose, stachyose, verbascose, and fructans). Aleurone cells contain bodies called aleurone grains; these consist of globoid composed of phytins, and crystalloids composed of proteins and polysaccharides. Deposits of  $\beta$ -(1 $\rightarrow$ 3) glucans (callose) are found at the aleurone–subaleurone interface.

The starchy endosperm makes up  $\sim 75\%$  of the barley grain. In the mature barley grain, starch, which constitutes  $\sim 80\%$  of the endosperm, exists in granular form, with two distinct populations of large (A-type) and small (B-type) granules. The small granules account for 90% of the total number of granules, but only 10% of their total weight. Starch is composed of two polysaccharides, amylose (AM) and amylopectin (AP), with AP constituting  $\sim$ 75% of the starch in normal barley. Two single-gene mutations can alter the AM/AP ratio. When the genetic trait of waxy starch is present, starch contains 95-100% amylopectin. The other mutation increases the amylose content up to 40%. The AM/AP ratio in different barley genotypes (waxy, normal, and high amylose) has a significant effect on the *in vitro* digestibility of these starches, with the high-amylose starch being the least and waxy starch the most susceptible to  $\alpha$ -amylolysis. However, the results of the *in vivo* studies are not yet conclusive. For example, when waxy barley starch is fed to rats, swine, or chickens, no increased digestibility is observed when compared with normal barley. The starchy endosperm also contains a relatively larger amount of proteins ( $\sim$ 9% of the tissue) and smaller amounts of lipids, minerals, and nucleic acids. The cell walls of starch endosperm (2 μm thick) are built up mainly of mixed linkage  $\beta$ -(1 $\rightarrow$ 3), (1 $\rightarrow$ 4)-glucans (70%), and arabinoxylans (20%), with only small amounts of proteins,  $\beta$ -(1 $\rightarrow$ 3) glucans, and other polysaccharides containing galactose, mannose, and uronic acids. The presence of waxy or high-amylose genes in barley affects the carbohydrate metabolism in the grain, and waxy and high-amylose barleys are consistently higher in  $\beta$ -glucans than normal starch genotypes.

# **Barley Pearling**

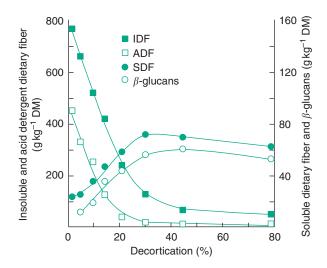
Pearling is one of the oldest practices of processing barley. Pearling is a process of abrasive scouring that gradually removes hull, pericarp, seedcoat, aleurone and subaleurone layers, and the embryo. One type of pearling machine consists of three to eight carborundum or emery stones, which revolve rapidly within a perforated cylinder. The hull and other fractions of the kernel are gradually rasped off by rubbing against the stones and the perforated cylinder. The time the kernel stays in the abrasion chamber determines the degree of abrasion. Depending on the pearling rate (degree), the process produces blocked (de-hulled) barley, pot barley, or pearled barley (Figure 2). Blocking removes only the husk. Removal of the next kernel layer, the pericarp, produces pot barley. Further abrasion, leading to formation of pearled barley, removes the seedcoat, aleurone, and subaleurone layers. The pearled barley represents usually  $\sim 40$ 50% of the grain, and may be adjusted as prescribed by the end use of the final product. Barley preferred for pearling should be uniform in kernel size and weight, plump, and free from discolorations. It has been proposed that two-rowed barley may be more suitable for pearling and milling because it is plumper and more uniform in kernel size and shape than sixrowed barley.

Pearling removes the components present in the outer layers of the barley kernel. Thus, the content of insoluble dietary fiber in pearled barley decreases with increasing pearling rates (Figure 3). The content of  $\beta$ -glucans, fat, protein, tannin, and phosphorus initially increases with increasing pearling rates, but declines after reaching optimum values between 15% and 30% decortication (Figure 4). In hulled barley, the hull and pericarp are usually present in the first

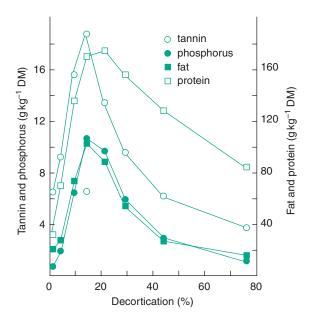


**Figure 2** Diagrammatic representation of the processing of barley into various fractions. (Courtesy of Frank Sosulski, University of Saskatchewan, Saskatoon, Canada.)

0-11% pearling by-product fractions, whereas the germ and aleurone constitute the subsequent 11-25% fractions. Due to the presence or absence of hull, the constitution of pearling by-products may vary for hulled and hull-less barley. The pearling



**Figure 3** Relationship between degree of pearling (decortication) and the relative concentrations of insoluble fiber (IDF), soluble dietary fiber (SDF), acid detergent fiber (ADF), and β-glucan fractions of barley. (Reproduced with permission from Pedersen B, Bach Knudsen KE, and Eggum BO (1989) Nutritive value of cereal products with emphasis on the effect of milling. *World Rev. Nutr. Diet.* 60: 1–91.)



**Figure 4** Relationship between degree of pearling (decortication) and the relative concentrations of tannin, phosphorus, fat, and protein. (Reproduced with permission from Pedersen B, Bach Knudsen KE, and Eggum BO (1989) Nutritive value of cereal products with emphasis on the effect of milling. *World Rev. Nutr. Diet.* 60: 1–91.)

by-products are potentially concentrated sources of bioactive components such as phytate, vitamin E (including tocotrienols), phenolic compounds, and insoluble dietary fiber. It has been shown that the pearling by-products of hull-less barley obtained at 20% pearling rate were enriched in tocols and contained 2.7–4.4 times more  $\alpha$ -tocotrienols,  $\alpha$ -tocopherols, and vitamin E activity than whole barley grain. Hence, pearling by-product is a nutrient-rich, healthpromoting food ingredient. Utilization of pearling – by-products for making pasta (by substituting 50% of standard durum wheat semolina) resulted in a product that was darker than durum wheat pasta but had good cooking characteristics with regard to stickiness, firmness, and cooking losses. Because of the high dietary fiber (15.2–16.1%) and  $\beta$ -glucan (4.3-5.0%) content, barley-enhanced pasta may be considered as a healthy food product.

Pearled barley contains mainly the central endosperm of the kernel, enriched in carbohydrates (starch and  $\beta$ -glucans) and proteins. Examination of the amino acid and protein composition in 16 consecutive layers of barley grain has revealed that the albumin protein fraction decreases from the outer layer toward the center, whereas the hordein and glutelin fractions increase from the outer layers to the middle ones, and then decrease toward the center. The globulin fraction exhibits a homogeneous distribution throughout the whole grain. The content of glutamic acid and proline increases towards the center, but other amino acids decrease or do not change. The distribution of  $\beta$ -glucans in hull-less barley grain has emerged from an examination of pearling by-products (pearling fines) collected at 10% intervals during successive abrasion of barley kernels up to 30% of their original weight. The low  $\beta$ -glucan genotypes of hull-less barley have a greater concentration of  $\beta$ -glucan in the subaleurone layer, which declines slightly toward the inner layers. The high  $\beta$ -glucan varieties, on the other hand, exhibit evenly distributed  $\beta$ -glucan throughout the entire endosperm. Because of specific localization of  $\beta$ -glucans in the endosperm cell walls, pearled barley contains more  $\beta$ -glucans on the weight basis than whole barley (Figure 3).

Blocked or pot barley is usually subjected to further processing, such as milling, grinding, or flaking, before incorporation into food products, even though pot and pearl barley are used directly in various soup, stew, and porridge recipes. Pearling of hulled and hull-less barley to 45% and 40% rate, respectively, results in a barley product which can be used as a rice extender. The superior nutritive value of barley has increased the demand for barley rice extender among increasingly health conscious consumers. The process is straightforward, but requires a specific quality of

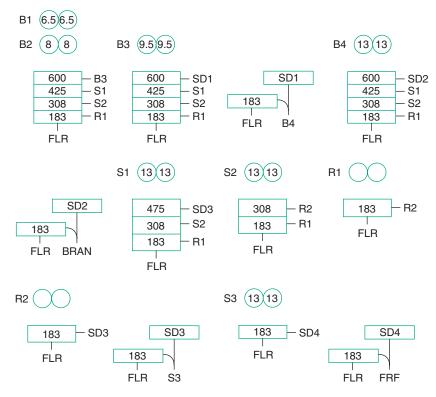
barley for trouble-free production and high-quality end product, which after cooking resembles white rice in color, shape, and texture. Waxy hull-less genotypes appear to have some advantages over covered or hull-less normal starch varieties because of their ability to pearl and split without breaking kernels or producing fines, and to produce desired color and texture after cooking.

# **Roller Milling**

Barley does not have a long tradition of being fractionated by roller milling, and the fractions derived by this process have not yet been standardized in terms of quality, composition, or terminology. Nevertheless, barley has been roller-milled to obtain flour and bran, using wheat milling equipment (Buhler mill, Allis-Chalmers mill, and Miag-Multomat mill). It is often assumed that barley bran, like wheat bran, consists of testa and pericarp, germ, aleurone, and the subaleurone layers; its composition may differ, however, depending on the degree of pearling implemented prior to milling, and needs to be better defined. It is generally agreed that barley bran is brittle and difficult to separate from the endosperm by roller

milling and, therefore, it increases the ash content and darkness of barley flour.

A wide range of flour yield, extending from 30% to 74%, has been reported for roller-milled barley. Roller milling of barley usually involves several break passages, sizing passages using corrugated rolls, and reduction passages with smooth frosted or corrugated rolls. Short duster passages, which utilize impact to separate starchy endosperm from the endosperm cell wall material, facilitate flour release. The number and sequence of passages in a mill affect the yield and composition of milling fractions. The Buhler laboratory-scale milling flow shown in Figure 5 consisted of four break (B), three sizing (S), two reduction (R), and four shorts duster (SD) passages. Following B4, the coarsest product after sifting (>600 μm) was passed through the shorts duster (SD2) and the coarse material collected over screens was designated as "bran." For barley samples pearled to >20%, this material is predominantly derived from endosperm cell walls rather than from outer coverings (pericarp, testa, aleurone, and subaleurone). Therefore for roller-milled pearled barley, the term "bran" may not correspond morphologically with bran as traditionally defined in wheat milling.

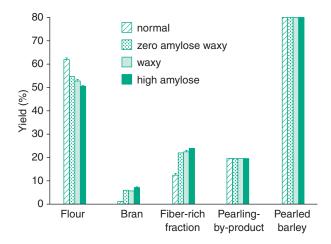


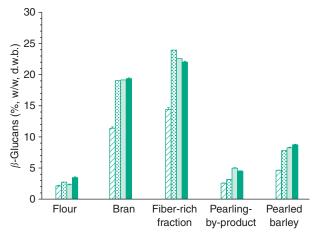
**Figure 5** Roller milling flow for barley. B = break passage; R = reduction passage; S = sizing passage; SD = shorts duster; FRF = fiber-rich fraction. Numbers on roll symbols indicate corrugations per centimeter. Numbers on sieves indicate aperture in  $\mu m$ . SD screen has 140  $\mu m$  aperture. (Reproduced with permission from Izydorczyk MS, Dexter JE, Desjardins RG, Rossnagel BG, Lagassé SL, and Hatcher DW (2003) Roller milling of Canadian hull-less barley: optimization of roller milling conditions and composition of mill streams. *Cereal Chemistry* 80: 637–644.)

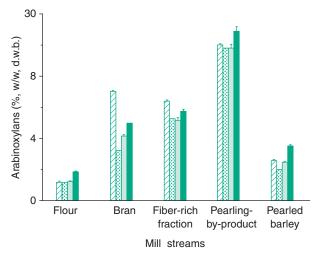
Coarse material derived from SD4 following S3, which would be designated as "shorts" (fine bran from reduction system) in wheat milling, originates mainly from endosperm cell walls regardless of whether barley is pearled before milling. This fraction, therefore, may be designated more accurately as a "fiber-rich fraction" (FRF) rather than shorts. Straight grade flour is obtained by combining all break, sizing, reduction, and SD streams.

One of the most important factors influencing flour yield is barley genotype or, more specifically, the interrelated contents of  $\beta$ -glucan and starch. The high  $\beta$ glucan genotypes, with waxy or high-amylose starch characteristics, may contain between 3% and 10% less starch than the low  $\beta$ -glucan varieties with normal starch characteristics. The high  $\beta$ -glucan barley genotypes yield, therefore, significantly lower amounts of flour but higher amounts of "bran" and "FRF" than the latter (Figure 6). High  $\beta$ -glucan barley flour has a higher proportion of large particles (150–  $250 \,\mu\text{m}$ ) than of small ones ( $<105-150 \,\mu\text{m}$ ), whereas the opposite is observed for low  $\beta$ -glucan barley. It appears that the endosperm cell walls of high  $\beta$ -glucan barley resist particle-size reduction during the milling process, which leads to lower flour yield and a higher proportion of large particles with adhered starch granules. Due to the plasticity of the thick endosperm cell walls of the high  $\beta$ -glucan genotypes, it is difficult to obtain clean separation of starch granules and other endosperm cell components from the cell wall material using successive break and reduction passages as in wheat milling. Impacting between grinding passages is necessary to optimize the process. Flour derived by impacting is also superior in color compared to flour produced by reduction grinding. Other preprocessing practices, such as tempering and pearling, may also affect the yield of milling fractions, their particle-size distribution, and composition (Table 2 and Figure 7). Today, the interest in obtaining better separation of starch from the cell wall material stems not only from the desire to obtain higher yields of whiter barley flour, but also from the demand for pure and highly concentrated barley fractions enriched in  $\beta$ -glucan and/or other bioactive nutrients.

Roller milling of barley has potential to fractionate the grain into various streams with unique composition and functionality. For example, waxy hull-less barley was fractionated using a Miag-Multomat roller mill equipped with three sets of break rolls and five sets of reduction rolls into six milling fractions: flour (obtained by combining the first break flour through the fourth middling), fifth middling, red dog, reduction shorts, break shorts, and bran. Among the milling fractions, the fifth middling, red dog, and reduction shorts were highest in total tocol concentration







**Figure 6** Yield (unpearled barley basis), β-glucan content, and arabinoxylan content of milled products from milling of hull-less barley genotypes. Barley was pearled 20% prior to milling. (Reproduced with permission from Izydorczyk MS, Dexter JE, Desjardins RG, Rossnagel BG, Lagassé SL, and Hatcher DW (2003) Roller milling of Canadian hull-less barley: optimization of roller milling conditions and composition of mill streams. *Cereal Chemistry* 80: 637–644.)

Table 2 Yild and composition of bran and flour obtained by Roller Milling of Regular and waxy starch hull-less barleys at different moisture levels<sup>a</sup>

Moisture (%)	Yield (%)		Ash (%)		Protein (%)		Starch (%)		β-Glucan (%)	
	Bran	Flour	Bran	Flour	Bran	Flour	Bran	Flour	Bran	Flour
CDC Candle (waxy)										
5	34.3	65.7	2.1	1.9	15.2	16.1	57.9	65.9	9.4	6.1
7	48.6	51.4	2.2	2	15.3	16.3	57.8	62.1	8.8	5.7
8	61.6	38.4	1.9	1.7	16.5	16.2	56.5	71	8.6	4.6
12	66.3	33.7	2.4	1.5	16.5	15.5	56.9	73.8	8.5	4.6
14	67.3	32.7	2.5	1.3	17.4	14.3	52.9	77.8	8.1	4.9
16	65.9	64.1	2.6	1.4	17.4	14.3	53.7	79.6	7.9	5.2
CDC Dawn (regular)										
5	14.7	85.3	4.2	1.4	18	12	44.2	74.3	5.3	4.4
7	22.8	77.2	3.8	1.3	17	11.6	45.4	73.5	5.4	4.1
9	40.4	59.6	2.6	0.9	15.9	10.9	54.5	81.4	5.5	3.5
12	43.3	56.7	3.2	0.9	17.3	9.9	51.5	80.3	5.5	3.2
14	50.9	49.1	2.9	8.0	16.9	9.6	50.6	80.9	5.6	2.7
16	52.6	47.4	2.6	0.7	15.4	9.2	60.2	86.8	5.6	2.7

<sup>&</sup>lt;sup>a</sup> Adapted from Bhatty RS (1997) Milling of regular and waxy starch hull-less barleys for the production of bran and flour. Cereal Chemistry 74: 693-699.

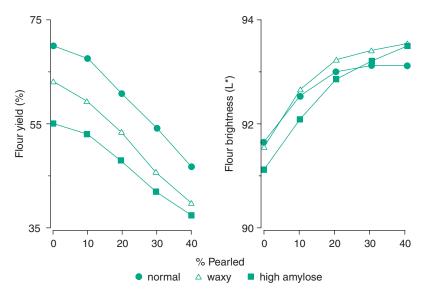


Figure 7 Effect of pearling level on flour brightness and flour yields (whole barley basis) for normal amylose (Falcon), waxy (CDC Candle), and high-amylose (CDC-92-55-06-48) hull-less barley genotypes. (Reproduced with permission from Izydorczyk MS, Dexter JE, Desjardins RG, Rossnagel BG, Lagassé SL, and Hatcher DW (2003) Roller milling of Canadian hull-less barley: optimization of roller milling conditions and composition of mill streams. Cereal Chemistry 80: 637-644.)

(Table 3). In another trial, where hull-less barley was pearled (20%) prior to fractionation in a Buhler laboratory mill, several nutritionally valuable milling fractions were obtained (Figure 6).  $\beta$ -Glucans and arabinoxylans from endosperm cell walls were highly concentrated in the FRFs obtained from the reduction system. For high  $\beta$ -glucan genotypes, yields of more than 20% (whole barley basis) of FRFs with  $\beta$ -glucan content of over 20% were obtained. Arabinoxylans, although especially concentrated in pearling byproducts (10-12%), still constituted a considerable portion ( $\sim$ 6%) of the FRFs.

Barley milling fractions have performed well in several baked products. Replacement of 20% of wheat flour in quick bread by both waxy and nonwaxy barley break flour or bran did not significantly affect loaf volume and produced a smooth attractive top crust surface and slightly softer texture of bread. Substantial increases of the total dietary fiber and  $\beta$ -glucan contents in bread have been obtained when fiber-rich barley milling fractions were blended with wheat flour. Satisfactory loaf volume and crumb structure can be achieved by choosing appropriate baking procedures, protein content in wheat flour, and/or

**Table 3** Concentration of oil, total tocols, and tocotrienols (T3) in whole grain and milling fractions of two waxy hull-less barleys<sup>a,d</sup>

Sample	Oil (g kg <sup>-1</sup> )	Total tocol <sup>b</sup> (mg kg <sup>-1</sup> )	$\alpha$ -T3 $(g kg^{-1})^c$	γ-T3 (g kg <sup>-1</sup> ) <sup>d</sup>	δ-T3 (g kg <sup>-1</sup> ) <sup>c</sup>
Whole grain	27.8 ± 3.5	71.0 ± 9.5	610±28	110 ± 14	10±8
Milling fraction					
Flour	$24.4 \pm 4.5$	$\textbf{42.7} \pm \textbf{16.9}$	$490\pm 7$	$100\pm28$	$10\pm 5$
Fifth middling	$\textbf{45.4} \pm \textbf{6.2}$	$76.9 \pm 31.3$	$\textbf{430} \pm \textbf{14}$	$80\pm7$	$10\pm3$
Red dog	$\textbf{37.9} \pm \textbf{1.6}$	$77.4 \pm 8.3$	$540\pm42$	$120\pm7$	$10\pm7$
Reduction shorts	$29.7 \pm 0.7$	$\textbf{78.9} \pm \textbf{2.5}$	$640\pm35$	$140\pm0$	$20\pm10$
Break shorts	$21.8\pm1.6$	$54.0\pm11.5$	$530 \pm 106$	$110\pm21$	$10\pm 6$
Bran	$19.1 \pm 0.8$	$46.9 \pm 3.5$	$\textbf{620} \pm \textbf{3.5}$	$130\pm7$	$20\pm11$

Means  $\pm$  SEM (mg kg $^{-1}$ ) of cultivar Azhul and Waxbar with two determinations for each cultivar.

<sup>&</sup>lt;sup>d</sup>Adapted from Wang L, Xue Q, Newman RK, and Newman CW (1993) Enrichment of tocopherols, tocotrienols, and oil in barley fractions by milling and pearling. Cereal Chemistry 70: 499-501.

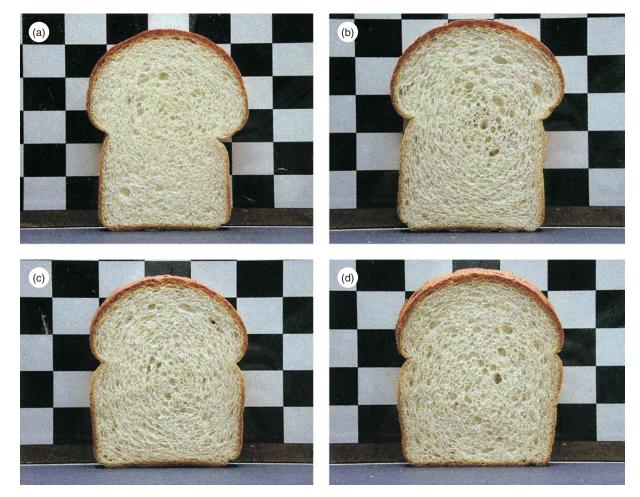


Figure 8 Effects of addition of fiber-rich barley fraction (15%) to wheat flour: (a) control wheat bread (CWRS, 14.5% protein); (b) wheat bread fortified with fiber-rich fractions from zero amylose; (c) high amylose; and (d) waxy hull-less barley. Bread baked according to the sponge and dough procedure.

enzymes (Figure 8). Fiber-enriched biscuits, sugar cookies, and muffins can also be produced with a waxy, hull-less barley milling fraction. Addition of barley flour (20-40%) to either white salted or

yellow alkaline noodles gave products with acceptable appearance, with only a slight decrease of brightness and yellowness of the noodles. It was also shown that modification of noodle texture can be achieved by

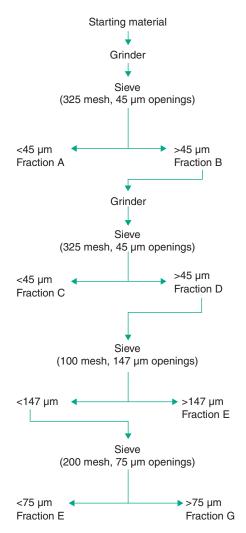
<sup>&</sup>lt;sup>b</sup>Including tocotrienols and tocopherols.

<sup>&</sup>lt;sup>c</sup>Of total tocols.

incorporating barley flour with altered starch characteristics; for example, addition of waxy barley flour produces softer, less chewy white salted noodles, whereas the addition of high-amylose barley flour results in firmer and chewier noodles.

# Milling and Sieving

Another approach to obtaining barley fractions rich in  $\beta$ -glucans involves milling of de-hulled barley, most effectively by an abrasion mill, and sieving the ground material through a series of sieves with openings of 45, 147, and 75  $\mu$ m (Figure 9). The resulting fractions, especially those with particles >147  $\mu$ m, may contain, depending on the variety, between 18.6% and 22.5% total  $\beta$ -glucan, which amount to 2.7–4.3 times enrichment of the original contents. This



**Figure 9** Milling and sieving procedure for barley to separate fractions by size. (Reproduced with permission from Knuckles BE, Chiu MM and Betschart AA (1992)  $\beta$ -Glucan-enriched fractions from laboratory-scale dry milling and sieving of barley and oats. *Cereal Chemistry* 69: 198–202.)

method, however, yields relatively small amounts of the  $\beta$ -glucan-enriched fractions (2.1–4.5%). The sieving process can be substantially shortened by implementing repeated sieving through only one type of sieve (150  $\mu$ m), but the  $\beta$ -glucan enrichment in this case is only doubled.

β-Glucan-enriched fractions can also be obtained when barley is pin- or hammer-milled and fractionated by air classifier and sifters. Coarse fractions obtained by these procedures are particularly rich in bioactive components and contain 37-45% total dietary fiber, including 16-18%  $\beta$ -glucan, and up to  $82 \,\mu\mathrm{g}\,\mathrm{g}^{-1}$  total tocols. Bread in which these barley fractions replaced 20% of the standard wheat flour contained 4.2 times the total dietary fiber, 7.6 times the total  $\beta$ -glucan, and 80% of the total calories compared to the control. The bread was judged acceptable by sensory panels, although loaf volume was reduced and color was slightly darker than the control. Pasta in which fiber-enriched barley fractions replaced 20% or 40% of wheat semolina provided 5.4-10.4 g total dietary fiber per serving; it too had acceptable sensory qualities but was darker than the controls.

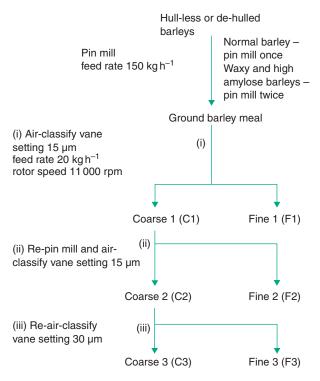
#### **Air Classification**

Application of air classification to barley also has potential for generating unique fractions enriched in specific components, and this technique should be explored and developed further, especially in light of current interest and demands for specific and functional grain fractions. Interest in using air classification to separate barley flours stemmed initially from the desire to develop nutritious highprotein fractions for food uses. Subsequent attempts at air classification of malting barley and barley flour were aimed at producing low-fiber, low-protein starchy flours with amylolytic activities, which are of special interest to the brewing industry. Recently, a method of preparing high soluble fiber barley fractions has been patented. The method involves a pinmilling step, followed by particle-size classification at 35-50 µm openings to separate the size-reduced flour into a starchy fraction and a fiber-enriched fraction with 1.2-5 times concentration of soluble dietary fiber compared to the original fiber level.

Air classification of waxy, regular, and high-amy-lose barleys has also been conducted with a view to obtaining fractions rich in large starch granules. Barleys were pin-milled to obtain meals passing through a 53 μm screen and fractionated into coarse and fine fractions by two passes at a vane setting of 15 μm and one at 30 μm (Figure 10). The first two fine fractions (F1 and F2) were protein rich and contained mostly small starch granules. In all three barleys,

air classification separated large starch granules from the small ones and concentrated them in the third fine fraction, F3. Most of the  $\beta$ -glucan in the ground barley appeared in the coarse fractions. The greatest enrichment of  $\beta$ -glucan was achieved in the third pass through the air classifier; the  $\beta$ -glucan contents were 23.8%, 13.1%, and 21.8% in C3 compared to 7.2%, 5.9%, and 7.8% in the whole grain for the waxy, normal, and high-amylose barleys, respectively. The yields of  $\beta$ -glucan-enriched fractions ranged from 7.6% for waxy and 10.4% for normal, to 20.9% for high-amylose barley.

Successful separation and concentration of grain components via air classification depends on such parameters as particle size distribution and fat content of the material to be air-classified. Substantial enrichment (62%) of  $\beta$ -glucan for air-classified high  $\beta$ -glucan barley variety Prowashonupana (originally containing 17–19%  $\beta$ -glucan and 6% fat) was obtained when de-fatting was conducted before processing. Parameters such as  $\beta$ -glucan content in barley, presence of hull, and starch characteristics may also affect the effectiveness of air classification. Further optimization of the process is required in order to obtain fractions concentrated in target component and to reduce the costs associated with power consumption.



**Figure 10** Diagrammatic representation of the procedure used for pin milling and air classification of barley. (Reproduced with permission from Vasanthan T and Bhatty RS (1995) Starch purification after pin-milling and air classification or waxy, normal, and high-amylose barleys. *Cereal Chemistry* 72: 379–384.)

# **Other Processing**

Other forms of processing of whole or ground barley have also been developed. The treatments may vary from simple cooking and drying of whole grain, to hydrothermal treatments, to even more sophisticated extrusion cooking or infrared heating. These more contemporary processing methods aim primarily at improving nutritional value of barley, and at creating more shelf-stable and convenient barley-based food items/ingredients which can be easily consumed and/ or incorporated in various food products. The processing of raw barley into products resembling traditional bulgur involves hydrating the whole grain to a moisture content between 45% and 70% by adding water and cooking the grain at temperature ranging from 60°C to 150°C. The temperature and time of this cooking step is important for obtaining optimal nutritional and functional properties of the final product. Following the cooking step, the grain is cooled and either dried or flash-frozen. If hulled barley is used for the production of bulgur, the grain has to be de-hulled after the drying step. Utilization of hull-less barley varieties eliminates this necessity. A cooked and dried grain product may be cracked, milled, or ground into flakes, granules, or powder and used as a ready-to-eat snack or cereal or further processed. Such processed barley may contain lower amount of vitamins, but can be an excellent source of dietary fiber. Increased water solubility, water binding, and adhering properties of this product improve its functionality and expand its potential as a multipurpose ingredient.

The hydrothermal processing of whole barley grain is shown to effectively degrade phytate (myo-inositol hexaphosphate) and, therefore, to increase the content of free myo-inositol (an important nutrient for infants) and the bioavailability of minerals such as phosphorus, calcium, iron, and zinc in barley. The treatment, comprising two wet steeps of barley grain in lactic acid solution alternated by dry steeps at elevated temperatures (45–70°C), and followed by successive drying (80°C), is thought to activate phytase, the endogenous enzyme which degrades phytate to free myo-inositol, and inorganic phosphate. Hydrothermally processed barley can be used in the manufacture of infant formulas or other applications such as gruels, flakes, or muesli products. Hydrothermal treatment is also used to reduce lipid oxidation during processing and storage of barley.

Another process aimed at improving the nutritional value of barley is extrusion. Extrusion cooking of high-amylose barley can facilitate formation of resistant starch in the extrudate. Resistant starch acts like dietary fiber in the gastrointestinal tract, resisting

digestion in the small intestine but undergoing fermentation in the large intestine, and resulting in formation of short-chain fatty acids (SCFAs), which are protective to colon mucosa. Formation of resistant starch increases the amount of total dietary fiber in barley after extrusion. Depending on the extrusion conditions (temperature, screw speed, and moisture content), desirable modification of other barley components may also take place during the process, including partial solubilization of  $\beta$ -glucans and formation of nondigestible oligosaccharides, thus improving the content of soluble dietary fiber in barley. Optimization of the process is necessary, however, to minimize vitamins losses. Another advantage of extrusion processing is improvement of palatability and technological properties of barley products. Due to good textural properties of barley extrudates, they may be consumed directly, used in breakfast cereals or as additives to other food products.

See also Barley: Genetics and Breeding. Consumer Trends in Consumption.

# **Further Reading**

- Bhatty RS (1993) Nonmalting uses of barley. In: MacGregor AW and Bhatty RS (eds.) Barley Chemistry and Technology, pp. 355-417. St. Paul, MN: American Association of Cereal Chemists.
- Bhatty RS (1997) Milling of regular and waxy starch hullless barleys for the production of bran and flour. Cereal Chemistry 74: 693–699.
- Bhatty RS (1999) The potential of hull-less barley (Review). Cereal Chemistry 76(5): 589-599.
- Briggs DE (1978) Barley. London: Chapman and Hall. Edney MJ (1996) Barley. In: Henry RJ and Kettlewell PS (eds.) Cereal Grain Quality, pp. 113-151. London: Chapman and Hall.
- Izydorczyk MS, Dexter JE, Desjardins RG, Rossnagel BG, Lagassé SL, and Hatcher DW (2003) Roller Milling of Canadian hull-less barley: optimization of roller milling conditions and composition of mill streams. Cereal Chemistry 80: 637-644.
- Izydorczyk MS, Symons SJ, and Dexter JE (2002) Fractionation of wheat and barley. In: Marquart L, Slavin JL, and Fulcher GR (eds.) Whole-Grain Foods in Health and Disease, pp. 47-82. St. Paul, MN: American Association of Cereal Chemists.
- Jadhav SJ, Lutz SE, Ghorpade VM, and Salunkhe DK (1998) Barley: chemistry and value-added processing. Critical Reviews in Food Science 38: 123–171.
- Knuckles BE, Chiu MM, and Betschart AA (1992)  $\beta$ -Glucan-enriched fractions from laboratory-scale dry milling and sieving of barley and oats. Cereal Chemistry 69: 198-202.
- MacGregor AW and Fincher GB (1993) Carbohydrates of the barley grain. In: MacGregor AW and Bhatty RS

- (eds.) Barley Chemistry and Technology, pp. 73-130. St. Paul, MN: American Association of Cereal Chemists.
- McIntosh GH, Newman RK, and Newman CW (1995) Barley foods and their influence on cholesterol metabolism. In: Simopoulos AP (ed.) Plants in Human Nutrition. World Review of Nutrition and Dietetics, vol. 77, pp. 89–108. Basel: Karger.
- Newman RK and Newman CW (1991) Barley as a food grain. Cereal Foods World 36: 800-805.
- Pedersen B, Bach Knudsen KE, and Eggum BO (1989) Nutritive value of cereal products with emphasis on the effect of milling. World Review of Nutrition and *Dietetics* 60: 1−91.
- Petersen PB and Munck L (1993) Whole-crop utilization of barley, including potential new uses. In: MacGregor AW and Bhatty RS (eds.) Barley Chemistry and Technology, pp. 437-474. St. Paul, MN: American Association of Cereal Chemists.
- Slavin J, Jacobs D, and Marquart L (2000) Grain processing and nutrition. Critical Reviews in Food Science and Nutrition 40: 309-326.
- Vasanthan T and Bhatty RS (1995) Starch purification after pin-milling and air classification or waxy, normal, and high-amylose barleys. Cereal Chemistry 72: 379-384.
- Wang L, Xue Q, Newman RK, and Newman CW (1993) Enrichment of tocopherols, tocotrienols, and oil in barley fractions by milling and pearling. Cereal Chemistry 70: 499-501.

#### **Relevant Websites**

http://www.ilswebdesigns.com. http://www.idahograin.org. http://www.albertabarley.com. http://www.ndbarley.net.

# Malting

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#### Introduction

Malting is the term used for the preparation of a brewing raw material, employing a controlled germination of grain in moist air. Barley is the preferred grain for malting; however, other grains such as wheat, rye, sorghum, triticale, or oats may be malted and subsequently used in brewing, distilling, or food production. Barley is the most common cereal used for the production of malt for brewing since it has a high starch to protein ratio and adhering husk that

contribute to the economic yield and ease of processing in brewing and produces the characteristic flavors associated with malt for this purpose.

Malting aims to convert or modify the physical structure of the barley grain and allow synthesis or activation of a series of enzymes such that the final product, malt, is more readily used in the next stages of brewing, distilling, or food manufacture.

During the malting process, hydrolytic enzyme production and/or activation is maximized leading to cell-wall degradation and protein solubilization with minimal starch breakdown. In order for this to occur, malting aims to both accelerate germination and retard embryo growth, essentially conflicting activities. Any shoot or root growth produced during the malting process is physically removed from the final product prior to storage and delivery and therefore minimizing embryo growth reduces losses incurred in the process.

The final product of malting, malt, physically resembles the original barley grain but is friable when crushed, revealing the complex biochemical changes that have occurred during the malting process.

# **Barley Grain Structure**

For the purpose of understanding the malting process, the barley grain can be divided into several major components: the embryo, the endosperm, the scutellum, the aleurone, and the husk (Figure 1).

The embryo is the living or metabolically active part of the grain. The husk covers the whole grain structure and consists of two parts — the lemma on the dorsal side of the grain (which is normally extended into the awn) and the palea on the ventral side. In most malting barley varieties, the husk is fused to the

testa at maturity. The husk acts to protect the grain from infection during development and as such is relatively water resistant. In intact grain where the husk has not been damaged during cropping or harvesting, water enters the grain and the embryo through the micropyle. It is the embryo that contains the shoot and root primordia, which will grow during germination.

The endosperm is the initial source of stored nutrition to fuel seedling growth prior to photosynthetic nutrient production. The majority of the endosperm tissue is not metabolically active and consists of large cells without nuclei packed with starch granules embedded in a matrix of storage proteins (Figure 2).

The scutellum is a shield-like structure that divides the embryo from the endosperm. It plays a role in both the degradation of the starchy endosperm and the nutrition of the embryo. The aleurone in barley consists of three layers of cells, which surround the endosperm. These cells are responsible for the production or activation of the key hydrolytic enzymes that will be produced during the malting process.

# **Evaluation of Barley for Malting**

Barley for malting must be of high viability, low dormancy, and uniform germination to produce a homogeneous malt product. Both two- and sixrow barleys can be used for malt production and their unique quality characteristics make them suitable for a range of malt products. Barley is generally processed into malt in batches of single pure varieties. This ensures uniformity in processing.

Structural grain characteristics such as grain size and shape, endosperm density, cell-wall thickness, and husk content all may affect malt homogeneity.

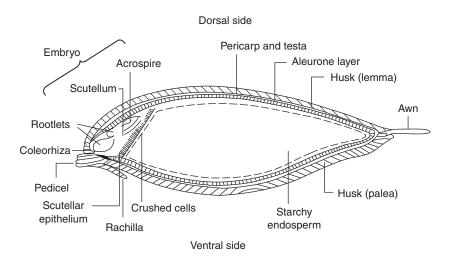


Figure 1 Major components of a barley grain.

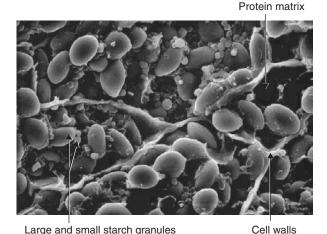


Figure 2 Endosperm tissue.

Malting barley should be of low protein and high starch content to maximize the potential for extract yield in the brewery. Extract is a measure of the amount of sugars extracted from the grain during the brewing process. The amount of sugars extracted is directly related to the level of alcohol produced and thus the value of the product in brewing and distilling. Protein levels must not be too low as proteins serve four basic functions in brewing. They are the origin of enzymes that catalyze the complex biochemical reactions involved in turning barley into malt and malt into wort. They are required for yeast nutrition; they contribute to foam and are involved in the flavor development that malt contributes to beer and whiskey.

Barley received for malting is cleaned by passing over a series of shaking screens with differing slot sizes to remove foreign material such as stones, nonbarley grains, and weed seeds. Broken grains, chaff or straw, and dust are also removed in this way and with aspiration. The cleaned sample is assessed for grain size, total nitrogen, and moisture to determine its suitability for producing quality malt. Critical tests for malting barley, however, are those that determine the viability, dormancy, germinative energy, and water sensitivity of the grain. These tests are carried out on every load of barley prior to processing.

#### **The Malting Process**

The malting process can be divided into three key stages: steeping, germination, and kilning. In the first stage, steeping, the acquiescent grain imbibes water and hydrates the embryo and endosperm. In the second phase, germination, enzymes are synthesized, activated, and mobilized, and the embryo begins to develop. In the final stage, kilning, grain growth is halted using a heat treatment (kilning), which dries the grain to constant and low moisture for storage.

#### **Steeping**

Harvested barley grain is generally stored at a moisture level between 10% and 15%. Storage of grain at this moisture level avoids mold growth and germination loss. Barley is generally processed into malt in batches, which may vary in size from 2 to 400T. The grain is cleaned and graded to remove foreign seeds, stones, straw, small and broken kernels, and dust prior to entering the steep vessels.

During steeping, barley is immersed in water to raise the moisture level to  $\sim$ 42-47%, which will allow the grain to germinate. The process also washes the grain removing any remaining loose debris and dust.

The embryo is the major living part of the grain and imbibes water rapidly through the micropyle while the endosperm (nonliving tissue) is hydrated by osmosis. Steeping aims to achieve a rapid and even uptake of water and oxygen in order to stimulate the embryo to respire and initiate hormone activity. The respiration of the embryo produces heat and carbon dioxide. Once the embryo is hydrated and metabolized, it is important to achieve a full and even distribution of water throughout the endosperm to produce a consistent quality in the malt.

Steeping involves a series of 2-4 immersions in temperature-controlled water, each followed by an air-rest period where the water in the steep is drained from the grain. The immersions allow the embryo to absorb water and the air-rest periods allow the water to be distributed into the nonrespiring endosperm. Steeping regimes are determined by plant design, barley characteristics, and condition and target malt specifications.

The steep vessels can take a number of forms (Figure 3a), but are generally either cylindroconical or flat-bottomed tanks. Design of steep vessels should ensure that hydrostatic pressure is not deleterious to grain health and this generally limits batch sizes to less than 100T in cylindroconical steeps.

In all cases the vessels should be well aerated with good water, temperature control between 12°C and 25°C, and efficient carbon dioxide removal. Aeration should be vigorous allowing a good rolling movement of the grain mass to optimize oxygenation and temperature control and removal of carbon dioxide (Figure 3b). During steeping, the embryo produces enzymes, which break down embryonic starch, initiating shoot and root growth. Hydration of the





Figure 3 (a) Cylindroconical steep vessels. (b) Close view of a steeping vessel.

scutellum and aleurone initiates hormone (gibberellic acid) production, the catalyst for hydrolytic enzyme production. In some cases in commercial production (when permitted by customer specifications) exogenous gibberellic acid (GA3) can be added to mimic or increase the effects of the naturally produced hormone. Exogenous GA is usually added either during transfer of the grain from steeping to germination or early in germination when the grain bed is turned.

At the end of the steeping, the grain should be evenly and fully hydrated with a high (%) chit count. Hydration is commonly determined by measuring the moisture content of the grain; however, this can be misleading as it does not necessarily give a measure of the level or evenness of hydration of the endosperm.

The "chit" counts measure the consistency of a sample of steeped grain for a visible protrusion (the chit) of the developing rootlet/cotyledon from the base of the grain (Figure 4). A high value

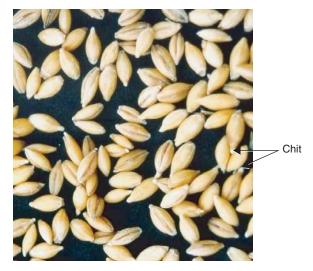


Figure 4 The "chit" counts used to measure the consistency of a sample of steeped grain for a visible protrusion (the chit) of the developing rootlet/cotyledon from the base of the grain.



Figure 5 Steeped grain spread on the floor for germination.

indicates that the grain batch is initiating germination consistently.

#### Germination

Steeped barley grain is transferred to germination vessels as either slurry or by dry transfer after draining.

The germination process aims to encourage enzyme synthesis and release, cell-wall breakdown in the endosperm, and the solubilization of stored nitrogen (proteolysis).

In the past, germination was carried out by spreading the steeped grain on the floor (Figure 5). Some manual floor maltings still exist today and may be particularly suited to producing specialized malts such as those used for the production of single malt whiskeys. In floor maltings, the bed of grain was turned over manually to allow fresh air to circulate and prevent overheating from grain respiration. Nowadays germination is carried out using a pneumatic system in vessels of various shapes and sizes (generally from 40 to 400T) such as drums (Figure 6) or more typically rectangular Saladin boxes (Figure 7), or round germination vessels. In the pneumatic system, humidified temperature-controlled air is circulated through the grain bed (generally 1 m deep). The grain rests on perforated plates (Figure 8) and is turned automatically by rakes, which travel slowly through the grain bed (Figure 9). Temperature, airflow, and small applications of water are used to maintain moisture, temperature, and oxygen levels in the respiring grain mass as it germinates and grows. Turning the bed of grain keeps the grain mass loose and allows adequate airflow and water distribution.

During the germination stage, grain moisture increases to ~45% and roots and shoots begin to grow. Germination is typically allowed to proceed for 3-5 days during which time enzymes are produced from the scutellum and aleurone, and regulated in response to stimulation from hormones such as gibberellic acid (GA), indoleacetic acid (IAA), and abscisic acid (ABA). Enzymes produced or activated during this stage include  $\alpha$ -amylase,  $\beta$ -amylase, limit dextrinase,  $\alpha$ -glucosidase,  $\beta$ -glucanase, xylanase, and

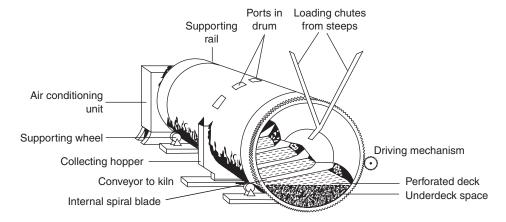


Figure 6 Cross-section of a drum used for germination using the pneumatic system.



Figure 7 View of a Saladin box typically used for germination using the pneumatic system.



**Figure 8** Grains resting on perforated plates used in the pneumatic system of germination.



**Figure 9** Grains resting on perforated plates automatically turned by rakes.

endo- and exoproteinases. Activated enzymes are produced in sequence and move through the hydrated endosperm degrading both cell walls and protein matrix in which starch granules are embedded. This process is often referred to as grain modification.

At the end of the germination process, the cells walls of the starchy endosperm should be evenly and fully degraded with little or no starch granule degradation (Figure 10). Approximately 40-50% of the proteins should have been solubilized and high levels of starch degrading enzymes, in particular  $\alpha$ - and  $\beta$ -amylase, should have been released. The grain moisture should have peaked at  $\sim$ 45% on day 2 and reduced to 38–40% at day 4. Once the grain has germinated it is referred to as "green malt."

#### **Kilning**

Transferring the green malt to the kiln halts the process of germination. Green malt is loaded to the kiln at  $\sim$ 40% moisture and is dried to 4–5% usually over a 24 h period. Kilning aims to prevent further root and shoot growth, to limit starch modification, to achieve a stable product for storage and transport, to preserve enzymes, and develop and stabilize color, aroma, and flavor. The process also removes undesirable flavors and chemical compounds. Once kilned, the malt is friable and can be milled for brewing or stored whole in cool dry conditions for up to 12 months without significant loss of quality.

The kilning process is the regulated removal of water from germinated grain. Moisture is removed from the grain by passing heated air through the grain bed. The air flowing to the kiln is gradually increased in temperature such that the grain

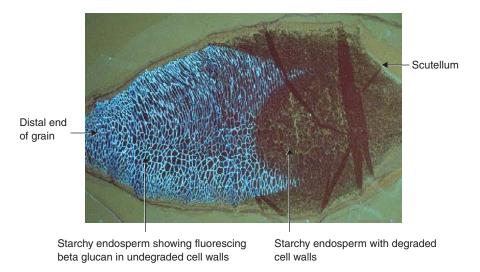


Figure 10 The cells walls of the starchy endosperm are degraded during germination.

temperature increases slowly and moisture within the grain diffuses to the surface of the kernel where it is absorbed and removed by the hot air stream. Since the grains form part of a grain, bed heating must be carefully controlled to allow moisture to be removed from the center of the grains in each of the layers in the grain bed without overheating the grains closest to the heat source.

There are typically three phases of moisture removal during kilning. In the "free drying" phase, moisture moves out of the grain relatively quickly, grain moisture drops rapidly and evenly to  $\sim 25\%$ , and kiln temperatures are relatively low (50-60°C). This stage is followed by the "intermediate" phase where removal of moisture from the grain without overheating is more difficult. In this phase, temperature is increased slowly in a stepwise fashion over several hours and moisture drops to 12%. In the third and final "bound water" or "curing phase," moisture falls to ~4% as air temperatures are increased to 80+°C. Careful regulation of temperature, moisture removal, and time is essential to ensure that as the grain is dried, the hydrolytic enzymes that will be required in the brewing or distilling processes are not denatured, color is controlled, and the formation of unacceptable chemical compounds such as

Table 1 Types of specialty malts

Green malt kiln dried	Vienna Munich Ale Pilsner
Green malt roasted	Caramalt
	Crystal
Dried malt roasted	Amber
	Brown
	Chocolate
	Black
Raw barley roasted	Roasted barley

nitrosodimethylamine (NDMA) and dimethyl sulfide (DMS) are inhibited or minimized.

Kilning conditions are one of the largest contributors to the final malt character and it is careful control of this stage that allows a range of speciality malt types to be produced from the malting process (Table 1). Malts with colors from 2 to 30°EBC can be produced in a normal malt kiln without the need for roasting. Roasted products are higher in color at 35–1500°EBC and may be produced from barley, malt, or green malt (Table 1). These products are kilned in a specialized drum roasting plant at very high temperatures, which destroy all enzymic activity, but produce very characteristic and strong flavors and colors (Table 2).

Conventional kilns can be constructed in various shapes and sizes and may be single or double decked. In some situations, both germination and kilning can be carried out in the same vessel usually referred to as a GK vessel. Historically, kilns were constructed in brick, however, today kilns are typically constructed from metal or concrete with a bed depth up to 1 m (Figure 11).

In order to minimize NDMA formation, which is undesirable due to its potential carcinogenic properties, kiln gases are low in nitrous oxide and/or heat is applied indirectly using heat exchangers to avoid direct contact of the kiln gases with the malt.

The hot air is forced through the grain bed, which rests on a perforated floor similar to that found in the germination beds.

Hot air exiting the grain bed can be recirculated and this process is used to control the drying process and conserve energy consumption. Regulation of the relative humidity of the air removed from the grain can be used to protect malt quality. During kilning, embryo viability is destroyed, sucrose levels increase, and sugar is caramelized. Melanoidins increase via complex nonenzymic reactions and low-molecular-weight flavor substances such as acids, alcohols, aldehydes,

Table 2 Specialty kilned malts

Product	Color EBC	Beer style	Description	Malting	Curing (°C)
Ale	4–8	Pale Ales Bitters	Malting	Well modified	90-95
Vienna	7-10	Vienna Marzen	Rich malting	Normal modification	90-100
Munich	15-25	Dark lager Bock	Rich malting full bodied	Over modified	100-110
		Roasted malts		Roasting	1
Brown	20-120	Porter I.P.A	Dry smokey	Roasted low color malts	s
Amber Chocolate	40-60 500-700	I.P.A Porter	Dry biscuity red hue Nutty roasted	Roasted ale mait  Roasted lager malt	
Black	800-1600	Stout	Roasted astringent	Roasted lager malt	
Caramalt Crystal	30-60 60-350	Lagers, ales Ales, dark lagers	Body, flavour sweet biscuity Sweet caramel roasted toffee	Stewed green malt Stewed green malt	

ketones, and esters are formed leading to the development of characteristic malt flavors and aromas.

# **Product Storage and Evaluation of Malt Quality**

Following kilning, malt is cleaned to remove rootlets and small and ungerminated grain. Cleaning and rootlet removal is generally carried out prior to storage as rootlets can add a bitter taste to malt and add considerable bulk to the material to be stored. Malt offal is a useful stock feed.

The malted barley is now friable and easily broken and damaged. Damaged malt generates dust, reduces the husk component required for lautering in brewing, and increases production losses. Malt movement should be minimized and conveying speeds optimized to reduce such damage.

Individual batches of malt are assessed during processing and on completion of kilning for key quality characteristics using standard methods of analysis (IGB, ASBC, or EBC) and according to the malt specifications negotiated with the customer.

The use of rapid in-process tests for green malt moisture, color,  $\beta$ -glucan, and soluble nitrogen allow process to be optimized to achieve the target malt specification.

# **Malt House Design**

Malt house design is varied and dependent on the site characteristics and production requirements. The decision to construct separate steeping, germination, and kilning vessels through various combinations to a full tower mating where the vessels are stacked on top of each other (Figure 12) is determined to a large extent by the range of products and customers to be serviced and the costs of construction in different locations.

In all cases modern plant design utilizes materials and layout that will take account of hygiene and energy efficiency.

# Hygiene

Malt is an ingredient in the food chain. It is important that the barley selected for the process is free from chemical residues and foreign material and that the malt processing vessels and environment are kept clean. The use of stainless steel vessels and streamlined plant design minimizes cleaning requirements and ensures a safe food quality production environment.

# **Energy Efficiency**

Malting is an energy intensive operation. Key utilities, gas, electricity, and water account for  $\sim 10\%$  of direct input costs. The major gas use is in kilning while electricity is used in driving fans, cooling, heating, conveying, and pumping. The knowledge and ability to monitor and continuously control temperatures and speeds in various areas of the malt house allows energy use to be optimized. Plant design can assist in minimizing the conveying, heating, and cooling requirements and therefore reduce utility usage and



Figure 11 Circular kiln.

Figure 12 Tower maltings.

waste generation. In the future, the use of alternative energy sources: recycling and cogeneration plants will drive further energy savings and environmental sustainability in malting plants.

See also: Barley: Genetics and Breeding; Agronomy; Harvesting, Storage, and Transport; Grading and Marketing. Beverages: Asian Alcoholic Beverages; Distilled. Cereals: Chemistry of Nonstarch Polysaccharides; Grain Defects; Grain-Quality Attributes; Protein Chemistry.

Enzyme Activities. Fermentation: Origins and Applications. Genetically Modified Grains and the Consumer. Grain, Morphology of Internal Structure. Grain and Plants, Morphology. Grains Other than Cereals, Nonstarch Polysaccharides. Starch: Chemistry.

#### **Further Reading**

Briggs DE (1978) *Barley*. London: Chapman and Hall. Briggs DE (1998) *Malts and Malting*. London: Blackie Academic and Professional.

Briggs DE, Hough JS, Stevens R, and Young TW (1981) Malting and Brewing Science, 2nd edn., vol. 1, Malt and Sweet Wort. New York: Chapman and Hall.

EBC Analytica (1998) European Brewing Convention Analytica. Zurich: Brauerei und Gertranke Rundschau. Institute of Brewing (1991) Recommended Methods of Analysis. London: Institute of Brewing.

Kunze W (1996) Technology – Brewing and Malting. Berlin: VLB.

MacGregor AW and Bhatty RS (1993) *Barley: Chemistry and Technology*. St. Paul, MN: American Association of Cereal Chemists.

Shewry PR (1992) Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. Oxon: CAB International.

#### **Relevant Websites**

http://brewery.org — Malting process in a nutshell. http://www.beer-brewing.com — The Brewers Handbook.

# **BEANS**

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#### Introduction

Throughout human history, more than 3000 species of plants have been used as foods. On a global basis plants provide 65% of food protein, over 80% of food energy, and account for 85% of gross tonnage. Excluding the large number of fruit and vegetable

species, only  $\sim 50$  crop species make a significant contribution to human diet. Of these, cereals are the largest group followed by legumes in terms of global production. However, because legumes contain almost 2-3 times more protein than cereals, their dietary importance as protein source is well appreciated. Of more than 1300 species of legumes, only  $\sim 20$  are most commonly consumed by humans. Among these, the common dry bean, *Phaseolus vulgaris*, is consumed in the largest quantity on a worldwide basis. Dry beans are low in fat (excluding oilseeds), low in sodium, and contain no cholesterol. They are a rich source of proteins, complex carbohydrates, fiber,

vitamins, and certain minerals. On a caloric basis, dry beans are more nutrient dense than cereals. Dry beans are less expensive than animal food products and when stored properly, have considerably longer shelf life than several animal, fruit, and vegetable products. Since legumes have the ability to fix atmospheric nitrogen and therefore add nitrogen to the crop-soil ecosystem, they are important in soil conservation and maintenance of soil quality.

# Global Distribution, Varieties, and **Commercial Importance**

The word "legume" is derived from the Latin word "legumen" which means seeds harvested in pods. The term "pulse" (from Latin word "puls" meaning pottage) is used for legume seeds that contain small amounts of fat, while for those containing large amounts of fat (such as soybeans and peanuts) the term "leguminous oilseed" is used. According to the Food and Agriculture Organization (FAO), the word "legume" is used for all leguminous plants. The most commonly used legumes as human food are listed in Table 1.

Although legumes have been cultivated for several thousand years, the chronology and origins of domestication of food legumes is almost impossible to reconstruct. Some legumes (lentils for example) have been dated back to 7000-6000 BC. Leguminosae (or Fabaceae) is the third largest family of flowering plants (after Compositae and Orchidaceae) in size and economic importance and is second only to the grasses (Gramineae). Current estimates indicate that Leguminosae has  $\sim$ 16 000–19 000 species in  $\sim$ 750 genera. The subclassification is somewhat controversial. Almost all of the domesticated legumes used as food are members of *Papilionoideae*. All of the common beans belong to the tribe Phaseolae.

In terms of global production, legumes (including oilseeds) rank fifth in annual world grain production. Dry beans account for  $\sim 23.39\%$  of the total world legume (pulses) production (Table 2). On a worldwide basis, the common beans (*Phaseolus* spp.) are the number one crop among dry beans (excluding oilseeds) in both production and consumption and are therefore economically an important crop. In 2001, the global dry bean supply was 2.1 kg per capita per year. Asia produces the largest quantity (44.90% of total world production) of dry beans followed by Latin America and the Caribbean (29.3%), South America (19.02%), North and Central America (17.23%), and Africa (15.26%). In 2001, Brazil (15.15%), India (13.58%), China (11.15%), Myanmar (9.06%), Mexico (6.56%), and the USA (5.49%) accounted for 61.00% of the global dry bean production. Europe, Asia, Latin America and Caribbean, and North and Central America lead world imports of dry beans in terms of dollar value (Table 2). Japan, US, UK, Italy, Mexico, India, Brazil, and France, respectively, accounted for 8.68%, 7.79%, 6.29%, 6.12%, 5.81%, 5.63%, and 5.19% of the total dollar value (49.39%) of worldwide imports (\$992 052 000) of dry beans. Asia and North and Central America lead the worldwide export of pulses and dry beans (Table 2). In 2001, the leading exporting countries of dry beans were Myanmar, China, US, Argentina, and Canada, respectively, accounting for 22.73%, 20.97%, 14.73%, 10.49%, and 9.29% of the total dollar value (78.21%) of worldwide dry bean exports.

# Morphology of the Pods/Seeds

Regardless of the fat content, most legume seeds have similar structure. Mature legume seeds have three major components: the seedcoat, the cotyledons, and the embryo axis. In most dry seeds, they account for 8-20%, 80-90%, and 1-2% of the seed weight, respectively. The majority of the nutrients are present in cotyledons. Typical seed structure and various anatomical parts of legume seeds are shown in Figure 1. The outermost layer of the seed is the seedcoat or testa. The external seed structure includes the hilum, micropyle, and raphe. The hilum is a scarlike structure (usually oval shaped) near the middle edge where the seed breaks away from the stalk. The micropyle is the small opening in the seedcoat where originally the pollen tube enters the valve. The raphe is the ridge at the side of hilum opposite to the micropyle and represents the base of the stalk that fuses with the seedcoat upon seed maturation. In most legumes, the endosperm is short lived and shrinks to a thin layer surrounding the cotyledons (or embryo). On soaking the seeds, the endosperm is easily removed along with the seedcoat. The remainder (embryo) of the seed consists of shoot (which contains two cotyledons), and a short axis above and below the cotyledons and terminates in the shoot tip. The plumule or embryonic stem is well developed in the resting seed and lies between two cotyledons.

The outermost layer of seedcoat is the cuticle, which has papillae or papillae-like growth in some legumes (e.g., green gram), but in most legumes it is a smooth structure. The thickness of the seedcoat is quite variable depending upon the type of bean. Generally, seeds containing thick seedcoats tend to have high fat content. Both hilum and micropyle are important in water imbibition by testa. Palisade cells derived from the outer epidermis of the outer integument, which are next to the cuticle, are either

Botanical name	Common name
Arachis hypogaea	Groundnut, peanut, monkey nut, goober pea, nguba
Cajunus cajan	Pigeon pea, arhar, red gram, tur, toovar Angola pea, gandal, ambre vade, alverja
Ca. indicus	Pigeon pea, Congo pea, yellow dhal
Canavalia ensiformis	Jack bean, horsebean, gotani bean, haba de burro, chickasaw, lima
C. gladiata	Sword bean, maxima
Cicer arietinum	Chick-pea, bengal gram, chana, deshi chana, kabuli, chiche
Ci. minotinum	Chana, garbanzo
Cyamopsis tetragonoloba	Cluster bean, guar, aconite, cyamopse
Dolichos biflorus	Horse gram
D. lablab	Hyacinth bean, bonavist, field bean, caballeros, Indian butter bean, val bean, Egyptian kidney bean
Ervum vulgaris	Lentils, masur dhal
Faba vulgaris	Windsor bean
Glycine max	Soybean, soja
G. hispida	,,
G. soja	
Lablab niger	Lablab bean
La. purpureus	Kidney bean, hyacinth bean, Indian bean, lubia bean
Lathyrus sativus	Grasspea, kesari dhal, vetch, chickling vetch, chicaro
Lens esculenta	Lentils, masur dhal, red dhal, lentille, split pea, lentija
Le. culinaris	Lerting, masur unai, reu unai, iertine, spin pea, iertija
Lupinus spp.	Lunine tanvi tarin nearl lunin wolf hean tromoco
Macrotyloma uniflorum	Lupins, tarwi, tarin, pearl lupin, wolf bean, tremoco Horse gram, Madras gram, Kallu, Kulthi bean
•	
Mucuna pruriens	Velvet bean, cowage, Mauritius bean, stizolobia
Phaseolus aconitifolius	Moth bean
Ph. acutifolius	Tepary bean, pavi, Yorimuni, dinawa
Ph. angularis	Adzuki bean, feijao
Ph. aureus	Mung bean, green gram, golden gram, chiroko, chicka sano pea
Ph. calcaratus	Rice bean, frijol arroz
Ph. lunatus	Lima bean, sieva bean, Madagascar bean, sugar bean, Burmabean, towe bean, pole bean, caraota, panguita
Ph. mungo	Mung bean, mungo bean, urd dhal, black gram, urad, woolly pyrol, kambulu
Ph. radiatus Ph. vulgaris	Mung bean, golden graham, green gram Dry bean, haricot, common bean, kidney bean, navy bean, pinto or snap bean, feijac opoca, rajma, French bean, chumbinho
Pisum sativum	Dry pea, green pea, garden pea, field pea
Pi. angularis Pi. arvense	Dry pea, green pea, garden pea, neid pea
Psophocarpus tetragonolobus	Winged bean (humid tropics), goa bean, asparagus bean, Colombo, four angled bear princess bean
Sphenostylis stenocarpa	Yam bean
Stizolobium spp.	Velvet bean
Tetragonolobus purpureus	Winged bean (Europe)
Trigonella foenumgraecum	Methi, fenugreek
Tylosema esculentum	Marama bean
Vicia faba	Broad bean, horsebean, faba bean, field bean, Windsor bean
Vic. sativa	Vetch
Vigna aconitifolia	Moth bean, matki, mouth bean, mat, math
Vig. aureus	Mung bean
Vig. radiata	·
Vig. mungo	Black gram, urd, urad, kambulu, pyrol
Vig. sinensis	Dry cowpea
Vig. umbellata	Rice bean, red bean, mambi bean
Vig. unguiculata	Black-eyed cowpea, black-eye pea, cowpea, kaffir bean, Hindu pea, asparagus pea
Voandzeia subterranea	Bambara groundnut, Madagascar groundnut, earthpea, Congo goober, kaffir pea, jug
. Januardin Garantin Maria	bean, haricot pistache

<sup>&</sup>lt;sup>a</sup> Data from Deshpande SS and Srinivasan D (1990) Food legumes: chemistry and technology. *Advances in Cereal Sciences and Technology* 10: 147–241; and Doughty J and Walker A (1982) *Legumes in Human Nutrition*. Food and Nutrition Paper 20, 152pp. Rome, Italy: Food and Agriculture Organization of the United Nations.

**Table 2** Acreage, production, yield, and import/export data for pulses and dry beans<sup>a</sup>

		arvested	Yi	eld		uction			Import				Export	
	(×10	0 <sup>6</sup> ha)	$(\times 10  \text{k})$	g ha <sup>-1</sup> )	(×1	10 <sup>6</sup> t)	(×1	0 <sup>6</sup> t)	(×10	D <sup>6</sup> \$)	(×1	0 <sup>6</sup> t)	(×1	0 <sup>6</sup> \$)
	P	D	P	D	P	D	Р	D	Р	D	Р	D	Р	D
World	67.15	23.85	78.96	67.90	53.02	16.19	9.40	2.02	3244.23	992.05	9.32	3.01	2985.21	1235.64
Africa	17.32	3.61	53.04	68.45	9.19	2.47	0.94	0.15	395.97	72.09	0.14	0.06	56.39	26.26
Asia	33.80	12.64	68.73	57.54	23.23	7.27	4.46	0.69	1468.60	281.35	2.64	1.83	1065.22	616.76
Europe	3.62	0.39	218.97	136.52	7.94	0.53	2.65	0.53	806.17	316.08	1.35	0.10	319.96	75.34
Latin America and Caribbean	7.09	6.49	77.98	72.32	5.53	4.70	1.04	0.47	394.50	217.52	0.60	0.37	333.21	196.13
North and Central America	6.03	3.08	111.77	90.46	6.73	2.79	0.74	0.39	350.41	207.89	3.70	0.62	1069.32	322.98
Oceania	2.03	0.05	125.78	105.93	2.55	0.05	0.03	0.01	18.54	10.17	1.15	0.05	299.08	24.42
South America	4.35	4.09	77.63	75.49	3.38	3.08	0.58	0.24	204.53	104.47	0.35	0.33	175.25	169.88

<sup>&</sup>lt;sup>a</sup> Data from FAO (2001) Statistical Databases. Rome, Italy: Food and Agriculture Organization of the United Nations (http://appsl.fao.org/serlet). P = Pulses and D = Dry beans.

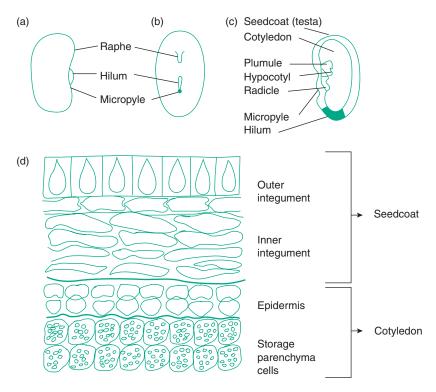


Figure 1 Dry bean (Phaseolus vulgaris) seed: (a) external side view, (b) external face or edge view (viewing at hilum side), (c) crosssection (one cotyledon removed), and (d) detailed cross-section across seedcoat and cotyledon. (Reproduced from Encylopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 407, Elsevier Ltd.)

loosely packed or densely packed, depending upon the seed maturity and may affect seed hydration. Next to palisade cells are the hourglass cells. The shape of hourglass cells can vary from bottle shape (guar) to dumbbell shape (broad beans) to hourglass shaped (soybeans). Only a few legume species (such as Dolichos, Cajanus, and Vigna) have more than one layer of hourglass cells. The remainder of the testa contains primarily mesophyll cells.

Legume cotyledons are primarily composed of parenchyma cells, which have variable size (70–100 μM) and act as storage sites for most nutrients. Each cell of the cotyledon is bound by the cell wall and the middle lamella. Vascular bundles in cotyledons generally are devoid of any filling material. Vascular bundles are often used as key structures in the identification of different plant types.

# **Chemical and Nutritional Composition**

The majority of nutrients in dry beans are primarily located in the cotyledons and account for up to 90% of the total nutritive value. Typically, dry beans provide 1255–1464 kilojoules (kJ) per 100 g of dry seeds. The majority of constituents of cotyledons are proteins and carbohydrates and respectively account for 15-25% and 50-75% of the total seed weight. The remainder consists of fat, minerals, fiber, and vitamins. With the exception of oilseeds, dry beans generally contain low amounts (1-3% of seed weight) of fat. Although most minerals are present in cotyledons, some (such as calcium and iron) may be present in seedcoat in significant proportion. Typical nutrient composition of several *Phaseolus* beans is shown in Table 3. Dry beans not only contain significant amounts of nutrients, but also several undesirable components and attributes: inhibitors of enzymes such as trypsin, chymotrypsin, subtilisin, amylases, and elastase; lectins; phenolic compounds including tannins; phytates; toxic amino acids mimosine and djenkolic acid; cyanogenic glycosides which produce HCN; flatulence causing oligosaccharides raffinose, stachyose, and verbascose; lipoxygenases, which catalyze the development of rancidity; and off odors often described as beany, grassy, painty, cardboardlike, and chalky.

#### **Proteins**

Phaseolus beans are not only important for their caloric contribution to human diet but are especially valued for their protein content since they are a major protein contributor to the human diet on a global basis. In certain parts of the world they are the sole source of dietary protein. Dry bean proteins can be classified as storage and metabolic proteins. The original protein classification proposed by Osborne was based on solubility of proteins in a series of solvents. In this scheme the water and dilute salt soluble proteins were termed as albumins and globulins, respectively. Dry beans contain 40-60% globulins and 20-40% albumins based on Osborne's protein classification. Globulins are exclusively storage proteins while albumin fraction contains both storage and metabolic proteins. The protein content of dry beans is usually calculated by multiplying Kieldahl nitrogen content by a factor of 6.25. Because dry beans contain 10–15% of total nitrogen as nonprotein nitrogen, most dry bean protein values are typically overestimated by 1-2%.

**Table 3** Proximate composition of Phaseolus beans<sup>a</sup>

Bean	Moisture (%)	Protein (%)	Carbohydrates (%)	Fat (%)	Ash (%)	Crude fiber (%)
Adzuki	11.00	20.20	49.80	1.90	4.39	4.90
Black beauty	10.41	22.87	70.79	4.48	1.86	
Black gram	10.2-10.9	19.7-24.0	56.6-63.4	1.3-1.6	3.2 - 3.4	4.4 - 6.4
California small white	9.65	25.90	58.00	0.25		
Cranberry	12.71	23.43	71.26	1.09	4.22	
Great Northern Lima	8.5-13.3	21.0-24.37	61.2-71.07	1.0-3.48	3.5 - 4.86	6.70
Baby	13.30-20.40	20.40	62.10	0.80	3.40	6.00
Large	8.90	22.30	63.80	0.80	4.20	7.40
Mung						
Green	17.92	27.12	62.85	1.53	4.01	4.50
Black	13.64	25.68	64.20	0.45	4.32	5.30
Navy	9.4-18.2	23.13-24.65	61.2-66.19	1.5-4.3	2.90 - 4.27	3.4-6.6
Pinto	9.05-14.70	18.8-24.97	61.8-69.47	1.2-3.6	3.07-4.10	3.9-6.3
Red kidney						
Light	10.52	20.89	73.20	1.52	4.39	
Dark	13.22	20.32	73.68	1.58	4.42	
Rice bean		18.0-25.0	60.0-77.0	1.0-1.6	3.8 - 4.3	3.3-4.8
Roshina G2	9.89-11.11	25.77-26.30	63.33-64.02	1.85 - 2.00	3.19 - 3.79	4.6-5.1
Roshina pink	4.90	19.40	68.80	3.50	3.40	4.60
Sanilac	11.61	18.98	75.09	1.65	4.28	
Tepary		21.0-25.0	70.0-73.0	0.8 - 0.9	4.1 - 4.8	
Small red	13.12	22.45	71.97	1.43	4.15	
Small white	13.03	19.73	74.34	1.99	3.94	
Viva pink	12.69	21.30	73.23	1.06	4.41	

<sup>&</sup>lt;sup>a</sup> Data compiled from Sathe SK, Deshpande SS, and Salunkhe DK (1984) Dry beans of Phaseolus: a review, Part 1, Chemical composition: proteins, CRC Crit. Rev. Sci. Nutri. 20: 1-46 and Salunkhe DK and Kadam SS (1989) Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization, vols. I (310pp.), II (294pp.), and III (323pp.). Boca Raton, FL: CRC Press. Data expressed on dry weight basis.

The major storage proteins in dry beans have been identified by several names and therefore their nomenclature is somewhat confusing. Based on the nomenclature using ultracentrifugation sedimentation coefficient (S), dry beans contain both 7S (vicilinlike) and 11S (legumin-like) storage proteins. Depending on the bean variety, the relative proportion of these two types of proteins varies considerably. The 11S-type proteins typically are nonglycosylated proteins with estimated molecular weight (MW) in the range 300 000-400 000. They are usually composed of six subunits (MW 60000), each consisting one acidic (MW 40 000) and one basic (MW 20 000) polypeptide linked by disulfide bond(s). Usually, 11S proteins are present in minor amounts in Phaseolus beans. The 7S globulin in *Phaseolus* beans has also been referred to as glycoprotein II, globulin 1, euphaseolin, globulin, and phaseolin. Depending on the type of bean, the 7S globulin type and quantity varies considerably. In *Phaseolus* beans the 7S globulins are, however, the major storage proteins and account for 40-60% of the total proteins. The three major types of 7S proteins that have been identified, biochemically purified, and characterized are: (1) Phaseolin, (2) Lectin (also called glycoprotein II, phytoagglutinins or phytohemagglutinins, and protein II), and (3) Arcelin in wild bean accessions from Mexico (named after town Arcelia in Mexico, where some of the accessions were collected). All the 7S globulins are glycosylated and contain D-mannose D-glucosamine as the major sugar constituents.

**Phaseolin** Phaseolin is the major globulin in domesticated *Phaseolus* beans. It is a trimeric, vicilin-like 7S globulin known to exhibit polymorphism. The polypeptide polymorphism is believed to be due to not only the differential glycosylation but also because phaseolin polypeptides are encoded by a small multigene family. Phaseolin is soluble in 0.5 M NaCl at all pH values. It undergoes reversible pH-dependent dissociation-association with sedimentation coefficients of 3.0S (pH 12.0), 7.1S (pH 7.0), and 18.2S (pH 3.6) known as peptides (MW 44 000), protomers (MW 163 000), and tetramers of protomers (MW 653 000). Phaseolin consists of a group of subunit polypeptides with MWs 43 000–54 000 and isoelectric points from pH 5.6 to 5.8, depending on phaseolin type. Among *Phaseolus* beans, three distinct types of phaseolins - named after cultivars Tendergreen (T), Sanilac (S), and Contender (C) – have been identified. Screening of 107 cultivars has revealed that S-, T-, and C-type phaseolins accounted for 69%, 25%, and 6%, respectively, of the total cultivars. These types can be easily distinguished by one-dimensional or two-dimensional gel electrophoresis using sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) for one-dimensional or isoelectric focusing in first dimension followed by SDS-PAGE in the second dimension for two-dimensional gel electrophoresis. The C-type phaseolin is believed to have originated from T and S types. Regardless of the type of phaseolin, phaseolin contains 3-5% carbohydrates and its amino acid composition is dominated by acidic amino acids (30–40% of total). Typically, the N-glycosylation sites in phaseolin polypeptides occur at amino acid residue numbers 252 and 341. Sulfur-containing amino acids (notably methionine) are the limiting amino acids in phaseolin as well as other major storage proteins in *Phaseolus* beans. The secondary structure of phaseolin in 0.5 M NaCl typically has a low  $\alpha$ -helix content (10%) and  $\beta$ -turns (9.0%) and large amount of  $\beta$ -sheet (48.0%) and random coils (33.0%). Native phaseolin is quite resistant to digestive proteases such as pepsin, trypsin, and chymotrypsin and is degraded to polypeptides with MWs 24 000–28 000. However, heat denatured phaseolin is easily digested by these proteases.

Lectin Bean lectins agglutinate erythrocytes due their ability to bind with cell surface glycoproteins and glycolipids. Although the precise function of lectins in beans is not known, they are thought to offer protection to the plant. Since lectins are toxic, they are of nutritional concern. Lectins occur in both albumin and globulin fractions. Certain bean cultivars lack lectins. When present, lectins represent 6–12% of total protein. In addition to agglutinating activity, many bean lectins have mitogenic activity. Most lectins of *Phaseolus* beans have subunits of MWs 29000-36500 with isoelectric pH in the range 4.9-7.9 (most are in the pH range 5-6). The majority of the native lectins have tetrameric nature (MWs 100 000–150 000) although some (lima beans for example) have dimeric nature. The majority of Phaseolus lectins have 4-6% carbohydrate, low sulfur-containing amino acids, and sugar specificity towards D-acetylgalactosamine. Lectins are very resistant to common digestive proteases and are slowly hydrolyzed in vitro even after extensive heat denaturation. Proper moist heat denaturation can completely inactivate the biological activity of lectins and therefore can render them nontoxic.

Arcelin Arcelin was first discovered in wild accessions of Mexican *Phaseolus* beans. It also occurs in lines that contain phaseolin as well as lectin. Because it is present in equal or greater levels than phaseolin in certain lines, it is one of the major storage proteins in *Phaseolus* beans. The MWs of arcelin subunit polypeptides range from 35 000 to 42 000 depending on

the variant, and are more basic than both lectin and phaseolin. The native arcelin has an MW of 89 000 and is therefore a dimeric protein. Arcelin has many similarities with lectin (including agglutinating activity) with respect to chemical composition.

Other proteins *Phaseolus* beans contain trypsin inhibitors (many of them also inhibit chymotrypsin), amylase inhibitors, lipoxygenases, and several other minor protein components. Most of these proteins are a part of albumins. Trypsin and chymotrypsin inhibitors in *Phaseolus* beans typically account for up to 10% of the total proteins and are generally rich in sulfur amino acids. The MWs of these inhibitors range from 2000 to 23 000. Most Phaseolus beans lack Kunitz-type (inhibitors with 170–200 amino acids with MW of  $\sim 20\,000$ ) trypsin inhibitors. Amylase inhibitors in dry beans have been characterized from only a few cultivars and therefore not yet extensively studied. The MW of kidney bean amylase inhibitor (a glycoprotein) has been shown to be 50 000. Appropriate moist heat treatment (such as cooking or autoclaving) can inactivate both the protease and amylase inhibitors.

#### **Allergenic Proteins**

With heightened concerns about food allergies and the increased frequency of food recalls due to actual or suspected presence of allergen in food, food-induced allergens are of increased interest to consumers and regulatory agencies alike. Since any food protein could be a potential allergen, it is important to recognize that dry bean proteins may be allergenic. Compared to aeroallergens, such as pollen, perhaps with the exception of peanut proteins, very little is known about seed protein allergens in general and dry bean proteins in particular. A few dry bean proteins have been identified to be allergens (Table 4) and even fewer have been characterized at molecular level. Phaseolin, the major storage globulin in dry beans, has not yet been reported to be an allergen. Among the legume proteins identified to be allergens, majority appear to be storage proteins such as 2S albumins (e.g., Ric c 1, Ric c 3, soybean MRP), 7S or vicilinlike proteins (e.g., Ara h 1,  $\beta$ -conglycinin, Len c 1), and 11S or legumin-like proteins (e.g., soybean glycinin, Ara h 3, Ara h 4), some of which are glycosylated (Ara h 1, Gly m Bd 28 k) while others are not (soybean glycinin). Since typical thermal food processing does not significantly hydrolyze food proteins, linear stretches of amino acids responsible for IgE binding, the linear epitopes, are expected to survive normal food processing operations. For these reasons, food processing is often unable to completely inactivate food allergens. It is therefore also not surprising that some investigators find many legume allergens to be acid and heat resistant.

#### **Carbohydrates**

Total carbohydrates in Phaseolus beans contribute 50-70% of the seed weight and include mono-, di-, and oligosaccharides; starch; and other polysaccharides. Starch is the most abundant nutrient in Phaseolus beans accounting for up to 70-80% of total carbohydrates. Among the simple sugars, oligosaccharides (raffinose, stachyose, verbascose, and ajugose) are the major constituents (up to 10% of seed weight) and are at least partially responsible for flatulence production. Crude fiber is primarily composed of cellulose, hemicellulose, lignins (not a carbohydrate), and other nonstarchy polysaccharides such as arabinogalactans, arabinoxylans, glucomannans, galactomannans, and pectins. The hypocholestermic effect of dry beans is partially attributed to the presence of nonstarchy polysaccharides. Phaseolus bean starch granules are quite variable in shape (round, oval, oblong, elliptical, spherical, kidney shape, and irregular) and size  $(5-60 \,\mu\text{m})$  and typically contain 10-45% (of total starch) amylose. The average degree of polymerization for amyloses and chain lengths for amylopectins of *Phaseolus* bean starches range from 1600 to 1900 and 22 to 26, respectively. Based on X-ray diffraction spectra, Phaseolus dry bean starches are mostly C type (mixture of A (typical of cereal starches) and B (typical of root and tuber and high-amylose cereal starches) types). These starches have restricted swelling, gelatinization temperature range of 60-89°C, high solution viscosities, and good thermal stabilities. Upon gelatinization they produce opaque gels. Dry bean starches (especially if cooked) are well digested (digestibility is comparable to those of many cereal and tuber starches) by humans. Because dry bean starches are digested slowly, however, they are hypoglycemic and therefore useful in the diets of diabetics.

#### **Vitamins and Minerals**

*Phaseolus* beans are a good source of B-vitamins, especially thiamin, riboflavin, niacin, and folacin. Typically, thiamine, riboflavin, niacin, and folacin contents (on a dry weight basis) of *Phaseolus* beans are, respectively, 0.5–1.14, 0.1–0.25, 0.4–3.14, and 0.037–0.676 mg per 100 g. Vitamin E content ranges from 0.72 to 1.97 mg per 100 g and B<sub>6</sub> content ranges from 0.2 to 0.659 mg per 100 g. *Phaseolus* beans are not good sources of vitamin A and C.

Phaseolus beans are excellent sources of several minerals including Ca, Fe, Cu, Zn, P, K, and Mg.

 Table 4
 Recognized legume allergens<sup>a</sup>

Legume	MW (kDa) SDS-PAGE	Identity	Allergen designation	Seq. <sup>b</sup>	Accession number	PubMed index
Dry beans						
Chickpea						
Cicer arietinum	10-106					11527247, 10705224
Cowpea						
Vigna sinensis	41, 55	Albumins				12546052
Grass pea						
Lathyrus sativus	21, 28, 46					11295670
Green pea						
Pisum sativum	$\leq$ 20, 20–30, 33, 50	Vicilin-like proteins				12589366
	1.8	Glycoprotein				991447
Lentil						
Lens culinaris	12–84					11080720, 10604559, 9893199
	48	Vicilin-like protein	Len c 1	С		
	66	Seed-specific biotinylated protein	Len c 2	Р		11080720
Oil seeds						
Castor bean						
Ricinus communis	11.2	2S albumin	Ric c 1	С	P01089	9430499
	12	2S albumin	Ric c 3	Р		9430499
		11 <i>S</i> crystalloid protein	Ric c 2			3392372
	47/51	Protein doublet				3392372
Lupin						
Lupinus albus	30-175					10359910
Peanut  Arachis hypogaea	63.5	Vicilin-like protein	Ara h 1	С	L34402, L38853,	7560062
					AF432231	
	17	Conglutin	Ara h 2	С	L77197, AY158467, AY117434	14538941, 11295663
	60	Glycinin	Ara h 3	С	AF093541, AF510854	10021462
	37	Glycinin	Ara h 4	С	AF086821, AF510854	10474031
	15	Profilin	Ara h 5	С	AF059616	10474031
	15	Hom: conglutin	Ara h 6	С	AF092846	10474031
	15	Hom: conglutin	Ara h 7	С	AF091737	10474031
	17	Pathogenesis related protein (PR-10)	Ara h 8	С	AY328088	
	30, 50, 68	Oleosin oligomers				12144563
	12	Oleosin monomer				12144563
Soybean						
Glycine max	7	Hydrophobic seed protein (HPS)	Gly m 1A/1B	Р	AAB34755, AAB34756	7603155
	8	Hull protein	Gly m 2	Р	A57106	
	14	Profilin 1, Profilin 2	Gly m 3	С	AJ223981, AJ223982	10589015
	17	(SAM 22) PR-10 prot.	Gly m 4	С	X60043	12417891
	21	Trypsin inhibitor				8836337, 11799388
	26	Vicilin-like glycoprotein	Gly m Bd 28k		AB046874	11267676
	34	Oil body associated glycoprotein	Gly m Bd 30k	С	AB013289	11227798
		Alpha-subunit of beta-conglycinin	Gly m Bd 60k			7787297
	22	G2 glycinin				11112856
	15	Glycinin G1 acidic chain				11146387
	7, 12, 20, 39, 57	Soy lecithins				11752879
	12	Methionine-rich protein		Р		11752879
	20	(MRP), 2 <i>S</i> albumin Soybean Kunitz		Р		11752879
	20	Trypsin Inhibitor (SKTI)		•		11102013

 $<sup>^</sup>a$ Compiled in part from the International Union of Immunological Societies, Allergen Nomenclature Sub-committee http://www.allergen.org/list.htm.  $^b$ Sequence source: P = Protein, C = cDNA.

Typically, raw beans contain 70–260, 0.5–1.40, 3.34–13.5, 160–320, 1.0–2.1, 380–570, 1320–1780, 4.0–21.0, and 1.9–6.5 (all expressed as mg per 100 g, dry weight basis) of Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn, respectively. Low sodium and high potassium content of raw beans makes them a desirable constituent of human food (especially for people with hypertension).

#### **Antinutritional Factors**

In addition to protease inhibitors and amylase inhibitors, Phaseolus beans contain several other antinutritional and antiphysiologic factors such as phytic acid, tannins, cyanogenic glycosides, saponins, allergens. Because these components are usually present in small quantities (less than 5% of the total seed weight), they do not pose a serious health hazard under normal conditions (that is when beans are a part of total diet and are properly processed prior to consumption). Of these antinutritional factors, phytates and tannins are of particular concern because both of them are heat stable and cannot be easily removed from beans during normal home processing. Phytate is a general term used for mono- to dodeca anions of phytate along with esters lower than hexaphosphate. Ca-Mg salts of phytic acid (myoinositol 1, 2, 3, 4, 5, 6-hexakis (dihydrogen phosphate)) are referred to as phytin. In most dry beans phytate phosphorus accounts for up to 80% of the total phosphorus. The amount of phytate in Phaseolus beans range from 0.6% to 2.1% (by weight) of the total seed weight. Because phytates are chelating agents, they may interfere in mineral utilization. Germination, fermentation, and soaking followed by cooking (if both soak- and cook-water are discarded) are effective methods of removing phytates (50–80% reduction).

Tannins (especially condensed tannins) are heatstable compounds and are present in *Phaseolus* seeds (especially in colored varieties) up to 2% of total seed weight. Because of their ionic character, they may interact with other constituents (notably proteins) and adversely affect the nutritional bioavailability of that constituent. Tannins are thought to offer protection to the plant from insects and pests.

# Lipids

Phaseolus beans contain 1–3% lipids (by weight) depending upon the species. Neutral lipids (30–50% of total) and phospholipids (25–35% of total) are the major constituents and glycolipids may account for up to 10% of the total lipids. Regardless of the variety, Phaseolus bean lipids primarily contain palmitic, oleic, linoleic, and linolenic acids. Polyunsaturated fatty acids and saturated

fatty acids typically account for 55-87% and 12-28% of total lipids.

#### **Beneficial Bioactive Compounds**

Continuing research on raffinose oligosaccharides, phytates, tannins, simple phenolic compounds, sterols, protease inhibitors, and several other major and minor constituents of dry beans suggests that some of these compounds, under appropriate conditions, may offer beneficial effects for human health. Long-term detailed and fundamental research is needed to fully realize the potential health benefits of dry bean consumption.

# **Grading, Handling, and Storage**

Dry beans are usually harvested at maturity. The seeds are removed from the pods either manually or mechanically and cleaned to remove dirt, stalks, leaves, blemished, and wrinkled seeds, and packaged prior to storage. The grading of seeds is usually based on external characteristics such as color, gloss, seed size, seed soundness, seed firmness, and presence of contaminating substances. The seeds are stored at farmer, trader, or government levels. Typically, farmers hold up to 8% of harvested seeds until next season so that they can be used as planting seeds if crop failures occur. In developed and developing nations, a majority of the seeds are stored by traders and/or governments to protect against subsequent crop failures (or low yields), price fluctuations, and fluctuations in supply (change in demand, shortages, and famines). Losses in seeds occur both pre- and postharvest. The normal preharvest losses are mainly due to birds and mammals feeding on bean plant seeds. Drought/floods, insects, and rodents can also contribute to preharvest losses. In developed nations, preharvest losses are usually small (as low as 1% of the crop). A majority of the losses occurs during postharvest handling and storage and can range from 8% to as high as 50% of total crop. It is estimated that as much as 48% of food produced in the world is lost (due to pre- and postharvest losses). Factors that influence postharvest losses of legumes include moisture, temperature, respiration rate, insect damage, microbial spoilage, and damage caused by mites and rodents. Properly packaged dry beans should be stored at low relative humidity and temperature conditions. High relative humidity and temperature favor the "hard-to-cook" beans. These conditions also favor the growth of molds and insects. Three major insect genera that cause much of the damage to stored legumes are Bruchus, Acanthoscelides, and Callosobruchus. De-husked, split stored pulses are

also damaged by Rhizopertha, Trogoderma, and Tribolium species. Usually, pests seem to have preference for the type of bean they infest although the basis for such preference (or the lack of it) has not been elucidated yet. The major microbial problem during bean storage is contamination by aflatoxinproducing molds (Aspergillus flavus and Aspergillus parasiticus). Mites can consume food up to their own weight  $(6-8 \mu g)$  and because of their large numbers can cause serious losses. Rodents cause twofold damage to stored legumes by not only consuming but also contaminating (up to 20 times the amount they would eat). Because rodents are carriers of many communicable diseases, they pose serious damage to stored beans. The species that most commonly cause damage include Rattus rattus, R. norvegicus, Bandicota indica, B. bengalensis, and Mus muscalatus.

# **Processing and Food Uses**

*Phaseolus* beans are processed and used in a variety of ways. The processing of beans is mostly at the household level in developing and underdeveloped countries, while in most developed countries the majority of the processing is done at the industrial level. Home processing methods include milling, soaking, cooking, frying, germination, fermentation (either alone or in combination with cereals), roasting, puffing, parching, extrusion and frying, and toasting. The method(s) used for home processing depend on regional preference for bean variety and the desired end product. For example, mung beans in sprouted form are popular on global scale and therefore germination is one of the preferred household processing methods used. Black gram, on the other hand, is extensively used for preparation of "idli," a breakfast food popular in India and Sri Lanka, after fermenting it with rice. Industrial processing includes freezing for such beans as green French beans, snap beans, etc., milling (production of flours and high-protein flours), baking (baked beans), cooking and frying (refried beans), and canning (alone in salt water or tomato juice or in combination with meats such as beef and pork). In developing countries, de-husking and splitting to produce "dhal" is also done at industrial scale.

In developing and underdeveloped countries *Phaseolus* beans are used in numerous ways depending on the type of bean and regional preference. They may be eaten as raw, immature seeds; cooked as green vegetables (such as French beans); consumed as part of salads; used in making curry; used as a soup ingredient; cooked, mashed, mixed with condiments and spices and used as gruels and porridges; prepared as pastes to be extruded to prepare fried snack products; sprouted; puffed or roasted and eaten as snack

foods; and fermented to prepare numerous fermented products.

In developed countries *Phaseolus* beans are consumed as salad and soup ingredient, sprouts, canned, frozen, and refried beans. They are also extensively used in the preparation of Mexican style preparations, such as burrito, chimichanga, taco, bean dips, tamale, etc., and often canned with meats such as beef and pork. In many South American countries cooked black beans are a preferred part of breakfast.

Although there is a good potential for the preparation of protein concentrates and isolates and the development of food starches, *Phaseolus* beans have not been used on large scale for such purposes. In many countries, especially the developed ones, *Phaseolus* beans have been extensively used as animal feed. In developing and underdeveloped countries, the green foliage, deseeded pods, and roots and shoots of bean plants are used as natural fertilizers especially after composting. Because the legume roots fix nitrogen, they help conserve soil quality. For this reason, in many developing countries they are extensively used for soil quality conservation.

See also: Chickpea: Overview. Cultural Differences in Processing and Consumption. Lentil: Breeding; Agronomy. Nutrition: Effects of Food Processing. Pea: Overview. Protein Chemistry of Dicotyledonous Grains. Pulses, Overview. Taxonomic Classification of Grain Species.

#### **Further Reading**

Broughton WJ, Hernandez G, Blair M, Beebe S, Gepts P, and Vanderleyden J (2003) Beans (Phaseolus spp.) – model food legumes. *Plant Soil* 252: 55–128.

Derbyshire E, Wright DJ, and Boulter D (1976) Legumin and vicilin, storage proteins of legume seeds. *Photochemistry* 15: 3–24.

Deshpande SS (1992) Food legumes in human nutrition. A personal perspective. CRC Critical Reviews in Food Science and Nutrition 32: 333–363

Doughty J and Walker A (1982) Legumes in Human Nutrition. Food and Nutrition paper 20, 152pp. Rome, Italy: Food and Agriculture Organization of the United Nations.

Geil PB and Anderson JW (1994) Nutrition and health implications of dry beans: a review. *Journal of the American College of Nutrition* 13: 549–558.

Guillon F and Champ MM (2002) Carbohydrate fractions of legumes: uses in human nutrition and potential for health. *British Journal of Nutrition* 88: S293–306.

Kami JA, Velasquez VB, Debouck DG, and Gepts P (1995) Identification of presumed ancenstral DNA sequences of phaseolin in *Phaseolus vulgaris*. Proceedings of the National Academy of Sciences, USA 92: 1101–1104.

Muntz K (1998) Deposition of storage proteins. *Plant Molecular Biology* 38: 77–99.

Osborn TC (1988) Genetic control of bean seed proteins. CRC Critical Reviews in Plant Sciences 7: 93-116.

Patwardhan VN (1962) Pulses and beans in human nutrition. American Journal of Clinical Nutrition 11: 12–30.

Reddy NR, Pierson MD, Sathe SK, and Salunkhe DK (1984) Chemical, nutritional and physiological aspects of dry bean carbohydrates: a review. *Food Chemistry* 13: 25–68.

Reddy NR, Pierson MD, and Salunkhe DK (1986) Legume-Based Fermented Foods, 254pp. Boca Raton, FL: CRC Press. Salunkhe DK and Kadam SS (1989) Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization vols. I (310pp.), II (294pp.), and III (323pp.). Boca Raton, FL: CRC Press.

Sathe SK (2002) Dry bean protein functionality. CRC Critical Reviews in Biotechnology 22: 175–223.

Sathe SK, Deshpande SS, and Salunkhe DK (1984) Dry beans of *Phaseolus*: a review. Part 1. Chemical composition: proteins. CRC Critical Reviews in Food Science and Nutrition 20: 1–46.

# **BEVERAGES**

Contents

Asian Alcoholic Beverages Distilled

# **Asian Alcoholic Beverages**

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For many centuries, China and other East Asian nations have used a mold-based system for the production of alcohol from grains. This mold-based system has also been adapted to the production of other fermented products such as soy sauce. The foundation of the system is "koji" (Japan), or "jiuqu" (China). Grain is prepared and mold allowed to grow on it. The mold enzymes include  $\alpha$ -amylase, gluco-amylase, as well as proteases. To produce rice wines such as "saké" and Chinese rice wines, the molded grains are mixed with rice, and in a complex process the rice is converted to an alcoholic beverage. The distilled sorghum liquors depend on a semisolid fermentation followed by steam distillation. This is done up to 8 times with the same batch of sorghum with "daqu" being added after each distillation.

# Introduction

Yeasts can only produce alcohol from soluble monosaccharides and disaccharides, most commonly from glucose and maltose. Two sources of these sugars are fruit juices and starch, but the starch is insoluble and the sugars are in polymeric form and so generally unavailable to yeasts. In the West, this unavailability problem was solved by using sprouted barley to release enzymes that breakdown the insoluble starch in the grain to sugars for yeast growth. However, in China, after an attempt ~3000 years ago to use sprouting grain to brew "Li" from "Nie" in a process similar to beer brewing, the idea was dropped in favor of a microbial-based system in which "Jiu" was brewed with "Qu" Modern beer making was only introduced to China in 1900 at Harbin by Russians. The ancient Chinese system depends on growing mold on grain, essentially a solid-state fermentation for enzyme production, mixing the molded grain with cooked grain and water, then soaking it in water to allow the enzymes from the mold to breakdown the starch, followed by a yeast fermentation to produce alcohol. Rice wine or Japanese saké are the modern descendents of this technological innovation.

The molded cereal-based system starts with wet grain, cooked or uncooked, so that molds grow on the grain to produce carbohydrate degrading enzymes. In Japan, this molded product is called koji, in China it is qu or jiuqu, in Korea it is "nuruk" (or "nuluk"). In Southeast Asian countries, a similar powdered-rice product is made and they go by the names "bubod," "ragi," "lakpaeng," "men," and "mochikouji," but rice is not necessarily the source of sugar. In the Philippines, the wet form of koji is called "binubudan" and sugar cane juice is fermented. Koji is used to produce a variety of foods, apart from alcoholic beverages, throughout east Asia. This microbial-based system of alcohol production has a long history, dating back to the Xia Dynasty, ~4000 years ago, but archeological artifacts associated with the technology have been dated to over 5000 years ago. Jiuqu has been specifically mentioned in Chinese literature dating from the Zhou Dynasty (770-221 BC). As with beer in the West, for centuries in China, and even now, some alcoholic beverages are thought beneficial to health.

In China, this mold-based system is used to make grain-based distilled liquors and wines, e.g., from rice, corn, millet, and sorghum. The Japanese have developed saké-brewing systems based on the scientific study of the organisms and processes while Chinese systems tend to be more traditional and far more complex.

#### Saké Brewing

The Japanese rice wine production began  $\sim\!300$  BC and today there are  $\sim\!10\,000$  brands of saké produced in  $\sim\!2000$  factories. The Japanese have developed pure fungal and yeast culture based systems for rice wine production as opposed to the traditional rice wine making systems mostly used in China. Saké is classified according to the degree of polishing of the rice used to make the wine (Table 1). Rice grain weight reduction is from 25-35%. Generally, "ginjo saké" is made with highly polished rice while "honjozo saké" is made with rice not polished below 60%. Less highly polished rice results in a higher level of inosinic acid that is responsible for the characteristic flavor of saké.

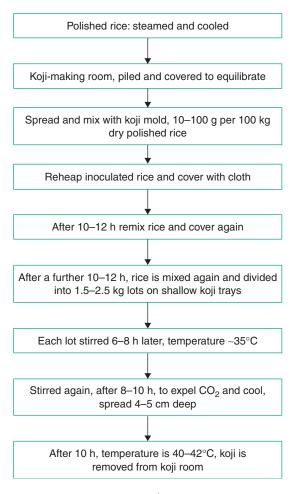
# Koji Production for Saké

Brewers favor large rice grains with soft kernels so that water uptake is rapid and mold mycelia can penetrate the kernels more easily (Figure 1). The mineral content of the water influences saké quality, iron levels must be low and potassium, phosphorus, and magnesium are required for mold growth, and calcium and chlorine improve fungal amylase extraction.

**Table 1** Types of saké are based on the Seimaibuai classification that relies on the percentage of original rice left after the rice has been polished

Type of saké	Seimaibuai classification (% of rice left after polishing)	Comment
Junmai dai ginjo	50	Highest grade of saké, light dry type
Junmai ginjo	60	Sweeter, heavier than above
Tokubetsu junmai	60	"Special Junmai," a marketing product
Junmai	70	Means "pure saké," no added alcohol, with heavier, fuller flavor

The lesser the percentage of original rice, the lighter and more delicate is the flavor of the saké.



**Figure 1** Koji production for saké manufacturing is an intensive process designed to maximize enzyme production.

The pH should be neutral to alkaline, of medium hardness (German scale 3–7) and highly chlorinated.

After washing off loose bran, the grains are steeped to take up water equal to 25-30% of the milled rice weight. The drained, steeped rice is then steamed in shallow open vessels for 30 min to 1 h, in batch systems, for a shorter period in continuous systems, so that the weight of the grains increase a further 8– 12%. The rice is then cooled to 34–36°C and transferred into the koji-making room where temperature and relative humidity can be controlled, and piled on an insulated bed and covered with cloth to allow equilibration with the room. The grains are then spread evenly and inoculated with "tane-koji," a seed culture of Aspergillus oryzae that has been grown for up to 6 days to maximize sporulation. It is inoculated at 10–100 g per 100 kg of polished rice, reheaped and covered with a cloth. The temperature rises as the fungus grows so the inoculated rice must be manipulated and mixed to control temperature.

The way the mold mycelia grow through the resulting molded grain influences the quality of the saké produced. If mold growth is confined to the outer layers of the grains, excessive breakdown of grain protein reduces saké quality. The fungus produces  $\sim 50$  kinds of enzymes including proteases and amylases. Of particular importance are  $\alpha$ -amylase (amylo-1,4-dextrinase) and S-amylase (amyloglucosidase), the former breaks down the starch molecules to smaller fragments to decrease viscosity, the latter removes glucose units (see Enzyme Activities).

#### **Moto Production**

In effect, this is the beginning of the brewing process because ingredients are added to the "moto," after it has been made, to make the saké. In moto production, yeast is prepared by a complex process that is, however, simpler than traditional methods. Water (2001), rice-koji (60 kg), and steamed rice (120 kg) are mixed and held at 13-14°C for 3-4 days with occasional stirring. The temperature drops to  $\sim 7-8^{\circ}$ C. During this stage, the koji enzymes begin the breakdown of the starch in the rice, and some wild bacteria and veasts begin growing in the mix. However, lactic acid bacteria (Leuconostoc mesenteroides var. saké and Lactobacillus saké) begin growing and lower the pH. These acid conditions, along with the high levels of sugars, eventually kill off these organisms. The temperature rises to  $15^{\circ}$ C and by  $\sim 15$  days a pure culture of saké yeast, a strain of Saccharomyces cerevisiae but with some unique characteristics (Table 2), is added to give a population of  $\sim 10^5$  $10^6 \,\mathrm{g}^{-1}$  that rises to  $\sim 10^8 \,\mathrm{g}^{-1}$  as temperature also rises to 20-23°C. A variation on this system is to

**Table 2** Some cultural characteristics that distinguish saké yeast and *S. cerevisiae* 

Characteristic	Response				
	Saké yeast	Saccharomyces cerevisiae			
Growth in vitamin-free medium	+				
Growth in biotin-free medium	+				
Tolerance to ethanol (% concentration)	20-23	16–19			
Habitat	Found only in saké brewing	Widely found in fruits and other environments			

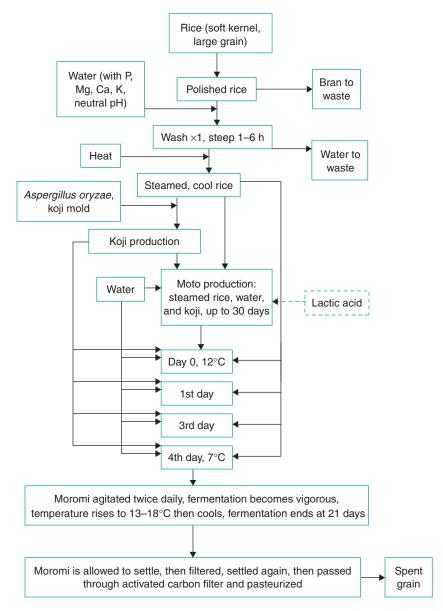
add lactic acid to the moto mix to bring the pH down to 3.6–3.8 to prevent the wild microbial activity but the saké yeast must be added soon after. The characteristics of the water phase at yeast inoculation are: specific gravity, 1.124–1.128; reducing sugar, 26–28%; amino acid as glycine, 0.5–0.8%; total acid as lactic acid, 0.3–0.4%. Traditionally, it took 25–30 days to produce suitable moto.

#### **Rice Wine Production**

The wine-making process is basically a batch-fed system, the fermentation process is begun and further raw materials are fed into the "moromi" as it progresses (Figure 2). (Moromi is a general term to describe a fermenting mash based on grains on which fungi have grown.) The moromi stage of the process involves putting moto, at 12–13°C, in a vessel 6–18 kl in size along with steamed cool rice, rice-koji and water (Table 3). By this stage, the water phase of moto has a specific gravity of 1.028–1.043, an ethanol content of 12–15%, amino acid content of 0.45–0.65% and total acid content of 0.9–1.0%. The saccharolytic enzymes from the koji release the glucose from the rice starch thus making it available for the yeast to ferment.

There are three additions of water, rice-koji, and steamed rice to the vessel containing the moto, and as each addition is made the temperature is lowered by adjusting the temperature of the steamed rice and water so that by the fourth day the temperature is  $7-8^{\circ}$ C. The lower temperature controls wild microbial growth. The moromi is then agitated twice daily and by 3-4 days, the water phase composition is: specific gravity, 1.051-1.058; ethanol, 3-4%; total acid as succinic acid, 0.06-0.07%.

The yeast concentration reaches about  $2.5 \times 10^8$  per g and the fermentation becomes more vigorous and temperature rises to about  $13-18^{\circ}$ C in



**Figure 2** Saké production is a complex process involving the production of koji that is used to make a relatively small batch of starter material, the moto, to which is added further batches of steamed rice, koji, and water. Acid conditions to prevent growth of spoilage organisms are important; so in some cases lactic acid is added instead of relying on the natural growth of lactic acid bacteria in the moto. Temperature control is also important.

**Table 3** The raw materials added progressively to the prepared moto to ferment the rice for saké

Preparation	Day		Cumulative totals					
stage		Total rice (kg)	Steamed rice (kg)	Rice-koji (kg)	Water (I)			
Moto <sup>a</sup>	0	180	120	60	270			
1st addition	1	510	360	150	500			
2nd addition	3	1020	830	210	1230			
3rd addition	4	1710	1350	360	2329			
Total		3420	2640	780	4329			

<sup>&</sup>lt;sup>a</sup> Moto production takes 2-4 weeks to complete before the actual wine production begins.

6–9 days, where the temperature is held for a further 5–7 days until the ethanol content is 17.5–19.5% and the temperature begins to decline.

In the final stage, further additions may be made to the moromi. Pure distilled ethanol may be added to bring ethanol to 20-22%, 7-8% steamed rice allows production of excess glucose to sweeten the wine but pure glucose may be added, lactic acid and succinic acid may be added as well as glutamic acid. By 21 days, the density of the water phase is 1.004-1.000, reducing sugar is 3-2%, ethanol is 18.5-19.5% and the acid content as succinic acid is 0.14-0.16%.

The moromi is allowed to stand for a few days to settle and it is then filtered and again allowed to settle for 5-10 days. If the period is too long, the saké quality begins to deteriorate. It is then passed through an activated carbon filter, pasteurized, and stored at  $13-18^{\circ}\text{C}$  to mature before bottling.

### **Scientific Basis of Process**

The wine-brewing process is conducted as a parallel fermentation, and is best conducted at low temperatures. Further, the development of low pH due to acid production by lactic acid bacteria or addition of lactic acid retards growth of spoilage organisms and permits the growth of yeasts. The parallel fermentation is unique in that while the enzymes are releasing the glucose from the starch, the yeasts are fermenting it to produce ethanol unlike beer where the soluble sugars are first released and then the fermentation is conducted.

From studies conducted in Japan, it is known that the release of the sugars initially from the steamed rice occurs rapidly. However, the increased glucose concentration suppresses  $\alpha$ -amylase activity, so release of glucose declines until significant yeast activity reduces glucose and produces ethanol. The breakdown of the rice-koji in the moromi is more complex. The mold growth in the outer layers of the rice grain solubilizes the outer layer of starch rather quickly leaving variable amounts of a core of the rice grain that breaks down much more slowly under the action of the  $\alpha$ -amylase. The breakdown of this inner core is dependent on the variety of rice used in the manufacturing.

#### **Chinese Rice Wine Production**

Unlike modern Japanese saké preparation methods, the Chinese traditional methods of preparation did not distinguish between the mold stage and the yeast stage, they were combined into one process that was very complex and varied enormously from place to place. However, pure cultures have been isolated and are increasingly being used but traditional methods are still used. In addition, constituents apart from rice may be used in the preparation so instead of steamed rice, barley, and millet are also used as the main source of starch.

Generally, Chinese rice wines are classified according to the residual sugar level, namely dry type, semidry type, semisweet type, sweet type, and extrasweet type (Table 4). They are also classified according to the production method. The four basic methods and their names (Table 5) are:

1. "Ling fan jiu," e.g., "yuan hong" wine is a dry type wine. The hot steamed rice is cooled by drenching

Table 4 The Chinese classification of rice wines

Wine type	Sugar (g per 100 ml, as glucose)	Ethanol (%, v/v)	Total acid (g per 100 ml, as succinic acid)
Dry type	<0.5	>14.5	0.45
Semidry type	0.50-3.00	>16.0	0.45
Semisweet type	3.00-10.00	>15.0	0.55
Sweet type	>10.00	>13.0	0.55

it with cold water then mixing the rice with jiuqu starter. It is commonly used as starter for inoculating more mash. It can be drunk after fermentation for several days.

- "Tang fan jiu," e.g., "jia fan" wine that is a semidry type wine. The steamed rice is spread onto bamboo rafts to cool and then mixed with jiuqu, and acidified rice-steeping liquid.
- 3. "Wei fan jiu," e.g., "san niang" wine that is a semisweet type wine. This rice wine is produced using a fed batch fermentation method similar to saké production. The fermentation is begun as in the first process, but then steamed rice is added intermittently during the fermentation period, usually in three batches.
- 4. Fortified rice wine, e.g., "xiang xue" wine that is a sweet type wine. Aged rice wine or spirits distilled from refermented spent grains from rice wine are added before the main fermentation ceases. The final alcohol concentration is above 20%, consequently the microorganisms in the mash are inhibited, but the saccharification of the remaining dextrin to sugar in the main mash continues slowly, resulting in high residual sugar contents.

A general outline of the manufacturing process of Chinese rice wine is given in Figure 3.

#### **Jiugu Production**

Chinese rice wine making begins with the preparation of a starter material called qu or jiuqu. The preparation is designed to encourage the growth of molds on prepared masses of grains and along with it yeasts also grow. The mold growth results in the production of enzymes in the jiuqu (see Enzyme Activities). The molded mass of grains is then shaped and dried for use as an inoculum in rice wine making. When the jiuqu is then added to prepared grains, such as steamed rice, the enzymes breakdown starch in the steamed rice just as malted grains can be used to breakdown adjuncts in beer production. However, unlike beer production, the jiuqu also fills the fermenting function, due to the presence of yeasts

Table 5 The ingredients and chemical composition of four kinds of Chinese rice wine

Component		Types	of rice wine	
	Yuan hong	Jia fan	San niang	Xiang xue
Proportions of components used to ma	ke the rice wine (kg)			
Glutinous rice	144	144	144	100
Wheat koji	22.5	25	25	10
Seed mash	8-10	8-9	15	
Acidified rice-steeping water	84	50	50	
Freshwater	112	68.6	100 <sup>a</sup>	
Distilled spirit		5		100 <sup>b</sup>
Composition of rice wines				
Specific gravity	0.992	0.995	1.0349	1.073
Ethanol (%, v/v)	15.6	17.8	16.7	19.4
Extracts (g per 100 ml)	3.325	4.450	15.65	24.44
Sugar (g per 100 ml)	0.38	0.78	6.50	20.00
Total acids (g per 100 ml)	0.48	0.46	0.46	0.28
Volatile acids (g per 100 ml)	0.06	0.027	0.054	0.056
Nonvolatile acids (g per 100 ml)	0.42	0.43	0.406	0.22

<sup>&</sup>lt;sup>a</sup>This is yuan hong wine.

Adapted from Xu G and Bao TF Grandiose survey of Chinese alcoholic drinks and beverages (http://www.sytu.edu/zhgjiu/umain.htm).

(S. cerevisiae) in the prepared jiuqu. The actual number of types of jiugu used for the production of alcoholic beverages in China is unknown. However, there are three broad types of jiuqu, based on the raw material used, and within each broad type there are subtypes based on the color, presence of additives, and size of the molded masses or molded pancakes produced. The names of various jiugus based on the raw materials rice and wheat are in Table 6. One is also made from wheat bran, called "fu qu," that is used for alcohol production. The manufacturing processes also vary which also influence the resulting jiuqu. These processes include the use of pure or mixed cultures, a high temperature or a medium temperature, incubation of jiugu in a thin or a thick layer, hanging in the air in a suitable small container, and wrapping the jiuqu in straw. Consequently, for a given mass of jiuqu, the enzyme activity varies widely. In addition, jiuqu is prepared to suit the product to be manufactured.

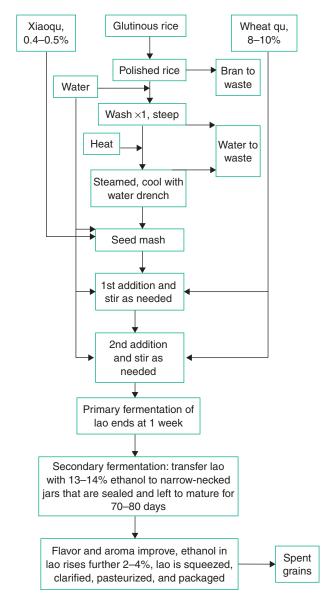
Shenqu Historically, the most active wheat-based jiuqu for making rice wine, was "shenqu." It was made by milling wheat, splitting it into three portions, one of which was roasted, another steamed, and the other left uncooked. The three portions were then mixed thoroughly with added water to a consistency that was less than a slurry. The mixture was transferred to an incubation room, and spread on the floor. Moisture content and temperature were critical and these were managed by opening windows to lower temperature and heaping the mixture to raise

temperature. The first three days required highmoisture content, to promote growth of mycelia, and lower-water content was required for the subsequent incubation period up to 49 days. After this time, the shenqu had a yellowish to yellowish-green color. The primary fungi for this preparation were *Aspergillus* species and pure cultures have been isolated and are now used.

Preparation methods today with pure cultures differ from the traditional method in that incubation may be on the floor, as in the traditional method, or in a compartment similar to the germinating compartment used for malting barley, or in trays similar to koji trays. Using the compartment, the steamed, cooked wheat grits are inoculated with fungal spores and piled in a heap for 3 h. The wheat is then spread in a 30 cm layer over the perforated plate in the compartment. The temperature and moisture content are controlled, and oxygen supplied for the fungi deep in the pile, by blowing air suitably conditioned for temperature and water content through the layer. Generally, the temperature is kept to a maximum of  $\sim 30^{\circ}$ C.

Daqu Daqu, meaning "large qu," is used for the manufacture of some Chinese spirits and its manufacture is different from shenqu. There are two basic kinds, depending on the temperature reached during manufacture. One kind is high temperature that is allowed to rise above 55°C, perhaps as high as 65°C, and the other is kept below 55°C. The raw materials are wheat flour, barley flour, and pea

<sup>&</sup>lt;sup>b</sup>This is 50% distilled spirit.



**Figure 3** The production of a Chinese rice wine. The name "lao" is equivalent to moromi in saké manufacturing. In this case, there is a secondary fermentation in which the lao is put into a jar and sealed up for months to allow the production of flavors and aromas.

flour mixed together in proportions, depending on the manufacturer. One manufacturer, Yanghe Distillery, uses the following proportions and procedures. The wheat:barley:pea proportions are 5:4:1. The moisture content is brought to  $\sim$ 40% and the resulting dough kneaded and eventually packed into a wooden mold with dimensions of  $\sim$ 195  $\times$  138  $\times$  75 cm. Traditionally, the packing was done by human treading by ten people with a gradation in body weight to get the dough at just the right firmness in the mold (Figure 4). In modern production systems, mechanical pressing devices are

used to prepare the blocks. The block is then removed from the wooden mold and placed in the incubation room, with other blocks, in two layers on bamboo rods giving a space of 2 cm between the layers covered with straw. Incubation is for  $\sim$ 4 weeks and has three phases. In the first phase of  $\sim$ 9 days, there is a rapidly rising temperature due to the growth of fungi and yeasts. The dominant fungi are Aspergillus species and Rhizopus species, namely R. oryzae and R. chi*nensis*. The humidity is initially high and is reduced by opening windows. The temperature of the whole batch is controlled to the temperature range 30-45°C by turning and rearranging blocks ∼6 times in the 9-day period. In the second phase, of  $\sim$ 8 days' duration, the temperature of the batch is allowed to rise smoothly a little further but within the range 30–45°C. In this phase, more of the moisture evaporates and its loss is controlled so that it is not lost too quickly. In the third phase, the room temperature is allowed to decline and moisture loss from the blocks allowed to increase so that they weigh less. If at the end the moisture has not been reduced to  $\sim$ 12%, the blocks are piled and recovered with straw to raise the temperature and accelerate moisture loss.

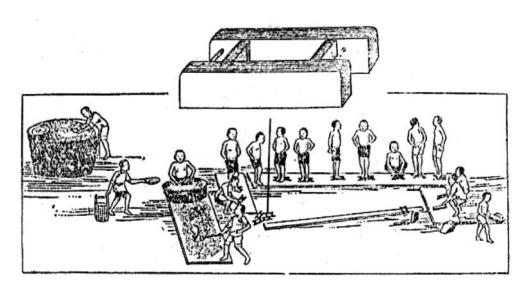
**Xiaogu** A further important jiugu is "xiaogu." The traditional method is similar to that used for daqu production except only steamed and powdered rice is used, the water used is flavored with steeped herbs, and the freshly made blocks are cut into smaller cubes and formed into balls. Selected old jiugu is used as a seed culture to inoculate the outer surface of the balls. The old jiuqu is a kind of selected starter culture. As the dominant fungi involved have been isolated, a pure culture method is now used in its preparation as follows. The *Rhizopus* species are grown on rice meal or wheat bran and the yeast, a Saccharomyces species, is cultured on an extract of a special jiugu and harvested by centrifugation. Then the Rhizopus culture and yeast concentrate are mixed in a 50:1 ratio. This mixture is used to make a seed mash for rice wine. This particular approach to rice wine making significantly reduces the time to make the wine.

#### **Chinese Rice Wine Making**

Although Chinese rice wines are usually made with rice, they are also made with maize and millet. When made with rice, ~230 kg of rice wine is produced from 100 kg of rice. The jiuqu in rice wine manufacturing has two functions: to supply amylolytic enzymes and to supply yeast for ethanol production. In some modern production systems, commercially manufactured enzymes are used to carry out the saccharification

	Rice	Wheat		
Qu name	Comment	Qu name	Comment	
Xiao qu	White, small dimension, made with Rhizopus spp.	Mai qu	Wheat qu, made with Aspergillus flavum	
Yao qu	Herbs added	Da qu	Dimension large	
Bai qu	White	Shu mai qu	Wheat steamed or roasted	
Hong qu	Red color, due to Monascus fungus	Seng mai qu	Fresh wheat used	
Hei qu	Black, due to Aspergillus niger	Bing qu	Pancake shape	
Wu yi hong qu	Red and black color	Dou qu	Added pea or bean, and rice or wheat flou	

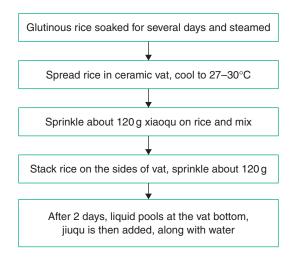
Table 6 Types of Chinese qu, based on the base grain employed to make alcoholic beverages from rice and other grains



**Figure 4** An ancient image of daqu production in China. In the early stage, the "dough" is shaped into rectangular blocks by men pressing the dough into a mold with their feet. The weight of the men pressing a block progressively increases. The block is then managed to encourage mold and yeast growth, before drying the blocks. (Data from Xu G and Bao TF Grandiose survey of Chinese alcoholic drinks and beverages (http://www.sytu.edu/zhgjiu/umain.htm))

step. For various types of wines different portions of ingredients are used (Table 5).

One of the ingredients in the mix is traditional seed mash, and the ingredients used to make this seed mash are 125 kg of glutinous rice that is soaked for several days and then steamed. The steamed rice is then spread in a ceramic vat and after it cools to  $\sim$ 27– 30°C, half of the xiaoqu jiuqu is mixed through the rice (Figures 3 and 5). The rice is then stacked around the sides of the vat to form a hollow in the middle of the rice and the rest of the xiaoqu powder is sprinkled onto the surface  $(0.187-0.25 \,\mathrm{kg})$  total). This maximizes the surface exposure to encourage growth of Rhizopus species (that produce lactic and fumaric acids) to lower the pH, discourage the growth of undesirable organisms, and encourage yeast growth. In addition, the amylase and glucoamylase in the jiuqu saccharify the rice. After about 2 days a pool of liquid containing sugars, etc., gather in the hollow. Then



**Figure 5** The production of seed mash used in the manufacture of Chinese rice wines.

#### Water and Acidified Rice Steeping Liquid

The water used in wine brewing should have a hardness in the range of 2–6, ~pH 7, and should have a low-iron content. The acidified rice-steeping liquid is traditionally prepared by steeping the polished rice in the water for periods of 10–20 days, preferably 13–20 days. During the steeping, lactic acid bacteria grow and lower the pH. This traditional liquid, when added to the final brew, helps to inhibit the germination of bacterial spores and growth of vegetative cells, provides amino acids and vitamins, lowers the pH for yeast growth, and helps improve the flavor of rice wines. However, alternatives to this include using diluted vinegar solution or a broth made with wheat flour, but lactic acid is now commonly added (see Fermentation: Origins and Applications).

# **Chinese Distilled Liquors**

Chinese distilled liquors are classified according to the jiuqu used, the type of aroma and flavor developed, the fermentation state for ethanol production, the quality, the ethanol content and the raw materials used. Important types are in Table 7 along with the characteristic flavor impact compounds. Many Chinese liquors are based on sorghum and contain 53–55% ethanol. They have characteristic aromatic fragrance due to the presence of 4-ethylguaiacol, vanillic acid, and vanillin. The hexanoic acid needed to result in ethyl hexanoate comes from the metabolic activity of anaerobic bacteria in the fabric of the fermentation pits. A difference between Chinese-distilled sorghum liquor and Western-distilled grain liquors is the use of external mold enzymes from koji-like products.

The production processes vary (see Beverages: Distilled). For rice spirit, the rice is washed, steamed, cooled, inoculated with xioaqu, and placed in a ceramic vat to allow the enzymes to breakdown the starch. Then water is added and the vat covered for 7 days to allow yeast growth and then the fermented liquid is distilled. For sorghum, the grains are crushed, mixed with hot water, steamed, cooled, and further water added. Daqu is added to the cooled mixture in a ceramic vat or mud pit or stone pit and sealed. The fermentation is allowed to proceed for 3—4 weeks and the steamed sorghum husks added and the mixture distilled. The spent sorghum is then remixed with daqu and sealed in a vat and left for

**Table 7** Some of the jiuqu mold preparations used in the manufacture of Chinese distilled liquors and the names of liquors based on distinctive aromas and flavors and their characteristic flavor impact compounds

Jiuqu used	Aroma and flavor	Flavor-impact compound
Daqu Xiaoqu Fuqu	Maotai Luzhou Fen Rice	Ethyl hexanoate and ethyl butyrate Ethyl acetate Ethyl lactate and ethyl acetate

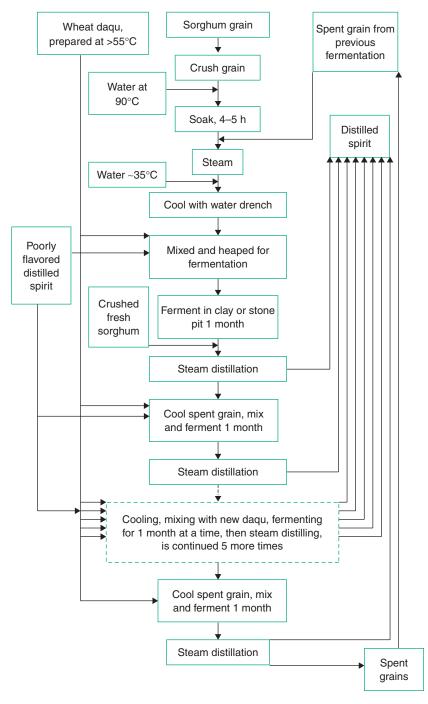
3–4 weeks whereupon steamed husks are added again and the mixture distilled again.

The production system in Taiwan is of rather shorter duration. The typical "wine cake" (or juiqu) for spirit production is produced with cracked wheat, contains up to five varities of yeasts (including *S. cerevisiae* and *S. fibuligera*); four molds (including *Mucor* and *Rhizopus* species); and six types of bacteria, including a *Clostridium* species. The level of activity of hydrolytic enzymes is usually low.

The steamed sorghum is spread out on the floor to a thickness of  $\sim$ 5 cm and powdered wine cake mixed through it. The moisture content of the sorghum is  $\sim$ 55%. The fungi grow on the cool and dry surface, whereas bacteria and yeasts grow within the wet mass of sorghum. After 24 h, the sorghum is piled into heaps that encourage growth of yeast in an anaerobic and warm environment (prefermentation). The size of the pile is restricted because the internal temperature of the pile must not be allowed to rise above 40°C, and the heat produced metabolically is not easily dispersed. The Taiwanese put the inoculated sorghum into stainless steel tanks or boxes and cover them with polythene sheet to prevent moisture loss and reduce aeration. Microbial growth and metabolism is allowed to progress for up to 10 days after which the mixture is put through a steam distillation to remove the ethanol. The pile of sorghum is reinoculated to encourage further growth and when ready the sorghum is distilled again. Each heat treatment, or steaming, causes partial hydrolysis of starch, carbohydrates, and protein. Steaming also removes the alcohol that prevents yeast cell growth in the thin film of water surrounding sorghum grains. Alcohol is collected by condensation of the distillate and the ethanol yield is  $\sim$ 22.53 kg per 100 kg of sorghum.

Microbial cells grow slowly on sorghum due to low availability of fermentable substrates, and this leads to the production of secondary metabolites mentioned above, which contribute to the characteristic strong aromatic fragrance of sorghum liquor.

In mainland China, the production of sorghum liquor is much more complex and it takes about 1 year



**Figure 6** The traditional Chinese method of preparation of sorghum-based distilled liquors. The amount of daqu and sorghum used is  $\sim$ 1:1. The traditional pit used for the fermentation is the source of particular flavors for some liquors due to associated bacterial activity. Eventually, the completely spent grain is discarded.

to complete the distillations. The process begins with sorghum that is crushed, soaked in hot water, spent grain from a previous fermentation added and then the entire mix is steamed. Spirit of lower quality and the inoculum of daqu is added to the cooled mixture (Figure 6). It is heaped to allow the fermentation to begin and then placed in a fermentation pit for up to a month to allow the fermentation process to take

place. The mixture is then steam-distilled, more daqu added, and the fermentation conducted all over again. With slight variation the process is conducted up to 8 times.

See also: Beverages: Distilled. Enzyme Activities. Fermentation: Origins and Applications.

# **Further Reading**

Fleet GH (1998) The microbiology of alcoholic beverages. In: Wood BJB (ed.) Microbiology of Fermented Foods, vol. 1, 2nd edn., pp. 217-262. London: Blackie Academic and Professional.

Kodama K (1993) Saké-brewing yeasts. In: Rose AH and Harrison JS (eds.) The Yeasts, 2nd edn. London: Academic Press.

Lisdiyanti P and Kozaki M (2003) Rice wine in Southeast Asian countries: Thailand, Laos, Vietnam and Myanmar. In: The First International Symposium on Insight into the World of Indigenous Fermented Foods for Technology Development and Food Safety, Organized by Department of Microbiology Faculty of Science Kasetsart University, and the National Research Council of Thailand International Cooperation, Ministry of University Affairs, August 13-14, 2003 at the Microbiology-Genetics (MG) Building, Department of Microbiology, Kasetsart University, Bangkok, Thailand (http://plantpro.doae.go.th).

Steinkraus KH (1995) Handbook of indigenous fermented foods. Food Science and Technology, series no. 73. New York: Marcel Dekker.

Xu G and Bao TF Grandiose survey of Chinese alcoholic drinks and beverages (http://www.sytu.edu/zhgjiu/ umain.htm).

Yoshida T (2003) Technology development of saké fermentation in Japan. In: The First International Symposium on Insight into the World of Indigenous Fermented Foods for Technology Development and Food safety, Organized by Department of Microbiology Faculty of Science Kasetsart University, and the National Research Council of Thailand International Cooperation, Ministry of University Affairs, August 13-14, 2003 at the Microbiology-Genetics (MG) Building, Department of Microbiology, Kasetsart University, Bangkok, Thailand (http://plantpro.doae.go.th).

#### **Relevant Websites**

http://www.enonline.sh.cn - Shanghai online. http://www.sytu.edu.cn - A site of the Southern Yangtze University in China. Contains an English translation of a wide-ranging survey of alcoholic drinks and beverages in China from an historical viewpoint to the technology used as well as aspects of the legal and regulatory side of the industry.

http://www.foodreference.com - A site dealing in general with all aspects of food. It includes daily food and beverage news, a culinary quiz and a "Today in food history" section. There is a wealth of information. Use of search function to search for "rice wine."

http://www.internationalrecipesonline.com – cipes for using rice wine are available on the site using the search function.

# **Distilled**

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#### Introduction

Distilled beverages that are sold commercially are produced from plant materials (Table 1) and their

Table 1 Raw materials used for the production of some major distilled beverages

Products		Raw materials			
1.	Scotch malt whiskies <sup>a</sup>	100% malted barley			
2.	Scotch grain whiskies <sup>a</sup>	Wheat or maize (+ malted barley)			
3.	Cachaça	Sugar cane juice only. Unlike "industrial" Cachaça, which is made from cane products (e.g., molasses) and sugar, only fresh sugar cane juice is used to produce traditional, "artesanal" Cachaça			
4.	Rum	Molasses mainly and sugar cane juice			
5. Irish whiskies		100% malted barley			
		Barley + malted barley			
		Maize + malted barley or enzymes			
6.	Gin	Maize or wheat (with malted barley or enzymes for starch conversion into sugars). Pot still distillation of Juniper berries and other botanicals or, addition of spirit to Juniper berries and botanicals or to the essence of these plants			
7.	Tequila (100%)	Agave ( <i>Agave tequilana</i> Weber Var.  Azul) plant			
8.	Tequila Vodka	Agave plant + sugar or syrup Cereals, grape juice, raisins, molasses, potatoes (with or without enzymes for starch conversion)			
9.	American whiskies (bourbon, corn, rye, Tennessee)	Maize + rye (with malted barley: plus or minus commercial hydrolytic enzymes)			
10.	Canadian whiskey	Maize, rye, and malted barley and malted rye + amylolytic and glucanolytic enzymes			
11	Brandy	g. addition to one yilloo			
	Cognac	Grapes			
	Armagnac	Grapes			
	Eau de vie	e.g., pears, raspberries, cherries, and plums			
12.	Aquavit	Grain or potato (with malted barley or enzymes for starch conversion).  Neutral spirit from grain or potato is then redistilled with flavorings.  Caraway is the main flavoring and citrus peel. Cardamom and anise can be incorporated as well			

<sup>&</sup>lt;sup>a</sup>Japan also produces malt and blended whiskies.

Table 2 Types of stills used in production of distilled spirit beverages

Products	Stills
1. Scotch malt whisky	Usually, two large copper pot stills (wash and spirit) - tube condensers
<ol><li>Scotch grain whisky</li></ol>	Continuous (patent) still (analyzer, rectifier)
3. Cachaça	Small copper pot stills with worm condenser coils. Traditional (e.g., "artesanal") Cachaça is produced using pot stills. Single distillation like Aramagnac. Continuous stills used to produce "industrial" Cachaça
4. Rum	Continuous still (Analyzer, rectifier) — Puerto Rican (light rum). Distillation in series of two or three copper pot stills (to avoid double distillation in one still). Cooling coil condensers present: Jamaican (high flavor (heavy) rum)
5. Irish whiskey	Three copper pot stills (viz: wash, low wine, and spirit). Triple distillation
6. Gin	Continuous still (analyzer, rectifier) then copper pot stills for distillation with botanicals
7. Tequila	Copper pot still (double distillation) or Continuous still (analyzer, rectifier)
8. Vodka	Continuous still (analyzer, rectifier)
9. American whiskey	Continuous stills or continuous still plus doubler for additional distillation
10. Canadian whiskey	Continuous still with analyzer column, extraction column, and rectifying column. Pot stills
11. Brandies	
Cognac	Small copper pot still with cooling worm, direct fired instead of internal heating coil. Double distillation
Armagnac	Small copper pot still or small single column continuous still. Single distillation in pot stills, like traditional Cachaça
Eau de vie	Copper pot still – double distillation (analyzer, rectifier)
12. Aquavit	Continuous still (analyzer, rectifier)

distillation processes (Table 2) are clearly defined. Each product has distinct aromas and flavors which reflect raw materials, distillation process, and postdistillation treatments such as maturation in wooden (e.g., oak) casks. The flavor compounds in distilled beverages are referred to as congeners. Different beverages can have small differences in congener levels but have large differences in flavor and aroma intensities. Flavor and aroma intensities may also relate to the complex modulating effects, which different congeners have on each other, and to the physiological and cultural differences of consumers. In distilled spirit beverages, ethanol is the main compound as regards quantity and the congeners can be regarded as "ancillary products" of the natural production of ethanol.

Ethanol of distilled beverages is produced from the different sugars derived from the raw materials used. The mechanisms of production of congeners are very complex. Some are produced by yeast during fermentation, others are produced during distillation, and some develop during maturation. In some distilled beverages such as gin, flavor materials are added. The term "distilled portable spirits" relate to distilled products that are eventually packaged and sold as beverages for human consumption (Tables 1 and 2). The production procedures of many distilled beverages are defined by law. For example, Scotch whisky means whisky distilled and matured in Scotland and Irish whiskey means whisky distilled and matured in Ireland. Scotch malt whisky is made from malted

barley. The blended brands of Scotch whiskies are blends of Scotch malt whiskies and Scotch grain whiskies. Scotch malt whiskies are produced in pot stills and Scotch grain whiskies are produced in continuous patent stills. Only enzymes from malted barley can be used in Scotch whisky production. In the United States, bourbon whiskey must be produced, from a mash conversion of not less than 51% corn grain and rye whiskey must be produced from not less than 51% rye grain respectively. Cognac or tequila can only be made in respective areas of France or Mexico. In many countries, a minimum age of postdistillation maturation in wooden barrels is designated by law. Also, in this regard, Scotch whisky can only be matured in Scotland in oak barrels and bourbon can only be matured in "new" (charred) oak barrels in the United States of America.

# **Historical Background**

The exact dates of development of many distilled beverages are not known. The Chinese may have developed the distillation process in ancient time. The ancient Egyptians had alembics which could have produced alcoholic drinks but it was the Moors who were responsible for distributing the technique and for the derivation of the word "alcohol" which is derived from the old Arabic word "Al-Kuhl." The distillation process is therefore an ancient-world technology and is known to produce a concentrated alcoholic essence of the initial ferment, which contained lower levels of

ethanol. A sugar solution of  $\sim 14-16\%$  will, after fermentation by yeast, yield  $\sim 7-8\%$  ethanol. According to Gay-Lussac, 100 lbs of glucose should, after fermentation by yeast in ideal conditions, yield 48.89 lbs of carbon dioxide and 51.11 lbs of ethanol. But, according to Pasteur, only  $\sim 95\%$  of these products can be expected in fermentation systems. Ethanol is a simple alcohol. It is a member of a large group of alcohols, some simple, others complex, but all containing carbon, hydrogen and oxygen, and hydroxyl groups, which have replaced hydrogen atoms.

Although it has been suggested that the Crusaders brought the technique of distillation to Europe in the eleventh to twelfth centuries, it was not long after this period that distillation activity was discovered in Ireland. The first written record of Scotch malt whisky appeared in 1494. The word whisky is spelt "whisky," when applied to Scotch but is spelt "whiskey" when applied to Irish, Canadian, and American whiskies. In 1931, the Coffey (continuous or patent) still was invented by Aeneas Coffey to produce Scotch grain whisky in large quantities. The first blended Scotch whiskies (i.e., mixtures of malt and grain whiskies) were produced by Andrew Usher in 1860. Blended whiskies are now ~93% of the Scotch whisky market worldwide.

Although the production of vodka, in Russia, dates back to the twelfth century, significant development of other major spirit beverages such as tequila (Mexico), Cachaça (Brazil), and cognac (France) took place in the sixteenth century. A large rum industry, operated by slaves in the West Indies, was very productive in the seventeenth century. Gin was being produced in Holland in the seventeenth and eighteenth centuries. By 1743, the annual consumption of gin in Britain was 70 million liters (Ml) for a population of 6 million people. The devastating effect of excessive drinking of gin was portrayed in Hogarth's (1697–1764) famous etchings of gin drinkers. American whiskies developed in the eighteenth century and Canadian whiskey appeared in the nineteenth century. Canadian rye whiskey came to prominence in the 1940s. Examples of other localized, smaller-volume products of the distilled spirit market are: aquavit (Denmark), arrack (e.g., Turkey), grappa (Italy), marc (France), okolehao (Hawaii), ouzo (Greece), pisco (South America, e.g., Chile and Peru), mao tai (China), and mezcal (with or without "worm" (Mexico)).

As regards the science and technology of distillation, the first study of the distilling process, where a liquid containing many compounds is vaporized, separated, and collected as desirable (drinkable) was made by the French alchemist Arnold de Villeneuve in 1310. Taxation of distilled spirits is an important source of revenue for many producing countries. For example, the first taxation on Scotch whisky was in 1644 at  $\sim$ 40 pence per gallon. That taxation persists today at the impressive figure of about £88 per gallon for pure alcohol. Of all the spirits in the world, vodka sells in the largest volume. Bacardi rum is the largest brand. Scotch whisky earns the largest income of the brown spirits and Cachaça is the spirit product that is consumed in the largest volume in one country (Brazil). It is produced in the largest number of production facilities (c. 18 000).

# **Malting and Mashing Processes**

#### Malting

Scotch malt whisky is produced from malted barley of known varieties. Malted barley is derived from the malting process (Figure 1). In this process,  $\sim 25-500\,\mathrm{t}$  of barley are steeped in water and air-rested for  $\sim 48\,\mathrm{h}$  at  $\sim 16^\circ\mathrm{C}$ . The water is drained and the grains allowed to germinate (grow) for a period of  $\sim 5\,\mathrm{days}$  at  $16-18^\circ\mathrm{C}$ . The germinated grains are then kilned (dried) at  $60-70^\circ\mathrm{C}$ . During kilning, peat may be burnt to "peat" the malt because the

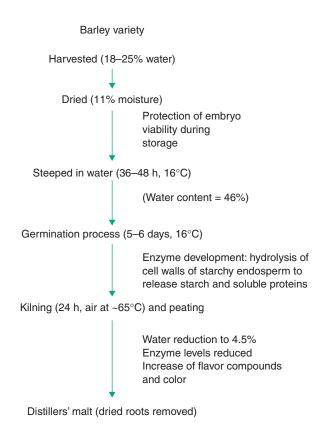


Figure 1 Production of the distillers' malt.

smokey-peaty (phenol: 0-50 ppm) flavor of malted barley is an important flavor note of many brands of Scotch whiskies.

During the germination process, the plant hormone gibberellic acid is produced in the growing embryo. This natural hormone is transported to the aleurone (bran) layer which encloses the starchy endosperm (Figure 2). The aleurone is induced by the hormone to synthesize endosperm-degrading enzymes such as cell-wall-degrading endo-β-glucanases; storage-protein-degrading proteases and starch-degrading αamylases. Other enzymes such as  $\beta$ -amylase (maltose producing) and carboxypeptidases (amino acid producing) develop in the starchy endosperm. Together, these enzymes modify (disrupt) the starchy endosperm so that subsequent milling and extraction (mashing) of the malt in hot water can occur readily. During this process, starch is converted into fermentable sugars, amino acids are extracted and produced. Vitamins and minerals are also extracted. These substrates are required, by growing yeast cells during fermentation, to produce ethanol and flavor compounds (Figure 4).

Distillers set laboratory specifications as regards the quality of the malt they require to produce their whisky products. For example, such malts must have a high fermentable extract potential of  $\sim 68\%$  and fermentability potential of  $\sim 87\%$ , amino acids (free amino nitrogen) should be 130-140 ppm and the modification of the malt, assessed in terms of friability should be at least 90% to enable easy milling and extraction.

Nitrosamines and ethyl carbamate are natural carcinogenic compounds, which develop during the kilning and distilling processes respectively. Very low levels of nitrosamines are specified (none or 1 ppb max.). Glycosidic nitrile is also specified at very low levels ( $3.0\,\mathrm{g\,t^{-1}}$  of malt) because it is the precursor of ethyl carbamate. Only malted barley can be used to produce Scotch malt whisky. In Scotch malt or grain whisky production, all the enzymes required to convert the starch and proteins to sugars and amino acids must be derived from the malt. By law, no extraneous enzymes can be added.

# The Mashing Process – The Starch Degradation and Sugar Extraction Process

Cereal grains (Tables 1 and 3) used in whisky (whisky) production contain significant quantities of starch and cell wall polysaccharides such as  $\beta$ -D-glucans and pentosans.

Cooking solubilizes both the large  $(10-30 \, \mu m)$  and small  $(< 5 \, \mu m)$  starch granules (Figure 3). Heating of raw starch granules beyond their gelatinization temperatures is necessary before they can be converted optimally into sugars by amylolytic enzymes. The large starch granules of wheat, barley, and rye, gelatinize between 63°C and 65°C. The gelatinization temperature of rye starches can extend to 70°C. Small starch granules will gelatinize at  $\sim 80$ °C. Maize and sorghum starches  $(10 \, \mu m)$  gelatinize above 70°C, and are usually cooked. Malt also contains starches which must be gelatinized to realize the extract that malt can contribute to the mash. Therefore, when

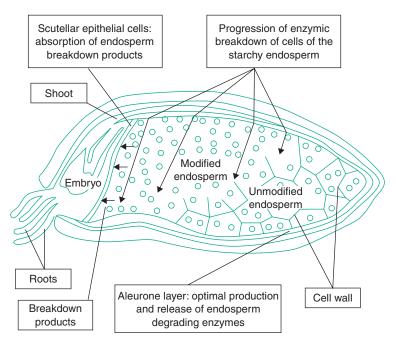


Figure 2 Progression of endosperm breakdown by endosperm-degrading enzymes during the malting process (×34).

Table 3 Constitu	ents of some cere	als (%)
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Cereals	Starch	Gelatinization temperature (°C)	Protein	Lipid	β-D-glucan	Pentosan
Barley	65	63	10.0	3.0	3.5	9.0
Wheat	64	60	11.0	3.0	0.5	8.5
Rye	60	65-70	13.0	2.5	1.5	9.0
Maize	70	>70	11.0	5.0	0.3	3.5
Sorghum	70	>70	10.0	3.0	0.3	3.0

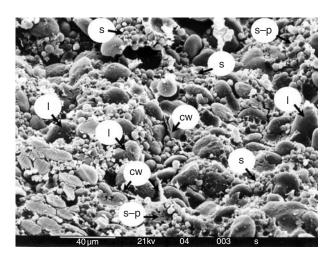


Figure 3 Starchy endosperm of wheat showing large and small starch granules and protein matrix. I - large starch granules; s - small starch granules; s-p - small starch granules and protein matrix; cw - endosperm cell wall.

malt is being used to convert cooked or gelatinized starch, the mashing temperature must be between 63°C and 65°C, temperatures at which malt starches (i.e., large starch granules) will gelatinize.

Malt contributes starch degrading enzymes such as  $\alpha$ -amylase, limit dextrinase, and  $\beta$ -amylase during mashing. α-Amylase liquefies gelatinized starch molecules,  $\beta$ -amylase hydrolyzes these molecules to maltose – the main sugar of cereal-derived worts. Malt is an important source of amino nitrogen which yeast requires not only for growth but also for the production of flavor compounds. Shortage of amylolytic enzymes in the mash can be corrected by adding commercial amylolytic enzymes, if permitted. Malt contains vitamin B and phytic acid. The latter helps to cause an acidic pH during mashing which favors the activities of amylolytic and protolytic enzymes. Amino acids also add buffering capacity to the wort. Unmalted cereals also contribute amino acids and small assimilable peptides to the wort, albeit at significantly lower levels than malted barley.

**Scotch whisky** By law, only the enzymes of malted barley can be used to convert (hydrolyze) the starches of all malt mashes for malt whisky production and the enzymes of cooked maize or wheat used for grain whisky production. Whole grains of maize or wheat are cooked at  $\sim$ 140°C under pressure. Grits of maize or milled wheat are cooked at ~105°C under pressure. After these cereals are cooked and then cooled to ~63°C, a slurry of 9-10% milled green or dried malt is added to effect starch conversion into sugars.

The sugary wort required for Scotch malt whisky production is derived from a mash of 100% milled malt (e.g., 8-10t). The malt is mashed in a water: malt ratio of  $\sim 4:1$ , at 63-64°C for at least 1 h. After the sugary wort is "run-off," the malt bed is washed, 2 or 3 times with hot water (80–100°C) to ensure that all the fermentable sugars are extracted.

Although some grain distilleries collect and ferment the sugary worts, others ferment the entire mash. To start the fermentation process, yeast is added to cooled worts (20°C) or to cooled unfiltered maize-malt or wheat-malt mashes (20°C). About 50% of the fermentable sugars of distillers' sugary worts are maltose. This sugar is mainly produced by  $\beta$ -amylase during mashing. However, α-amylase and limit dextrinase enzymes also assist in the hydrolysis of gelatinized (cooked/heated) starch – these enzymes facilitate the activity of the  $\beta$ -amylase enzyme.  $\beta$ -Amylase is more heat labile than  $\alpha$ -amylase.  $\beta$ -D-glucans are present in the cell walls of the endosperm. Barley contains  $\sim 3.0\%$  $\beta$ -glucan. Malt should contain less than 0.4%  $\beta$ -glucan to ensure that during mashing, wort "run-off" rate and starch release are optimal. Cell wall  $\beta$ -D-glucans and pentosans can limit water removal from spent grains required for animal feed production. Appropriate treatment of spent grains with  $\beta$ -glucanase assists dewatering.

**Irish whiskey** In Ireland, a mash can contain only malt for malt whiskey production or the mash may have 60% of hammer-milled barley and 40% malt or it may comprise 90% hammer-milled maize and 10% malt. The 10% malt may be replaced by hydrolytic enzymes. Commercial enzymes can only be added to the production of grain whiskey. The different spirits

produced are used to produce a wide range of distilled products such as malt whiskies or blends of malt and grain whiskies made in pot stills or continuous stills.

American whiskies Bourbon must be produced from a mash of not less than 51% corn but typically a bourbon mash contains ~70% corn, 15% rye, and 15% malted barley. A rye whiskey mash can contain 51% rye, 39% corn, and 10% malted barley. Tennessee whiskey can be made from a mash of 80% corn, 10% rye, and 10% malt. Different milling systems are used to mill the raw grain. The milled grains and malt or enzymes are heated and cooked and then cooled. An additional quantity of milled malt is mashed in at temperatures not exceeding 64°C. Highly enzymic malts are used. The quantities added are usually lower than those used in Scotch grain whisky production. During mashing, natural enzymes of the malt and commercial enzymes produce optimal conversion of starch to sugars. Conversion (mashing) time varies from 15 min to 60 min. The term, "sour mash," describes the acidic nature of the mash. Long mashing times can encourage the development of microbial infection. At the end of the mashing process, the mash bed (spent grains) is washed with hot water to optimize sugar release, as in other whisky-making processes.

Canadian whiskies For Canadian whiskey, maize grains are milled and cooked at  $140^{\circ}$ C. The mash is cooled to  $100^{\circ}$ C and amylase is then added. Malts containing amylases and commercial amyloglucosidase are added at  $63-65^{\circ}$ C to optimize fermentability. For Canadian rye whiskey, the mash contains milled rye alone or more commonly, rye and other cereals such as maize and/or wheat. Heat-stable amylase and  $\beta$ -glucanase enzymes are added as the mash is heated up to  $\sim 85^{\circ}$ C where the grain meal is held for  $\sim 20$  min. It is then cooled to  $63-65^{\circ}$ C and amylases and amyloglucosidases are added to give optimal release of sugars. Finally, the mash is cooled and yeast is added. Fermentation starts at  $20-30^{\circ}$ C. Yeast may have been subjected to lactic souring.

Gin and vodka The spirits used for the production of gin or vodka are usually neutral spirits. These are mainly highly distilled ethanol (minimum 96%) and are almost free of congeners. The main flavor product is ethanol. The lowest limit of ethanol for neutral spirit (96%) is usually higher than the 94% taken for the production of Scotch grain whisky. In this regard, Scotch grain whisky has more flavor congeners than the spirits used for gin and vodka production. Neutral spirits are produced from mashes of milled/cooked cereals, potatoes, molasses, fruits, or

sugars. Mashes containing cereals and potatoes require the addition of malt enzymes or external enzymes to convert starches into sugars as described for whisky/whiskey production.

Cachaça For traditional Cachaça, e.g., "artesanal," no mashing process is necessary because the fermentation sugar, sucrose, is squeezed from washed, freshly harvested, stems as sugar cane juice. Many varieties of sugar canes are used; some reflect crosses of standard varieties such as *Saccharum officinarum* L. with other varieties. Sugar content of the juice is  $\sim 12-16\%$ .

Rum The fermentation sugar, sucrose, is derived primarily from high or low sugar molasses. Sugar cane juice is also used. Molasses have a higher mineral and nitrogen content than sugar cane juice and impart a different flavor to the final product. Molasses of high sugar content ( $\sim$ 22–24% sucrose), tend to produce a spirit of 10–12% ethanol, whereas sugar cane juice (12–16% sucrose) tends to produce a postfermentation alcohol content of  $\sim$ 6–8% alcohol. Rum produced from sugar beet molasses gives a very different flavor from rum produced from sugar cane molasses. Tasting trials showed that the sugar cane product is generally preferred.

Tequila Tequila and mezcal (with or without the "worm" in the bottle) are both produced from the Agave plant. However, tequila is produced in Tequila and its vicinity from *Agave tequilana* Weber var. Azul, whereas mezcal is produced from *Agave patatorum*. Agave head is harvested and cut into pieces for cooking. During cooking, inulin polymer is hydrolyzed to produce fructose which is sweeter than sucrose — this process can take 2 days. Autoclave cooking will produce a sugar syrup of  $\sim 10\%$ . The cooked agave is milled in small sugar cane mills and the milled product and sugary juices are collected.

For the production of 100% tequila, only agave sugar can be used. For other kinds of tequila, cane sugar, molasses, or corn syrups can be added to levels of 49%. The sugary worts used to produce 100% tequila have sugar concentrations that range from 4% to 10%. For other tequilas, sugar concentration can range from 8% to 16%.

#### **Brandies:**

Cognac derives its name from the town Cognac.
 The base wine for cognac production comes
 mainly from the St. Emillion grape although grapes
 from Folle Blanche and Columbard are authorized. The glucose sugar of the acidic wine is

- 2. Armagnac comes from the Armagnac region and there is written reference to this brandy in 1411, two centuries before cognac was produced. Grape sugar (glucose) is fermented to produce, like cognac, low-alcohol wines. These are then concentrated by distillation without prior removal of grape residues (lees).
- 3. Eau de vie soft fruits are mashed (unheated or preheated) and their sugars are converted to alcohol during fermentation. Alcohol is concentrated in pot stills. Eau de vie is associated with the Alsace region. Calvados brandy, which is made from apple mashes, specifically comes from the provinces of Brittany, Normandy, and Maine.

#### **Fermentation**

Cereal (malt) worts can have the following composition of sugars and dextrins: fructose 1%; glucose 10%; sucrose 5%; maltose 50%; maltotriose 15%; maltotetrose 6%; and dextrins 13%. Excluding sucrose, these sugars are the products of amylolytic hydrolysis of gelatinized starch during mashing. In contrast, sucrose is the only major sugar of cane juice. In fruit mashes and juices, glucose and sucrose are the major sugars and in tequila mashes, fructose is the dominant sugar. Total sugar levels of distillers'

wort range from 10% to 16%. Yeast requires a nitrogen source to effect expected growth from which ethanol and flavor compounds are produced (Figure 4). The raw materials used usually satisfy the nitrogen requirements of the fermenting yeast.

A malt whisky wort can contain  $250 \text{ mg l}^{-1}$  of  $\alpha$ amino nitrogen, whereas a grain whisky mash of 90% wheat can contain  $\sim 100-120$  mg l<sup>-1</sup>. In malt whisky production, there is an exceptionally high level of amino acids which may contribute to the production of esters and other flavor compounds. The role of amino acids in the formation of flavor compounds is complex. However, optimal levels of these nitrogenous compounds will assist in optimal production of esters. Suboptimal levels of amino compounds may encourage the production of higher (fusel) alcohols. However, even at optimal levels of amino nitrogen compounds, different levels of esters can be produced suggesting that other factors influence ester production, such as yeast type, wort concentration, lipid content, and fermentation temperature. Special mention is made of esters because they are important flavor compounds that contribute different flavors to distilled spirits. In this regard, the total ester content does not convey the individual or the complementary contribution of different esters to overall flavor. Insufficient nitrogen nutrients can limit yeast growth, resulting in the production of unexpected or undesirable flavors.

One of the main distinctions in fermentation systems is fermenter size. Cachaça and brandies

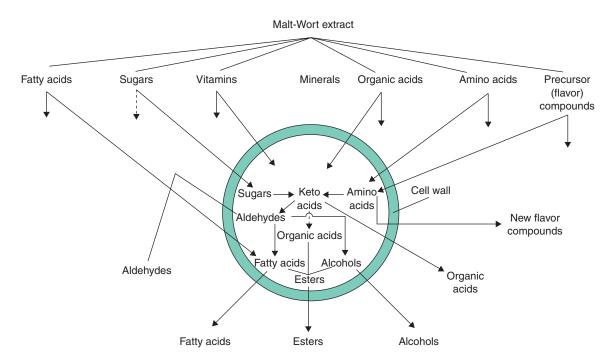


Figure 4 Fermentation: production of low percentage of spirit (6–8%) from plant sugars and nutrients prior to distillation.

such as cognac and armagnac are produced from fermentation volumes  $(\sim 1500 - 30001)$ whereas rum and tequila are derived from larger volumes in the order of 10 000-12 000 l. In malt distilleries, fermentation vessels can range in size from 4000 to 20 000 l. In grain distilleries, these vessels can be as large as 250 000 l. Yeast is usually added to initiate and advance fermentation in gin, vodka, rum, and whisky production. Residual postfermentation yeasts may be used to start new fermentations of spirits such as Cachaça, tequila, and some brandies. However, in cognac, wine is allowed to self-ferment for 3-5 weeks. For "artesanal" Cachaça, naturally trapped yeast is added initially to effect fermentation. In the production of heavy Jamaican rum, natural inoculation is encouraged but can be supplemented by yeast additions. In Scotch whisky production, fresh yeast is added to new fermentations.

Fermentation temperatures vary. Unlike beer fermentation, which usually takes place below 15°C, distillery fermentations range from 20°C to 40°C. Since there is no temperature control, whisky/whiskey fermentations can start at 20°C and rise to 30°C over a short 48 h period of fermentation. Most distillery fermentations are usually completed by 48 h, compared with beer fermentations which can extend to 5 days. However, in tequila and American whiskey production, slow fermentations may exceed 72 h. The high fermentation temperature and the high yeastpitching rate are mainly responsible for the fast fermentation rates of most of these spirits. Pitching rates for yeast (Saccharomyces cerevisiae) vary and can range from  $\sim 10$  to 20 million cells per ml. Distillers use either residual postfermentation yeast, companycultured yeast, or purchased yeast to effect fermentation. Yeast under cold storage tends to be more viable that yeast stored at warm temperatures. Although, great efforts are made to control bacterial infection during fermentation, the by-products of the metabolism of lactic acid bacteria and Clostridium saccharobutyricum in heavy rum production, can be essential congeners of the expected flavor profile of fermented worts and mashes. In general, during fermentation, sugars such as sucrose, glucose, fructose, maltose, maltotriose, and maltotetrose are fermented to ethanol, and, nutrients (e.g., zinc and amino acids, vitamins, fatty acids), and some malt flavor compounds are used by the yeast to grow and form a wide range of flavor compounds characteristic of the spirit to be produced. These flavor compounds include simple alcohols and higher alcohols (fusel oils), esters, organic acids, aldehydes, ketones, sulfur compounds, and aromatic flavor compounds such as acetals which tend to develop in acid conditions and high ethanol medium.

At the end of the fermentation process, the chemical composition of mashes and worts could be similar. However, intraspirit and interspirit differences become distinct after the distillation process has isolated, separated, and combined the congeners that define particular spirit products. Fermented worts, derived from different raw materials, will produce different spirit products. However, within a product type, differences will be caused by differences in still sizes, shapes, and modes of operation. In this regard, distillation is as important as raw material in defining spirit type and spirit quality. Here, quality is defined as meeting the expectations of the customer.

## **Distillation**

# **Distilling Using Copper Pot Stills**

The fermented worts or mashes of many distilled spirits contain  $\sim 6-8\%$  ethanol. The purpose of distillation is to vaporize the compounds present in worts and mashes and effect their separation and concentration. Still size can vary from 5000 to 25 0001 in capacity. Volatile compounds are derived from the contents of the still. New volatile compounds are also formed during distillation. If specific cyanidecontaining precursors are present, unacceptable compounds such as ethyl carbamate are produced. Cereal spirits should have an upper limit of  $150 \,\mu g \, l^{-1}$  of ethyl carbomate; fruit spirits should have no more than  $500 \,\mu g \, l^{-1}$ . Most stills are heated by heating coils. Cognac stills are fired directly. In the production of Scotch malt whisky, double distillation is involved but triple distillation occurs on a limited scale. The situation is similar for rums. For Irish whiskies, triple distillation is standard. Single distillation is also used to produce products such as Cachaça and armagnac. Double distillation usually takes place in wash and spirit stills. The distillate of the wash still (the low wine) is very high in congeners and ethanol is concentrated from 6-8% to  $\sim 23\%$ . In the spirit still, the low wine is fractionated into "foreshots" (heads), "middle cut" (spirit), and "feints" (tails). Single distillation pot stills operate in a similar manner to spirit stills, separating the ferment into three factions. After maturation in wooden (e.g., oak) casks, the "middle cut" (spirits) will be transformed into the distilled product (Table 2). In the triple distillation system, as used for Irish whiskey, the late portion of the distillate of the wash still is removed and redistilled in a low-wine still. The early portion of the wash still distillate and the early portion of the low-wine still are added to the spirit still for fractionation in "heads," "middle cut," and "tails." As is the case for spirits such as Scotch whiskies and brandy, the congener

levels of traditional Cachaça are higher than those of rum or bourbon. However, this may not be the case for all "industrial" Cachaça, produced in continuous stills.

The overlap of "foreshots" into the "middle cut" (i.e., the spirit) and the overlap of the "middle cut" into the "feints" can change the expected flavors of the spirit. High levels of "foreshot" compounds in the spirit can be detected by making a 50/50 mixture of spirit and water. If cloudiness develops, the "foreshot" content of the spirit is usually too high and adjustments to the "spirit cut" time should be made. "Foreshots" contain very volatile compounds which can confer "solvent-like" character to the spirit. In contrast, "feints" contain low volatile compounds that give the spirit a distinct stale, metallic odor.

During pot still distillation, ethanol concentration rises during "foreshot" collection but declines during the "middle cut" collection. Depending on still type, the total "middle cut" spirit fraction can contain 50-70% alcohol. Extended collection of the spirit fraction will lower its alcohol content and increase the possibility of having "feints" in the spirit. As regards the volatility of congeners: acetaldehyde, diacetyl, methanol, and some sulfur compounds are congeners of high volatility; esters of various kinds are congeners with medium volatility; fusel oils (i.e., higher alcohols) such as propanol, isobutanol, furfural, and isoamylalcohol have lower volatility than many esters but are usually more volatile than fatty acid congeners such as propionic, isobutyric, isovaleric, hexanoic, and octanoic acids. Despite differences in volatility, all these congeners can be found in the spirit, at levels that characterize the spirit type. The duration of collection of fractions containing these compounds is crucial as regards the distinctiveness and quality expectations of the spirit product.

Still size, shape, and distillation volume also affect the composition and levels of congeners present in the "middle cut" (i.e., spirit). The insertion of a water-cooled "purifier" between the neck of the still and the condenser also acts to reduce congener levels and lightens the spirit. The single distillation of Cachaça into "foreshots," "middle cut," and "feints" in small pot stills with cooling worms makes the middle cut spirit of Cachaça a distinctly different product from rum which is double distilled or triple distilled. Rums tend to have lower levels of flavor congeners. In general, in many spirits the fusel oils are in high concentration but do not dominate the overall aroma and taste because they are counter-balanced by esters and aldehydes.

Esters confer fruity and floral aromas to spirits and although esters are lost in the "foreshots," the levels

retained in the "middle cut" (the spirit) are vitally important. In this regard, butanol, propanol, and aldehydes play important parts in giving distinctiveness to different spirits but when these congeners are balanced with esters and other flavor compounds, a more complex spirit is achieved. Phenol, cresol, and guaicaol compounds are derived from peated malts. These substances are distilled into the spirit and confer medicinal, smokey, and phenolic aromas and taste to Scotch malt whisky. The intensities of these compounds are balanced by congeners such as esters.

Methanol is regarded as an undesirable compound in spirits but is produced in high quantities in noncereal mashes of plant tissue (e.g., potato, agave and fruits, Table 2). This highly volatile compound is dangerous to health and is absent from most distilled spirits but is present in "safe levels" in Eau de vie. It is a very volatile compound and should be removed in "foreshots" but fruit mashes contain high levels of pectin from which significant quantities of methanol are derived during mashing (extraction). Preheating of fruits to destroy the enzymes that release methanol or the fermentation of fruit juices instead of mashed fruits significantly reduces the levels of methanol produced.

Properties of copper in distilling The patina, which forms on the internal surface of pot stills, can function to reduce the levels of off-flavor sulfur compounds i.e., sulfides (e.g., dimethyl sulfide and mercaptans) from the spirit. Although copper can be detected in some spirits, the levels are within safe food levels.

#### **Distillation Using Continuous Stills**

Continuous stills are large vertical constructions. They contain internal sheets of copper which perform the same function as the copper in pot stills. In the Coffey stills there are two columns, the analyzer and rectifier. The 6-8% ethanol ferment or mash is heated as it passes down the rectifier. This heated mass then passes to the analyzer where steam is blown through it. Ethanol and flavor congeners are driven off. These pass back into the bottom of the rectifier and are collected at their designated heights from the lower plates to the higher plates of the rectifying column. For example, plates may ascend from 1 to 39: plate 7 is rich in isoamyl alcohol (fusel oils), plate 14 is rich in isobutyl alcohol, plate 23 is rich in npropyl alcohol, plate 32 is rich in ethanol at  $\sim$ 94%. Ethanol concentration increases from plate 1 to plate 32. Volatiles such as methanol, sulfur compounds, and acetaldehyde are found in plates 35-39. An alternative type of continuous still is the

column still. This comprises of a beer stripper where the 6-8% ethanol ferment is heated using stem, as in the analyzer. The vapors pass to the bottom of the concentrator which, like the rectifying column, fractionates the vaporized compounds at appropriate plates, for example: plate 5 (fusel oils, e.g., isoamylalcohol), plate 8 (propanol), plate 35 (ethanol, 95%), and the topmost plate 40 (methanol and volatile sulfur compounds). In all stills, condensers are connected to condense vapors that leave the still.

American whiskies are produced in continuous stills. They cannot be distilled above 80% ethanol and therefore contain more congeners than Scotch grain whisky at 94% ethanol and vodka and gin spirit at 96% ethanol. Canadian whiskies are produced from maize, rye, malted barley, and malted rye. Spirit fractions are collected between 65% and 95% ethanol and are used to produce different distilled products. Vodka and gin spirit (94% alcohol) may be passed through an extractive (water/steam) column still, which removes traces of volatiles such as methanol and diacetyl. The spirit is then concentrated in a rectifier where fusel oil is drawn off lower down the rectifier. The spirit is now at least 96% ethanol and is passed finally through a demethylizer to remove the last traces of methanol. In some plants, neutral spirit for vodka production is passed through charcoal to assist the purification process. During mashing, glycerol can be converted by lactic acid bacteria to acrolein which escapes during distillation causing an irritating pepper odor in the distillery.

#### Maturation

As indicated in Figure 5, distilled spirits are usually matured in wooden barrels for minimum periods of time. Some spirits are not matured such as vodka and gin, whereas, by law, Scotch whisky spirit and Irish and Canadian whiskey spirits are matured in oak casks for a minimum period of 3 years. American whiskies are matured for a minimum time of 2 years. The maturation periods for rums are variable – from, not at all (e.g., some light rums), to weeks or years, for heavy rums. Cachaça of the highest quality is matured in wooden barrels, especially oak and for at least 2 years. Brandies (e.g., cognac) are matured in oak barrels and, like Scotch whisky, can be matured for over 20 years. Maturation can take place in new, charred, wooden barrels (e.g., cognac or American bourbon whiskies) or in oak barrels previously used for sherry or bourbon. Port, madeira, and a variety of sherry barrels are now being used by some distillers to "finish" the maturation periods of their whiskies for wider brand appeal. Legally, tequila must be matured in oak casks for 2 months (rested tequila) or 1 year (aged tequila).

Different oak species are used for barrel construction, e.g., *Quercus ruber* (English), *Quercus petraea* (Spanish or French), or *Quercus alba* (USA). Barrel staves are sawn so that the normal tangential loss of water from the tree stem is avoided. Different spirits are filled into barrels at different strengths of ethanol. Spirit strengths of ~63.5% ethanol are most

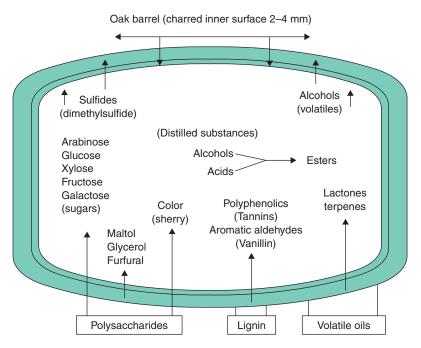


Figure 5 Maturation of distilled spirit ("middle cut") in oak barrels.

extractive. Temperature conditions influence maturation rate and maturation results. High temperature accelerates ethanol loss from barrels. For Scotch whisky, which can only be matured in oak casks, a loss of  $\sim 2\%$  ("the angels' share") of the contents of the barrel occurs each year. In such maturation conditions, from an initial fill of 63.5% ethanol,  $\sim$ 58% ethanol will remain after a maturation period of 12 years. During maturation, unpleasant compounds are extracted by the barrel; pleasant compounds are released into the spirit and various important flavor compounds such as esters are formed in the spirit (Figure 5). The active charred inner surface of the barrel contains carbon which absorbs the unpleasant flavors of dimethylsulfide, dimethyldisulfide, methional, and methional acetate from the whisky, but not dimethyltrisulfide which, even at low concentrations of 4 ppb can be detected organoleptically (i.e., by taste or smell). From the scorched subsurface, important wood complexes are released into the spirit, adjusting its flavor, aroma, and increasing its color. Hydrolyzable tannins released into the spirit promote oxidative reactions such as ester formation between alcohols and acids.

Lignin is the strengthening material of wood and is a complex polymer which, when scorched by charring, releases aromatic aldehydes into the spirits such as vanillin and coniferaldehyde. Vanillins confer vanilla-like aromas to the spirit. Vanillin levels are very high in new barrels after charring and are characteristic flavors of bourbons and cognac. Other spirits that are matured in ex-bourbon casks also have detectable levels of vanilla-like aroma and taste. Low-level sweetness is conferred by the release of simple compounds such as glycerol and monosaccharide sugars such as xylose, arabinose, and glucose. Dry toasted malting flavors come from the release of furfural and maltol. American oak barrels release more lactones into the spirit than Spanish oak barrels. Cis-lactones confer desirable woody aromas and sweet, smooth, coconut flavors. Components of tannins such as gallic acid and ellagic acid can contribute astringency and can be oxidized to give color and fragrant compounds. The solid matter content of whisky increases by  $\sim 2.5 \,\mathrm{g}\,\mathrm{l}^{-1}$  over a period of 12 years. Some of this material is lipid material (fatty acid esters) that will form hazes when water is added to matured spirit products, if such products are not cold filtered. Cask strength Scotch whiskies which are not filtered tend to form hazes when water is added. Metal ions can also form flocs, which are removed by filtration of the product. Pieces of charcoal that are dislodged from the charred surface of the barrel are also removed during filtration. The sherry flavors and vanilla flavors of distilled products are derived from the barrels in which they are matured. Sherry barrels also contribute color to maturing spirits.

Repeated use of a barrel will deplete its reservoir of color-producing materials and flavor compounds, and its ability to remove undesirable compounds such as sulfides. Such barrels are rejuvinated by scraping away the inner carbon surface and recharring the newly exposed wood. A barrel may last as long as forty years. However, its effectiveness declines over these years. Maturation moderates the volatility and increases the smoothness of the spirit. This is particularly important in high congener spirits.

# **Blending and Bottling**

Before blending or vatting, the spirit is checked before the casks are emptied and collected in tanks. In general, whiskies, Cachaça, rums, brandies, or tequila are vatted and left to "marry" before being reduced with water of low mineral content to bottling strength. The ethanol levels of vodka and gin are also reduced using low mineral water. Judicious vatting (mixing) of cognac of different ethanol concentrations is effected to arrive at the bottling strength required. The mixing of generic spirits can be referred to as vatting. However, blending is used to describe the mixing of different kinds of spirits (e.g., malt and grain whiskies). In this regard, malt whiskies can be described as "pure" single malts (malt whisky from one distillery), "pure" malts (mixture (vatting) of different malt whiskies), or "blends" of malt and grain whiskies. Here, pure is taken to mean of one kind. The mixing of Cachaça products from different production operations is equivalent to the vatting of single malts because the Cachaça products used are different in character but are produced traditionally and are therefore of one kind. In contrast, in America, blended whiskies will contain grain whiskey, neutral spirit or light whiskey and a complement of heavy whiskey or whiskies such as bourbon or maize whiskey. These heavy whiskies are called "straights" if matured for more than 2 years. As in Ireland, the mash bill and still type determine the types of whiskies that are blended: such as products of high congener levels (malt or high malt whiskies) and products of low congener (high grain whiskies) levels. In Canada, light whiskies, made from rye and malted barley and malted rye, in continuous stills are blended with high flavor corn whiskies (65–80% ethanol), made from  $\sim$ 60% corn (maize), rye, and malted barley in either pot stills or continuous stills. Some Canadian are blended with wines, sherries, brandies, rums, bourbons, and malt whiskies to create different flavors and aromas. Lighter whiskies may be used in

larger quantities than heavier spirits; they may not only add lightness but also add flavor notes of freshness, which are crucial to the "drinkability" of blended whiskies.

Since blended whiskies, e.g., Scotch blends, can contain as many as 30-40 malt whiskies and  $\sim 6-$ 8 grain whiskies, the overall complexity is greater than any comparable single malt. Blending extends product range and provides the possibility of arriving at new products which the public may prefer. This can take a long time; but the opportunity of developing new products should not be ignored. The levels of important congener compounds (e.g., esters) may not be reduced by blending because very highly flavored whiskies in the blend can compensate for reduction of esters by dilution with grain whiskies which have low congener levels. In this regard, some blended Scotch whiskies can have similar ester contents to malt whiskies, even though a malt whisky may have 4-5 times as many esters as a grain whisky. Blending is the high art of distilled beverage production, providing the customer with consistency and complexity without losing the high traditional qualities of a wide range of individual products which are blended together to extend drinkability.

# Conclusion

Reference has not been made to the by-products of the distillation process. In some plants (e.g., Scotch grain whisky) carbon dioxide is collected and sold, spent grains from the mashing process and spent materials from the distillation process (stillage) are dried and sold as animal feed. Wastewater (backset) is kept hot (e.g.,  $65-95^{\circ}$ C) and reused as part of the mashing-in liquor or part of the cooking liquor. This liquor contains lactic acid. It is acidic, nutritious, and is a contributing factor to the term "sour mash" in American whiskey production. In addition, fusel oils are collected during the production of grain whisky and sold to the perfume industry. Waste sugar cane skins and fiber (bagasse) are used as fuel. These practices show that the distilling process is "environment friendly" because most of the products of the process are recovered and sold. Copper in the stillage can be problematic in animal feed but this is primarily a problem for single gut animals. The use of caramel of the highest quality is permitted in the industry to adjust the color of spirit products to expected levels. In most cases, very small levels are used compared with the very high levels used in, for example, chocolates, cola, and gravy mixes. In general, distilled beverages are ethanol-based products which are made with sound science and high art and, from the levels of their sales worldwide, are not only pleasant to drink, they also play an important part in defining the culture from which they originate. Those who produce them are proud of their distinctiveness and quality. An example of such pride can be found in Robert Burns' poem, "John Barleycorn," which accurately describes the production of Scotch whisky and glorifies its virtues — virtues Burns described collectively as a "cup of kindness" in the universal New Year's song, "Auld Lang Syne." He believed passionately that whisky and "freedom" went together. Maybe the same beliefs could be extended to other spirit beverages that reflect historical custom and practice (culture).

In order to protect standards of production and cultural heritage, the essential features of production of distilled spirit beverages should be enshrined in the law, as is the case for many of the distilled beverages described in Tables 1 and 2. In this regard, original place of production, raw material used, production process employed and overall aroma and taste should be taken into consideration. The spirit industry has always encouraged moderate drinking because it is fully aware that excessive alcohol consumption can cause health and social problems. The value of moderate consumption of alcohol, as regards improved cardiovascular function, is still being researched but the results so far are encouraging. Although, the production of distilled spirit beverages sustains many jobs and creates high tax returns to various governments, their contribution to the culture of celebration is probably their most important merit in society.

See also: Beverages: Asian Alcoholic Beverages. Fermentation: Foods and Nonalcoholic Beverages.

# **Further Reading**

(1981) International Guide to Drinks. Hutchinson.

Craig HC (1994) *The Scotch Whisky Industry Record*. Scotland: Index Publishing Ltd.

Inge R (2003) Whisky: Technology, Production, Marketing. Handbook of Alcoholic Beverages Series. London: Academic Press.

Jacques KA, Lyons TP, and Kelsall DR (1999) *The Alcohol Textbook*, 3rd edn. Nottingham: Nottingham University Press.

Lee MK-Y, Paterson A, and Piggot JR (2001) Origin of flavours in whiskies and a revised flavour wheel: a review. *J. Inst. Brewing* 107: 287–313.

Nykanen L and Lehtonen P (1984) Flavour Research of Alcoholic Beverages, vol. 3. Foundation for Biotechnical and Industrial Research: Helsinki, Finland.

Palmer GH (1989) Cereal Science and Technology. Scotland: Aberdeen University Press.

Palmer GH (1997) Scientific review of scotch malt whisky. *Ferment* 10: 367–397.

#### **Relevant Websites**

No specific websites are listed. Most companies producing distilled beverages include some technical

information on their product. In particular, interesting images of production processes can often be found.

# **BREADS**

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# Introduction

Bread, in one form or another, has served as mankind's Staff of Life for centuries. Its ability to provide sustenance to the human body is remarkable, as evidenced by analysis of its dietary components. While many people around the globe think of bread as simply a staple of the daily diet, it is much more than that to many others. To the wheat breeders, farmers, millers, and bakers of the world, bread is a means of providing for themselves and their families, a product to be proud of, and practically a way of life.

As would be expected of a product that has been in existence for so long, bread has evolved over the years such that it means different things to different people. Indeed, the geographical and cultural differences in the product known as "bread" are quite broad, as evidenced by the tremendous array of bread varieties available today in just the North American market. Pan breads, hearth breads, buns and rolls, and flat breads are increasingly available around the world, even if they are not indigenous to the consumer's homeland. Simply put, the differences between breads are largely a function of the ingredients used, and the manner in which these ingredients are processed into the final product.

# **Ingredients**

Various ingredients are used in bread formulas to manipulate the characteristics of the finished product. As man has learned over time, only two ingredients are absolutely necessary to make a palatable loaf of bread, those being wheat flour and water. As time went by and man learned more about his surroundings, he discovered that salt and yeast added flavor and lightness to his bread. Today, additional ingredients

are used in bread to meet the demands of the modern-day consumer, namely, better keeping qualities, better flavor, softer (or firmer) texture, and other physical and sensory improvements. As mentioned previously, geographical and cultural differences exist between breads, meaning that ingredients that are commonly used in North American, British, and Australian pan breads may not be used in German rye bread, French baguettes, or Arabic bread, for example. See Table 1 for common ingredients and their functionality.

# **Equipment**

As detailed in **Bakeries**, the modern commercial bakery houses many pieces of specialized equipment. The goal of using modern automated equipment is to carry out the basic steps of the dough production process in bulk quantities, and to do it profitably. Equipment design, construction, and usage is truly global, with bakers all over the world buying and using equipment made in foreign countries. See **Table 2** for an overview on equipment used for modern bread production.

# **Dough-Making Processes**

Whether bread is made by hand or by machine, certain steps of the bread-making process are the same. Bread owes its uniqueness not only to the ingredients and equipment used in its manufacture, but also to the different processes by which dough is made. Most of these processes are of geographical or cultural origin, and are still practiced in the areas of their founding today. However, with globalization of trade and world travel, consumers now demand products that were not familiar to them in the past. It is not difficult to find authentic French bread in America, flour tortillas in Australia, or pita bread in Hong Kong. However, achieving the unique characteristics of different breads often requires that a specific dough-making process be used.

Table 1 Functions of bread ingredients

Ingredient	Function(s)
Wheat flour	Forms structure and provides body Contributes gluten-forming proteins Contributes starch for heat-induced formation of crumb
Water	Hydrates flour protein and damaged starch Acts as solvent for other ingredients Controls dough temperature Contributes pan flow Contributes to shelf life
Salt	Enhances flavor of product Strengthens gluten network Controls fermentation rate
Yeast	Provides leavening Metabolizes fermentable sugars into CO <sub>2</sub> and alcohol Fermentation products contribute to bread flavor
Sweeteners	Serve as food source for yeast Enhance flavor of product Contribute to crust coloration Act as product tenderizer Contribute to shelf life
Shortening	Lubricates dough system Facilitates easier expansion of gas cells in dough Tenderizes crust Facilitates easier slicing Contributes to shelf life
Dairy products	Contribute to nutrition profile of product Provide dough buffering Enhance flavor of product Contribute to crust coloration
Wheat gluten	Strengthens dough Increases water absorption Provides greater baked loaf volume
Yeast food	Enhances fermentation process Provides yeast nutrients Acts as water conditioner Conditions dough (with optional oxidant)
Enzymes	Extend shelf life (amylase) Convert starches into fermentable sugars (amylase) Shorten dough mixing time (protease) Strengthen dough (glucose oxidase, xylanase) Provide greater baked loaf volume (fungal amylase, xylanase)
Preservatives	Retard mold growth Contribute to shelf life

#### **Straight Dough Process**

As noted in Figure 1, this is an uncomplicated process whereby all the ingredients are loaded "straight" into the mixer. The dough is mixed, then allowed a bulk fermentation period of 60 min to 4 h, during which the dough is "punched down" to control expansion.

After the fermentation period, the dough proceeds to "make up" a bakery term for the process of cutting and forming smaller dough pieces.

# **No-Time Straight Dough Process**

This process is a variation of the straight dough method. It does away with the bulk fermentation period, substituting in its place chemical dough development, or the use of oxidizing agents and mix time reducers. A slightly modified version of this process is used for the manufacture of frozen dough products, which are used extensively by supermarket bakeries in North America, Europe, and elsewhere.

# **Sponge-Dough Process**

This process is dominant in North America for the production of pan breads, and also finds use in other countries where pan breads are popular. The sponge is a thick stiff mass, "plastic" in texture, which contains most of the formula flour, water, and yeast. After mixing, the sponge is allowed to ferment for 3-4 h , and is then remixed with the remaining dough ingredients. A short rest period, or "floor time," is given to the finished dough before it proceeds to the makeup stage, as shown in Figure 2. Breads made in other parts of the world use a similar process, but the names are different. For example, Italian breads such as ciabatta and pugliese can be made with a biga, which is very similar to a North American sponge except for the longer fermentation time it is given. French breads, including baguettes and batards, can be made with poolish, a liquid version of the sponge that actually originated in Poland, or with levain, a slightly modified version of sourdough.

# **Liquid Sponge Process**

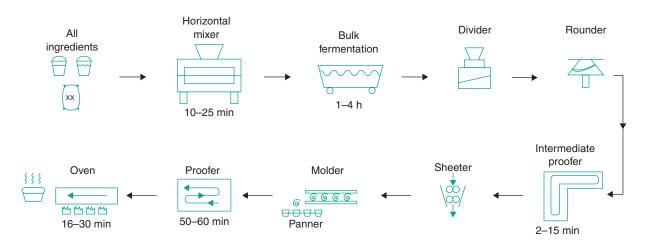
This is a close relative of the sponge-dough process, but as the name implies, it is liquid in nature, containing at least as much water as flour. This enables the fermentation process to take place in stainless steel tanks, as shown in Figure 3, with the chilled liquid sponge pumped to the dough mixer, a great production advantage. The fermentation time is shorter for liquid sponges than for plastic sponges, usually on the order of 60 min to 3 h.

# **Continuous Mix Process**

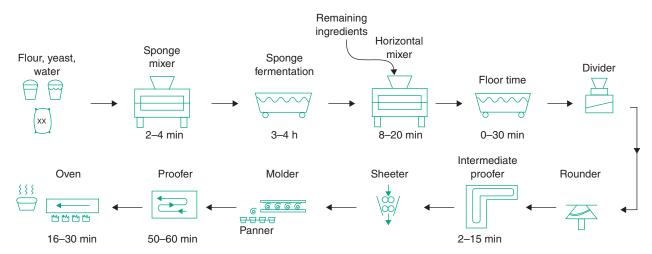
This process enjoyed great popularity in North American commercial bakeries in the 1960s and 1970s, particularly for sliced white pan bread. As the name implies, this process produced bread dough continuously, rather than in individual batches. Ingredients were metered into a high-speed developer head, which mixed the dough and

Table 2 Common wholesale bread bakery equipment

Equipment	Purpose	Comments
Dough mixer	Imparts mechanical shear to flour proteins and water to form gluten and develop dough mass	Capacity of up to 1400 kg. Can be horizontal or vertical.  Mixer bowl can be refrigerated
Divider	"Divides" large dough mass into loaf-sized pieces	The bakery's "cash register"
Rounder	Forms divided dough piece into smooth, tight ball	Skin on exterior of dough ball helps to retain gas
Intermediate proofer	Relaxes rounded dough balls	Dough balls ride on trays pulled by moving chain
Sheeter moulder	Expels gas from and flattens dough balls with sheeting rollers, curls dough sheet into cylinder, and seals seam	Straight grain, cross grain, and curling molders all commonly used
Panner	Drops molded dough pieces into pans on conveyor below	Can index dough pieces into pans with seams on bottom
Proofer	Allows panned dough to rise to proper height prior to baking	Employs controlled temperature and humidity
Oven	Bakes dough, kills yeast, gelatinizes starch, and colors the crust	Tunnel, lap, rotary, deck, rack Steam required for hearth bread
Cooler	Cools baked loaves prior to slicing	Traveling tray, belt, spiral, rack
Slicer	Slices loaves prior to packaging	Band, reciprocating
Bagger	Pulls poly bag over sliced loaf	Bags closed with tie or clip

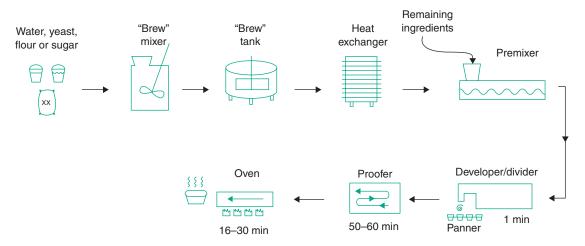


**Figure 1** The straight dough process. (Adapted from *AIB Science of Baking Correspondence Course, module III Breads and Rolls* (2001) Dough Mixing and Development, pp. 6–8. Manhattan, KS: American Institute of Baking.)



**Figure 2** The sponge-dough process. (Adapted from AIB Science of Baking Correspondence Course, module III Breads and Rolls (2001) Dough Mixing and Development, pp. 3–5, 7. Manhattan, KS: American Institute of Baking.)

**Figure 3** The liquid sponge process. (Adapted from *AIB Science of Baking Correspondence Course, module III Breads and Rolls* (2001) Dough Mixing and Development, pp. 5–7. Manhattan, KS: American Institute of Baking.)



**Figure 4** The continuous mix process. (Adapted from *AIB Science of Baking Correspondence Course, module III Breads and Rolls* (2001) Dough Mixing and Development, pp. 6–7. Manhattan, KS: American Institute of Baking.)

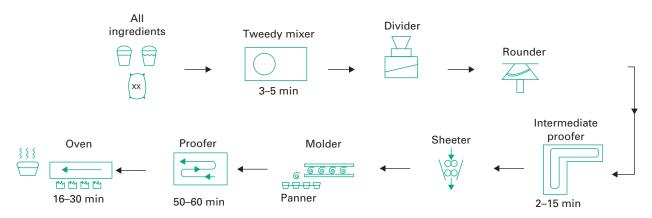
deposited it into baking pans. This process, while economical, eventually fell out of favor due to the lack of flavor and crumb strength in the bread. See Figure 4 for a schematic representation.

#### **Chorleywood Bread Process**

Developed by the British Baking Industries Research Association at Chorleywood, England, this process is used around the world to produce bread from lower protein flours. For this reason, it has not gained great acceptance in North America, where higher protein flours are the norm. It relies on a short time, intensive mixing, sometimes with partial vacuum in a Tweedy mixer, after which the developed dough is sent to the makeup stage, as depicted in Figure 5.

# Sourdough Process

This is the oldest and most time consuming of the methods discussed, and is used to produce breads with rich and complex flavor and texture. More European in nature, sourdough breads are leavened with a sour "starter," which consists only of flour and water. The starter is allowed to ferment spontaneously, i.e., with only the naturally occurring microorganisms present in the flour (mainly wild yeast and lactic acid-producing bacteria). There is no added



**Figure 5** The Chorleywood process. (Adapted from *AIB Science of Baking Correspondence Course, module III Breads and Rolls* (2001) Dough Mixing and Development, pp. 7, 9. Manhattan, KS: American Institute of Baking.)

bakers' yeast. To keep the starter active for daily use, it must be fed, or replenished, periodically with added flour and water. Given proper feeding, a starter can last many years.

# **Bread Products**

As previously discussed, bread can take many shapes and sizes, and can have extremely diverse sensory characteristics. In addition to the ingredients, equipment, and processes just mentioned, the various physical and organoleptic differences between bread products available today are attributable to cultural, geographical, and philosophical differences between the people who consume them. Generally, certain bread types are prevalent in certain cultures, and not in others. This is changing, however, with the world gradually becoming smaller with the advent of jet travel, wireless communication, and international trade.

#### **Pan Breads**

Loosely defined, these are simply breads that are baked in pans, either open or closed. These breads are very common in North America, Eastern and Western Europe, Australasia, and other industrialized areas of the world. Most of the pan bread baked on these continents is produced in large wholesale baking plants, using automated or semi-automated equipment. It is almost always sold presliced in polyethylene bread bags, and can be found in supermarkets, convenience stores, and other retail outlets.

In many of the aforementioned regions, pan breads are available as either "round-top" or "Pullman" loaves. Round-top bread is baked in a pan with an open top, so that the baked loaf has the characteristic round crown when viewed in profile (Figure 6). Pullman bread, commonly called "sandwich"



**Figure 6** A round-top "strap" pan, background; sliced white bread, left; unsliced white bread, center; sliced cracked wheat bread, right.

bread, is baked in a rectangular pan, usually longer in length than a round-top pan, and has a lid placed over it after proofing and before baking (Figure 7). The lid prevents expansion of the dough in the oven, and results in a baked loaf having a nearly square profile. This bread is usually sliced slightly thinner than round-top bread for the North American market, the thought being most sandwich consumers want more of the filling per bite, and less of the bread.

Both round-top and Pullman loaves are available in several different varieties in most global markets. The most common include white bread, made with white refined wheat flour (Table 3), and wheat or "whole grain" bread, made with whole grain or whole meal wheat flour. In the USA, "whole-wheat" bread may be made only with 100% whole-wheat flour, while the more generic "wheat" bread may be made with a blend of white and whole-wheat flours (Table 4). These types of pan breads often need to be supplemented with dough strengtheners, as the inclusion



**Figure 7** A Pullman "strap" pan and lid, background; sliced white sandwich bread, left; sliced honey bran sandwich bread, center; sliced multigrain sandwich bread, right.

Table 3 Sponge and dough white pan bread

Ingredient	Sponge (%)	Dough (%)
Bread flour <sup>a</sup>	70.0	30.0
Water	40.0	22.0 (variable)
Yeast, compressed	3.0	, ,
Salt		2.0
Sweetener (solids basis)		8.0
Shortening		3.0
Yeast food	0.5	
Milk replacer		2.0
Fungal protease	0.5	
Mono- and diglycerides		0.5
Calcium propionate		0.2

 $<sup>^{</sup>a}$  All ingredients are given in bakers % (flour = 100%).

Table 4 Sponge and dough wheat pan bread

Ingredient	Sponge (%)	Dough (%)
Bread flour <sup>a</sup>	60.0	
Whole wheat flour	15.0	25.0
Water (variable)	42.0	18.0
Yeast, compressed	2.5	
Yeast food	0.5	
Salt		2.0
Shortening		3.0
Molasses		4.0
Sweetener (solids basis)		4.0
Vital wheat gluten	2.0	
Calcium propionate		0.2

<sup>&</sup>lt;sup>a</sup> Wheat flour + whole wheat flour = 100%.

of significant amounts of whole-wheat flour weakens the gluten network and results in bread of lower baked volume. Other types of pan breads fall under the "variety" bread category — including multigrain bread, made with several cereal grains in whole-wheat dough; fiber bread made with varying types of highfiber ingredients; and the ever-popular raisin bread (Figure 8). To respond to the consumer's desire for greater selection and to gain market share, bakers have developed pan breads that contain a wide array of specialty ingredients, including oats, crushed wheat, flaxseed, sunflower seeds, cinnamon, fruit, honey, and nuts. Additionally, toppings can be added to the surface of the proofed loaves prior to baking. Some of these include wheat bran, wheat flour, crushed wheat, sesame seed, flaxseed, sunflower seed, and other differentiating ingredients. A common practice for North American round-top breads is to split the surface of the proofed dough lengthwise with a water jet, and apply melted butter in the cavity, thus creating the "buttertop" variety (Figure 8).

Pan breads can be formulated to be lean, rich, or somewhere in between, depending on the consumer's taste. Most white breads tend to be soft and somewhat sweet, containing high amounts of water, shortening, and crumb softeners, along with higher sweetener levels. Wheat breads tend to be slightly dry, chewy, and not as sweet. Common in many markets around the globe is the practice of producing similar bread varieties at different price points, or perceived quality levels, for supermarket sale. Terms such as "value priced," "private label," "branded," "premium," and "superpremium" illustrate the practice. The differences are usually higher-quality ingredients, a richer formula, possibly the use of a pan with a slightly different shape, and more elaborate packaging graphics.

#### **Hearth Breads**

Historically, hearth breads, also referred to as artisan breads or rustic breads, have been made from relatively stiff and dry doughs so that they could be baked directly on the hearth, or floor, of the oven, without the benefit of a pan to impart shape or structure to the loaves. Today, the best-quality hearth breads are still made this way, especially by European bakers. While French and Italian bakers have traditionally lead their overseas counterparts in hearth bread quality, excellent product can increasingly be found in all corners of the world. In North America, this type of baking has enjoyed a true renaissance, with hundreds of small and medium-sized bakeries turning out French baguettes, batards, and boules, Vienna bread, and crusty hard rolls. Bakeries in Japan, Korea, Hong Kong, and Thailand offer their customers similar product, as do those in Ireland, Israel, and Argentina.

The best known of the hearth breads is generically referred to as French bread. However, the term does

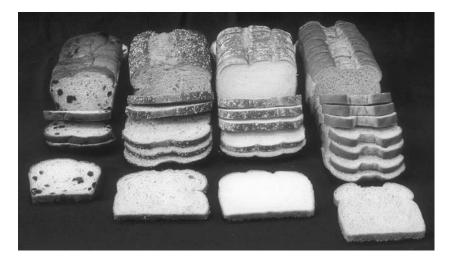


Figure 8 Left to right: raisin bread; split and topped seven-grain bread; double-split and topped fiber bread; buttertop wheat bread.

not do justice to the broad array of bread products produced in France. Indeed, the French take their bread so seriously that the government found it necessary to legally define baguette, and to regulate the various methods of bread making that are employed there. In France, French bread is made with wheat flour, salt, and water, with fermentation carried out by levain, direct addition of fresh yeast, or the addition of prefermented dough. In North America, certain compromises have been made to adapt French bread to the consumer's palate and to the producer's balance sheet. For example, shortening and a small amount of sweetener are sometimes added, to make the bread softer and sweeter tasting, and to extend the shelf life beyond the usual few hours (Table 5). Interestingly, the inclusion of these two ingredients in French bread is not legal in France.

True French bread, whether it is made in France or elsewhere, is available in several shapes and sizes. Baguette is the term for a long, narrow loaf with rounded ends, measuring 60-70 cm in length and weighing 250 g. It has seven characteristic slashes applied to the top surface after the proofing step, which helps control bursting during baking. A batard is similar to a baguette in weight, but measures only 35-40 cm in length and carries only three slashes. Ficelle is smaller still, weighing 125 g with a length of 40– 45 cm. It usually has five surface slashes. Parisian is a large loaf weighing 400 g and measuring 60–70 cm in length. It has five to seven surface slashes. Boules are circular loaves, and can be of varying weights and diameters. Various surface designs are applied via slashing in order to give the boule a distinctive appearance (Figure 9). The crust of these breads should be relatively thin, crispy, and blister free, courtesy of the steam present in the oven chamber during baking.

Table 5 Sponge and dough white hearth bread and hard roll

. (0()
ıh (%)

<sup>&</sup>lt;sup>a</sup> All ingredients are given in bakers % (flour = 100%).



Figure 9 French-style stick loaves and boule.

Crust color can vary from pale brown to practically black, depending on the region and consumer taste.

Internal characteristics are just as unique as the external appearance. The crumb color is usually creamy to pale yellow, with a very open and irregularly shaped grain structure. There are normally several moderately sized holes in each slice, giving the

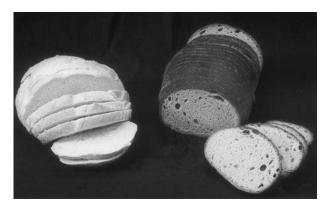


Figure 10 San Francisco sourdough boule, left: European sour rye bread, right.

crumb surface a rough texture. The crumb will be slightly chewy in the mouth.

Several different processes can be used to produce French bread, including the previously mentioned straight dough, sponge-dough, and sourdough processes.

In the USA, a close relative of French bread originated in the San Francisco, California region during the Gold Rush period of the late 1840s and early 1850s. Called San Francisco sourdough bread, it was a mainstay of the diets of gold miners. Today, it is still a fairly unique product in the American marketplace, in that it has a very characteristic sour flavor, open grain, and substantial crust (Figure 10). Many specialty sourdough bakeries have opened across the USA to capitalize on the growing consumer demand for this product. As the name indicates, the bread is made with a sour starter, also called a mother sponge (Table 6).

Another type of hearth bread is rye bread, which takes its name from the inclusion of flour from the cereal grain rye. Rye bread has been produced around the world for centuries, and has gained great popularity for its distinctive flavor and dense, chewy crumb. In northern and eastern Europe, rye bread is a highlight of the meal, rather than an accompaniment. The types of rye bread are many, including American rye, Jewish rye, German rye, Russian rye, and Swedish rye, to name a few (Figure 10). The differences are largely due to the ingredients and consumer taste indigenous to the producing region.

Rye flour differs greatly from wheat flour in that its protein does not form gluten. As such, it contributes nothing to the strength of the dough or the volume of the bread. Therefore, the amount of rye flour that can be used in rye bread dough is limited, with the majority being wheat flour. Rye flour is available as light rye, medium rye, and dark rye; rye meal, rye flakes, and cracked rye are also available. Generally, rye

Table 6 Sourdough bread

Dough starter		Dough		
Ingredient Amount (kg)		Ingredient	Amount (kg)	
High gluten flour Water (variable) Starter	45.4 22.7 11.4	High gluten flour Water (variable) Starter Salt Yeast, compressed <sup>a</sup>	136.0 81.6 25.0-34.0 2.7 0.17	

a Optional.

Table 7 Sponge and dough rye bread

Ingredient	American (light)	Swedish	Jewish
Sponge			
Flour: clear <sup>a</sup>	70.0	66.0	
Patent			40.0
Light rye			20.0
Water	34.0	36.0	36.0
Yeast, compressed	2.5	2.5	2.0
Yeast food	0.5	0.5	0.25
Diastatic malt	0.5	0.5	0.5
Dough			
Flour: clear	5.0		10.0
Light rye	25.0	24.0	30.0
Dark rye		10.0	
Water	30.0	28.0	32.0
Yeast			0.25
Salt	2.0	2.0	2.0
Shortening	3.0	4.0	1.0
Molasses	2.0		
Caraway seed	1.0		
Syrup		12.0	
Nonfat milk solids		4.0	

<sup>&</sup>lt;sup>a</sup> Wheat flour + rye flour(s) = 100%.

breads are differentiated based on the amount of wheat flour used and the type of rve flour that is chosen (Table 7). This creates rye breads having different crumb color, crumb density, and flavor. In some types, it is common practice to include ground or whole caraway seed in the formula to further accentuate the flavor of the bread.

Rye bread is generally made with the sponge-dough process. Traditional pumpernickel bread, a dark and heavy version of rye bread, uses a sour made of whole rye meal. German sour rye bread is also produced with a sour.

#### **Buns and Rolls**

These are simply small individual units of baked bread, normally eaten with a meal or as the bread component of a sandwich. In North America, buns are usually sweeter and richer in formulation (Table 8), while rolls tend to be made from leaner formulas (Table 5). Both products can be panned and baked either as single units or in clusters, depending on the intended users' desires. Buns and rolls can

Table 8 Sponge and dough hamburger and hot dog buns

Ingredient	Sponge (%)	Dough (%)
Bread flour <sup>a</sup>	70.0	30.0
Yeast, compressed	3.5	
Water	40.0	24.0
Sweetener (solids basis)		12.0
Salt		2.0
Vegetable oil		4.0
Protease enzyme	0.5	
Yeast food	0.5	
Vital wheat gluten	2.0	
Crumb softener		0.5
Calcium propionate		0.3

<sup>&</sup>lt;sup>a</sup> All ingredients are given in bakers % (flour = 100%).

be made from the same types of dough as used for bread loaves. White, wheat, rye, and sourdough buns and rolls are common. The most popular usage of buns is for the ubiquitous hamburger and hot dog, as their respective buns are shaped and sliced specifically to accommodate those specialty meat items (Figure 11). Hamburger and hot dog buns are most usually made in wholesale bakeries on automated high-speed equipment in large quantities. Kaiser rolls, made from a lean bread dough formula (Figure 11), are also popular in North America. The dough pieces are flattened slightly after forming, then have a characteristic "star" pattern stamped into the top surface. The proofed rolls are baked with steam in the baking chamber to form the light, slightly crispy crust that is expected of the product.

As opposed to the North American taste, European rolls are traditionally of the hard type, made with lean formula dough and baked with steam in the oven to facilitate the formation of a crispy crust (Figure 12). Hard rolls are also available in other areas of the



Figure 11 "Cluster" hamburger buns, left rear; "cluster" hot dog buns, right rear; assorted kaiser rolls, front.



Figure 12 Assorted hard rolls surrounded by croissants.

world, and are of good quality as long as two things are present in their manufacture – strong protein flour and oven steam.

Other types of rolls include croissants and brioche, both of European origin and very popular all over the world. The croissant (Figure 12) is made with laminated Danish pastry-type dough, i.e., the dough is actually comprised of many individual layers of dough and fat, usually unsalted butter. The dough is continually reduced in thickness through a series of sheeting steps, after each of which it is folded and sheeted again. This procedure is repeated until the proper number of laminations has been made. It is common for croissant dough to have anywhere from 36 to 72 dough/fat layers. These layers serve to trap moisture and carbon dioxide produced during fermentation. On exposure to oven heat, the moisture turns to steam which, in conjunction with the carbon dioxide gas, causes the croissant dough to "bloom" in the oven. This blooming action results in the formation of the light and flaky texture for which croissants are known.

Brioche is a light, sweet, yeasted roll that is made from a rich formula high in eggs, sugar, milk, and butter. As such, it finds favor as a dessert item, and is popular for holiday celebrations. It is usually made with the sponge-dough process, but can also be made with either the straight dough or sourdough methods. As the term "brioche" can apply just as easily to the dough as to the individual baked product, the rolls can take on many different shapes and sizes. In Europe, the appearance of brioche is often based on the regional preference of the baker and his clientele.

#### **Flat Breads**

These are the most widely consumed of all bread types, eaten daily by hundreds of millions of people all over the globe. While this number may seem questionably large at first, it is put into proper perspective by considering the wide selection of products available, many of which are not known or available to those outside of certain regions. Generally, flat breads can be divided into the single-layered and double-layered types.

Single-layered flat breads are consumed universally. For most North and Central Americans, the flat bread of choice is the tortilla. Indeed, for many people it is the bread accompaniment at most every meal. While the corn tortilla is the more traditional product, wheat flour tortillas have gained great acceptance in the USA and Canada, especially for the fast food trade (Figure 13). See Tables 9 and 10 for representative formulas. In many non-Hispanic homes in the USA, tortillas have become as common as sliced white pan

bread. See Tortillas for additional information on tortillas.

Tanoor bread is another single-layered flat bread that is widely consumed, especially in parts of the

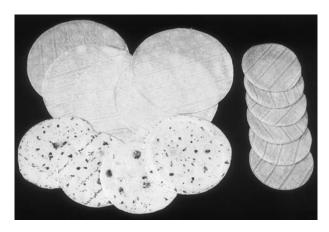


Figure 13 Large and small wheat flour tortillas, left; corn tortillas, right.

Table 9 Straight dough wheat flour tortilla

Ingredient	%
Bread flour <sup>a</sup> Vital wheat gluten Soy flour Water Salt Shortening Gums Yeast, compressed Baking powder Oxidizers <sup>b,d</sup>	100.0 0-5 0-5 45-60 1-2.5 5-12 0-4 0.1-1 1-1.5 Variable
Mold inhibitors <sup>c,d</sup>	Variable

<sup>&</sup>lt;sup>a</sup> All ingredients are given in bakers % (flour = 100%).

Table 10 Corn tortilla

Ingredient	%
Yellow corn <sup>a</sup>	85-100
White corn	0-15
Water	25-30
Lime	1–2
Soy flour	0-10
Sodium hydroxide	1-4
Calcium hydroxide	0.25-1
Buffers/preservatives <sup>b</sup>	Variable

<sup>&</sup>lt;sup>a</sup> Yellow corn + white corn = 100%.

 $<sup>^{\</sup>it b}$  Can include azodicarbonamide (ADA), potassium bromate, and/or ascorbic acid.

<sup>&</sup>lt;sup>c</sup>Can include potassium sorbate surface spray, calcium sorbate, sodium propionate, calcium propionate, 100-grain vinegar.

<sup>&</sup>lt;sup>d</sup> Please note that not all specified additives are permitted for food use in all countries.

<sup>&</sup>lt;sup>b</sup>Monocalcium phosphate, sorbic acid, citric acid, fumaric acid, calcium propionate, potassium sorbate, etc.

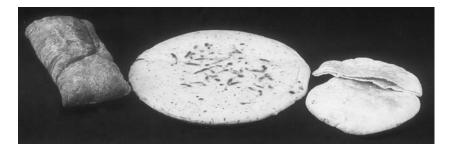


Figure 14 Ciabatta, left; focaccia, center; pita bread, right.

Middle East, India, and Pakistan. While it may be given slightly different names in different regions (taftoon in Iran, tanouri in Afghanistan), it is essentially the same product, with similar ingredients and processes used. It is made of wheat flour, soda (sodium bicarbonate), yeast, and water, with optional ingredients being salt and date syrup in certain types. Sourdough can be used in place of the yeast. It is baked into thin, oblong shapes.

Ciabatta is Italian in origin, and has gained a large following in Europe and North America (Figure 14). It is baked from lean formula sponge dough, usually consisting of wheat flour, water, yeast, and salt. Olive oil is sometimes added, especially in Italy. High amounts of water are added to the dough to give the baked bread its characteristic open and irregular crumb structure.

Similar to ciabatta, focaccia (Figure 14) uses the same basic ingredients but is prepared with the straight dough procedure, often with a long bulk fermentation period for added flavor. The dough is traditionally flattened in a shallow pan with the fingertips, then topped with various items such as onions, peppers, tomatoes, and seasonings. A short proof is given after which the dough is baked. Topped focaccia is seen as an upscale type of pizza in North America, and is a very popular item in boutique bakeries and cafes.

Pizza is a universally popular type of flat bread enjoyed all over the world. While pizza is usually distinguished by the toppings applied to it, the crust itself is subject to variation in thickness, texture, and added flavorings, depending on the particular geographical region in which it is served. Thickness can range from very thin and cracker like (0.5 cm) to thick and hearty (upwards of 5 cm). Crust texture is manipulated by the ingredients chosen, including flour strength, and the process used to manufacture the dough. Toppings vary widely, and are limited only by the imagination. Traditional US toppings include tomato sauce, mozzarella cheese, olive oil, and various herbs, vegetables, and meats.

Table 11 Straight dough arabic (Pita) bread

%
100.0
0.5
1.5
50.0

<sup>&</sup>lt;sup>a</sup> All ingredients are given in bakers % (flour = 100%).

Chapati, also known as roti, is estimated to be the most widely consumed flat bread in the world. The people of Tibet, India, Pakistan, Afghanistan, and other countries eat it as their daily bread. It is unleavened, round in shape, and made with whole-wheat flour and water.

The most recognizable double-layered flat bread is Arabic bread, also known as pita bread (Table 11). This product is lean in formulation, round in shape, and has a characteristic "pocket" inside that defines the product (Figure 14). This pocket is formed largely by a second proofing step that is not given to singlelayered flat breads. The second proof allows the formed dough surface to dry slightly. The resultant dough skin allows the product to balloon during baking, forming the internal pocket. Baking is carried out at high temperatures, normally 400°C, for short periods of time, usually 90–100 s. The combination of high temperature and short time helps to emphasize pocket formation by quickly converting internal dough moisture to steam, which, along with residual CO<sub>2</sub>, accentuates the separation between the top and bottom of the bread.

## **Future Considerations**

As it has with most things, technology will continue to affect the baking industry. Further advances in ingredients, equipment design, product shelf life, and distribution techniques will almost certainly give the bread baker advantages his ancestors never had. Implementing this technology to create wholesome and

appetizing breads that are also safe and profitable will prove advantageous for bakers and consumers alike.

See also: Bakeries. Cakes, Pastries, Muffins, and Bagels. Enzyme Activities. Fortification of Grain-Based Foods. Gluten and Modified Gluten. Tortillas. Wheat: Dry Milling.

# **Further Reading**

Bilheux R, Escoffier A, Herve D, and Pouradier J (1989) Special and Decorative Breads. New York: Van Nostrand.

Calvel R (2001) *The Taste of Bread*. Maryland: Aspen Publishers.

Doerry WT (1995a) Baking Technology, vol. 1, Breadmaking. Manhattan, KS: American Institute of Baking. Doerry WT (1995b) Baking Technology, vol. 2, Controlled Baking. Manhattan KS: American Institute of Baking.

Ito R *Quality Breads of Japan*. Tokyo: Century Enterprise. Jenkins S (1975) *Bakery Technology Book 1, Bread*. Toronto: Lester and Orpen Limited.

Kulp K and Lorenz K (eds.) (2003) Handbook of Dough Fermentations. New York: Marcel Dekker.

Kulp K and Ponte J, Jr. (eds.) (2000) Handbook of Cereal Science and Technology, 2nd edn. New York: Marcel Dekker.

Matz S (1999) Bakery Technology and Engineering, 3rd edn. McAllen, Texas: Pan-Tech International.

Pyler EJ (1988) Baking Science and Technology, 3rd edn. Marriam, KS: Sosland.

Pyler EJ (1994) *Bakers Handbook*. Merriam, KS: Sosland. Qarooni J (1996) *Flat Bread Technology*. New York: Chapman and Hall.

Williams A (ed.) (1975) Breadmaking, The Modern Revolution. London: Hutchinson Benham.

#### **Relevant Websites**

http://www.bbga.org — The website of the Bread Bakers Guild of America, offering content of interest to artisan bakers, along with information and formulas from the Team USA baking team which placed first in the 1999 Coupe du Monde de la Boulangerie, and second in the 2002 competition.

http://www.aibonline.org — The American Institute of Baking, located in Manhattan, Kansas, USA, was founded in 1919 as a service and education organization for the American wholesale baking industry. Today, the Institute's influence is worldwide, with services ranging from seminars and short courses to contract research facilities to food safety auditing.

http://www.baking.ca — The Baking Association of Canada serves members of the wholesale, retail, and in-store baking segments, as well as those in the allied trades.

http://www.bri.com.au — BRI Australia Limited, formerly the Bread Research Institute, was founded in 1947 with the origin goal of improving bread quality. It has evolved over the years into one of the world's most respected sources of research and technical information on grains, grain processing, milling, and baking technology.

http://www.campden.co.uk – Located in the United Kingdom, the Campden and Chorleywood Food Research Association conducts research and development on food and beverage products for clients worldwide, and is the UK's largest independent membership-based organization.

# BUCKWHEAT

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### Introduction

Buckwheat originates in China and is widely cultivated as a minor crop in many places of the world. It is a pseudocereal crop, belonging to the genus Fagopyrum of the family Polygonaceae. There are two cultivated species, i.e., common buckwheat (Fagopyrum esculentum) and tartary buckwheat (Fagopyrum tataricum). Common buckwheat is most widely cultivated and utilized, while tartary buckwheat is mainly grown and consumed in China. The major buckwheat-producing and -consuming countries include China, Japan, Korea, Nepal, India, Russia, Ukraine, Poland, Hungary, Slovenia, Italy, Canada, Brazil, and the United States. Buckwheat is mostly consumed in the form of groats and flour, which are used as material for porridge, noodles, bread, pancakes, spaghetti, and other food items. Many traditional buckwheat foods are still popular in some countries, such as Japanese "soba" and "wantuo" and "helao" in China. Buckwheat is rich in protein of excellent quality as well as starch with special and different properties from other cereals. In addition, it also contains high levels of fiber, minerals, vitamins, and flavonoids with positive therapeutic effects on the human body. Buckwheat has increasingly attracted attention because of its positive effects on some chronic disease conditions, such as hypertension, hypercholesterolemia, diabetes, and other cardiovascular diseases. Buckwheat has further potential for use as an important functional food source.

# **General Aspects of Buckwheat Production**

Buckwheat grows mainly in the northern hemisphere, notably in Russia, China, Japan, India, Poland, Ukraine, Canada, and USA. Buckwheat is also planted in the southern hemisphere, e.g., in Brazil, Australia, and South Africa. In 1993, Russia was the biggest producer of buckwheat in the world, with a planting area of more than 4 million hectares (Mha), mainly in the eastern and north eastern parts of the country. It is reported that in 1990, buckwheat yield per hectare reached 3400 kg in Russia. China has the longest history (since the seventh century AD) of buckwheat cultivation, and now ranks second in world production with an average annual cultivation area of  $\sim 1.33$  Mha, and production fluctuating in the range of 0.5-0.9 million tons (Mt). Average yield per hectare is 527 kg for common buckwheat and 1294 kg for tartary buckwheat in 1986. In China, major production areas are in Inner Mongolia, Shanxi, Gansu, Shaanxi, Sichuan, Yunan, and Guizhou. Buckwheat is also cultivated to support the production of honey, and is considered one of the highest yielding honey plants.

# **Biological Aspects of Buckwheat**

Buckwheat, a dicotyledonous plant, belongs to the genus Fagopyrum of the family Polygonaceae. Fagopyrum has 15 species, including two groups: (1) annual species: F. esculentum Moench (common buckwheat), F. tataricum L. (tartary or bitter buckwheat), Fagopyrum giganteum Krotov, etc.; (2) perennial species: Fagopyrum cymosum Meissn, Fagopyrum suffruticosum Fr. Schmidt, etc. Among these species, only F. esculentum and F. tataricum are cultivated species while others are wild species. Buckwheat has broad ecological adaptability and can grow well in adverse soil and climatic conditions; in particular, tartary buckwheat can grow in poor soils even at low temperature and low precipitation, such as in high altitude mountainous regions.

Common buckwheat is characterized by petiolate leaves, terminal and axillary flowers, and with corymbose or paniculate cyme. Tartary buckwheat has narrow-shaped leaves, more branches, and smaller flowers than common buckwheat, with inconspicuous greenish-white sepals. Buckwheat flowers are indefinite inflorescences that are white, pink, or yellow. Buckwheat grain is actually a fruit (achene) whose color ranges from a glossy or dull gray to brown or black. Buckwheat grain (achene) consists of hull (pericarp) and kernel, or seed (testa, aleurone layer, embryo, and endosperm). Such achenes are mostly triangular,  $\sim 4-9 \,\mathrm{mm}$  long with 1000-grain weight ranging from 15 to 35 g. Common buckwheat grain is generally bigger and huskier than tartary buckwheat grain. Common buckwheat has a softer hull

than tartary buckwheat. Common buckwheat grain tastes harsh, and tartary buckwheat tastes slightly bitter.

# **Chemical Composition of Buckwheat**

Chemical components of buckwheat include protein/ amino acids, starch, lipids, fiber, minerals, vitamins, and other functional components (e.g., flavonoids, phytosterols, and fagopyrins). These are distributed in different parts of the buckwheat grain, e.g., protein mainly occurs in the aleurone layer and embryo, starch in the endosperm, and fiber, ash, and flavonoids are normally in testa and pericarp. Milling procedures, fineness, and flour yield influence chemical composition in the final products of buckwheat milling (Table 1). Different buckwheat varieties (genotypes) and plant conditions (soil and climate) also cause compositional differences.

#### **Protein and Amino Acids**

Buckwheat grain contains not only a high level of protein but also a good quality or nutritional balance of protein. Protein content of buckwheat grain and flour is similar to or slightly higher than that in wheat grain and flour (Table 1), but significantly higher than those in many other cereals (e.g., rice, maize, sorghum etc.). Amino acid composition of buckwheat protein is nutritionally well balanced, and is rich in essential types (e.g., lysine and arginine), compared with most cereals. Table 2 shows that all amino acids in buckwheat grain are higher than in wheat grain, except for glutamic acid and proline.

**Table 1** Proximate composition of buckwheat compared with wheat<sup>a</sup>

Composition	Buckwheat seeds	Buckwheat groats	Buckwheat crude flour	Buckwheat fine flour	Wheat grain	Bread wheat flour
Carbohydrate	58.5-73.5	70.9	67.4	70.7	66.9	74.7
Protein	10-14.5	9.7	12.0	10.9	14.0	11.8
Fat	2.0 - 2.6	1.8	2.3	1.7	2.1	1.1
Fiber	9.3-10.9	3.7	7.2		2.6	0.3
Ash	2.0-2.5	1.7	2.1	1.6	1.9	0.4
Moisture	12-14.0	12.3	10.3	13.1	12.5	11.7

<sup>&</sup>lt;sup>a</sup> Percentage on dry weight basis: data from Lin RF (1994) *Buckwheat in China* (in Chinese). Beijing: Chinese Agriculture Publishing House, and Li SQ and Zhang QH (2001) Advances in the development of functional foods from buckwheat. *Critical Reviews in Food Science and Nutrition* 41: 451–464.

**Table 2** Amino acid contents of grains from 1505 buckwheat genotypes compared to wheat grains<sup>a</sup>

Amino acid	Common buckwheat		Tartary bud	Wheat	
	Range	Mean	Range	Mean	
Ala	0.28-0.75	0.51	0.26-0.79	0.50	0.33
Arg <sup>b</sup>	0.69-1.72	1.11	0.11-1.61	1.09	0.51
Asp	0.10-2.34	1.09	0.60 - 1.84	1.06	0.43
Cys	0.03-0.33	0.17	0.03-0.44	0.16	0.11
Glu	0.67 - 3.49	2.21	0.60-3.12	2.05	2.44
Gly	0.44-0.95	0.65	0.07-2.54	0.64	0.33
His <sup>b</sup>	0.17-0.40	0.26	0.03-0.50	0.27	0.20
lle <sup>b</sup>	0.04-1.65	0.46	0.05-0.71	0.46	0.27
Leu <sup>b</sup>	0.49-1.83	0.77	0.08-1.07	0.75	0.50
Lys <sup>b</sup>	0.06-1.96	0.66	0.12-1.86	0.64	0.29
Met <sup>b</sup>	0.02-0.33	0.17	0.03-0.34	0.17	0.16
Phe <sup>b</sup>	0.31 - 1.79	0.57	0.05 - 0.99	0.55	0.36
Pro	0.20-1.06	0.44	0.11-1.22	0.41	0.92
Ser	0.35-0.91	0.57	0.05-0.90	0.58	0.36
Thr <sup>b</sup>	0.16-0.67	0.44	0.24-0.62	0.43	0.21
Trp <sup>b</sup>	0.06-0.18	0.12	0.06-0.18	0.13	
Tyr	0.09-0.55	0.32	0.07-0.55	0.33	0.23
Val <sup>b</sup>	0.38-1.24	0.58	0.26-1.18	0.58	0.36

<sup>&</sup>lt;sup>a</sup> Percentage on dry weight basis: data from Lin RF, Zhou MD, Tao YR, Li JY, and Zhang ZW (eds.) (1992) *Proceedings of the 5th International Symposium on Buckwheat* (20–26 August, 1992, Taiyuan, China). Beijing: Agricultural Publishing House and Wei YM (1995) *Quality and Processing of Buckwheat Grain* (in Chinese). Xi'an, China: World Publishing Corporation.
<sup>b</sup> Essential amino acids.

## Carbohydrate

Carbohydrate (mainly starch) is the major component (59–74%) of buckwheat grain (**Table 1**). Amylose content in starch generally ranges from 20% to 26%. Starch granules are 2–15 µm in diameter, round, oval, or polygonal in shape with a few holes and pits on the surface. Buckwheat starch exhibits a typical A-type X-ray diffraction pattern and the crystallinity varies from 38.3% to 51.3%. Buckwheat grains also contain 0.65–0.76% reducing sugars, 0.79–1.16% oligosaccharides, and 0.1–0.2% non-starchy polysaccharides.

# Lipids

Buckwheat grain contains 2.0-2.6% oil. Major fatty acids are palmitic (16:0), oleic (18:1), and linoleic (18:2) acids, with average values of 14.8%, 36.5%, and 35.5%, respectively. Unsaturated fatty acids constitute  $\sim$ 75-80% of total fatty acids.

#### **Fiber**

Buckwheat grain contains 9.3-10.9% crude fiber,  $\sim 20-30\%$  of which is soluble dietary fiber. Common buckwheat grain generally has higher crude fiber than tartary buckwheat. Content of crude fiber in buckwheat flour commonly depends on the milling process because fiber is mainly distributed in testa and pericarp. Crude flour contains more fiber (3.4-6.9%) than fine flour (0.1-1.6%).

#### **Minerals**

Buckwheat grain is rich in Mg, Se, Fe, K, Ca, Cu, Mn, Zn, etc. Some studies show that buckwheat flour contains more Mg (11-13 times), Sr (5-36 times), Li (5-7 times), Fe (3-4 times), and K (2.9-3.6 times) than wheat flour. Additionally, a high level of Se is detected in tartary buckwheat flour (0.43 mg per kg).

# **Vitamins**

Buckwheat grain contains higher levels of vitamin B<sub>1</sub> (thiamin), B<sub>2</sub> (riboflavin), E (tocopherol), and PP (nicotinic acid/amide) than most cereals (**Table 3**). Generally, tartary buckwheat has more vitamin B<sub>1</sub>, B<sub>2</sub>, and PP, but less vitamin E than common buckwheat.

#### **Therapeutic Components**

Buckwheat is also rich in components with therapeutic effects, including flavonoids, phytosterols, fagopyrins, and cinnamic acid.

1. Flavonoids: Several flavonoids have been identified in tartary and common buckwheat, i.e., rutin,

**Table 3** Comparison of vitamin contents between buckwheat flour and some cereal flour<sup>a</sup>

Vitamin <sup>b</sup>	Buckwheat	Wheat	Rice	Maize	Oat	Sorghum
	0.22	0.19 0.06 1.6	0.21 0.06 2.2	0.09 1.6	0.29 0.17 0.8	0.09

<sup>a</sup> Data from Wang LM, Zhong SH, and Cai SL (1991) Contents of vitamin PP and vitamin E in Chinese (common and tartary) buckwheat. *Crop Genetic Resources* 2: 23–25 and Wei YM (1995) *Quality and Processing of Buckwheat Grain* (in Chinese). Xi'an, China: World Publishing Corporation.

quercetin, quercitin, kaemferol, orientin/isoorientin, and vitexin/isovitexin. Rutin, also called vitamin P, is the major and most important flavonoid component in buckwheat, which is not found in cereals. All parts of the buckwheat plant (seeds, hulls, leaves, stems, flowers, and even roots) contain flavonoids. The flavonoid components and content in buckwheat differ among growth periods and among species and genotypes. Generally, the flavonoids in tartary buckwheat are significantly higher than that in common buckwheat. It is reported that common buckwheat grain contains 4-13 mg per g flavonoids, whereas tartary buckwheat grain has  $\sim$ 40 mg per g. In tartary buckwheat flowers, leaves, and stems, total flavonoid content reaches over 100 mg per g.

- 2. Phytosterols: Buckwheat contains several kinds of phytosterols, e.g.,  $\beta$ -sitosterol, campesterol, stigmasterol, and isofucosterol.  $\beta$ -Sitosterol is the major sterol component, accounting for  $\sim$ 70% of total sterol content. Some studies report that de-hulled buckwheat groats after lipid extraction have 667-753 mg per kg  $\beta$ -sitosterol, 89-97 mg per kg campesterol, and a trace of stigmasterol. Phytosterols also occur in buckwheat pollen.
- Fagopyrins: Fagopyrins normally occur in buckwheat grain in low quantity and are difficult to separate. The research projects in the field of fagopyrin isolation and development are still underway.
- 4. Cinnamic acid: Tartary buckwheat contains 2,4-dihydroxy-*cis*-cinnamic acid, which can be helpful for inhibiting black pigment formation on skin and preventing old age skin specks and freckles.

#### **Antinutritional Inhibitors**

Antinutritional factors have been found in buckwheat grain, such as trypsin inhibitors (I, II, and III) and

bmg per 100 g dwb (dry weight basis).

<sup>&</sup>lt;sup>c</sup>Mean of 565 common buckwheat genotypes.

<sup>&</sup>lt;sup>d</sup>Mean of 491 tartary buckwheat genotypes.

tannin. They may influence digestibility of buckwheat protein. Also, a high level of fiber in buckwheat may be considered as an antinutritional factor.

# **Nutritional Value and Properties** of Major Components

#### **Protein**

Buckwheat is one of the best sources of high biological value protein in the plant kingdom. The biological value (BV) of buckwheat protein is 93, compared to egg albumin (100), soybean protein (68), and wheat protein (63). The high BV of buckwheat protein results in much higher utilizable protein value (20–30%) compared to other cereals, although its digestibility is relatively lower. Buckwheat protein complements other food proteins to improve the dietary amino acid balance. In buckwheat protein, threonine and methionine are the first and the second limiting amino acids, respectively, but they are quite abundant in other plant proteins.

In buckwheat grain, protein albumin is the major fraction (28–42%), and glutelin (11–21%) and globulin (14–20%) are next, whereas prolamin is the minor fraction (1.7–2.3%). Comparatively, wheat grain is high in prolamin and glutelin (70–75%). These differences between buckwheat and wheat lead to differences in food functional properties and processing quality. Because of the very low level of prolamin in buckwheat (i.e., free from gluten) and based on chemical and immunological studies, buckwheat protein is a valuable source of dietary protein for gluten-sensitive individuals and can be used for patients with gluten sensitivity (celiac disease).

Interesting findings on thiamin-binding protein (TBP) in buckwheat grain were reported in 1986. TBP can stabilize thiamin during storage through forming a complex between protein and thiamin, ordinarily at a 1:1 binding stoichiometry. After complexing, it can be digested by proteases and release the thiamin. Therefore, TBP can be used for patients who suffer from lack of thiamin and cannot store thiamin.

Studies at the University of Hong Kong found that buckwheat protein exhibited much higher solubility, better emulsifying activity, and slightly lower foaming ability, compared to soy protein, and poorer foaming ability than amaranth protein (Table 4). Recent Japanese studies indicated that the physico-chemical properties of buckwheat protein were obviously different from those of soy protein and casein. Buckwheat protein affected the rheological properties of maize starch. An increase in the peak viscosity due to the addition of buckwheat protein concentrate was

**Table 4** Functional properties of common buckwheat protein concentrates, compared to other protein isolates

Protein concentrate	Emulsifying activity at pH 7.0 (%)	Solubility (%)	Foam stability (ml)
Buckwheat Soybean Amaranth <sup>a</sup>	63.8 50.6 73.3	64.2 33.3 56.5	14 20 56

<sup>&</sup>lt;sup>a</sup> From A. cruentus genotype (K350).

observed, but a lesser increase from the addition of buckwheat protein hydrolysates. From the established relationship between pasting viscosity and granule swelling during gelatinization, it was concluded that the observations were due to the protein exerting a stabilizing factor on starch granule integrity. It was also observed that buckwheat protein addition weakened starch gel structure. This phenomenon was most likely due to the preferential interactions between starch granules and proteins during gelatinization and retrogradation.

Furthermore, buckwheat protein concentrate was examined for direct utilization in an emulsion-type meat product comprising lean beef, pork fat, salt, and water. It was found that the use of buckwheat protein concentrate considerably affected both the emulsion and the cooked meat gel properties. Buckwheat protein showed favorable effects to be suitable as a meat extender, as good as soy protein. Also, buckwheat protein concentrate was tested for direct application in other food systems (wheat dough and noodles). Addition of the buckwheat protein concentrate could improve the mixing properties of wheat flour and produce a positive influence on noodle formulation and final product quality.

However, some reports pointed out a problem of the low digestibility of buckwheat protein, which might result from antinutritional factors: trypsin inhibitors, tannin, and a high content of dietary fiber. Buckwheat trypsin inhibitors are relatively thermo stable, acid resistant, and partially susceptible to pepsin action. In addition, allergens in buckwheat proteins have attracted attention. During buckwheat grain milling and food processing, some workers have allergic reactions, such as nose itching, sneezing, rhinorrhea, dyspnea, asthma, and even more serious symptoms. It is necessary to know more about the behavior and processing properties of specific buckwheat allergenic proteins.

#### Starch

Some reports suggested that buckwheat starch normally has higher peak viscosity, higher gelatinization

temperature, greater swelling power, and higher susceptibility to acid and enzymatic degradation when compared with cereal starches (maize and wheat). Some studies indicated that buckwheat starch retrogradation is generally slower than that of maize and wheat starch. Buckwheat starch has lower syneresis when stored at 4°C for 3-10 days and has better stability to syneresis after freeze-thaw cycles at  $-12^{\circ}\text{C}-25^{\circ}\text{C}.$ 

The authors investigated the starch properties of common and tartary buckwheat compared to wheat starch. Clear differences were found in physico-chemical properties between buckwheat and wheat, but less variation was observed between three common and three tartary buckwheat genotypes. Buckwheat starch had higher peak gelatinization temperature (average  $T_p = 68.7^{\circ}$ C) than standard wheat starch ( $T_p = 63.7^{\circ}$ C). The important pasting properties of buckwheat starch were higher hot paste viscosity and cool paste viscosity, better resistance to shear thinning, and less effect of NaCl on peak viscosity than for wheat starch. Starch swelling volume is 27.4-28.0 ml for common buckwheat and 26.5–30.8 ml for tartary buckwheat, compared to the reference (20.1 ml) for wheat starch.

Investigations on the nutritional properties of buckwheat starch showed that it has potential use in the design of foods with lower glycemic index properties. The in vitro rate of starch hydrolysis and resistant-starch formation in boiled buckwheat groats and in a series of buckwheat-wheat breads and noodles (30–70% buckwheat flour) were evaluated. The highest level of resistant starch (6% total starch basis) and the lowest starch hydrolysis index was found in boiled buckwheat groats. The rate of in vitro amylolysis was much lower in all buckwheat products compared to the reference whole wheat bread or noodles. Consumption of boiled buckwheat groats or bread and noodles containing more buckwheat induced significantly lower postprandial blood glucose and insulin responses than with whole wheat bread.

Additionally, several researchers reported the effects of processing treatments (steaming or autoclaving, extruding, etc.) on buckwheat starch properties. The results showed that hydrothermal treatment increases gelatinization temperature, swelling power, and solubility of buckwheat starch. Autoclaving/cooling treatment of buckwheat groats resulted in improved availability of buckwheat starch and led to more apparent amylose and true amylose in the buckwheat starch and more resistant-starch formation in comparison to the untreated samples. It was also reported that extrusion influenced the physicochemical properties of starch and formation of starch-protein and starch-lipid complexes in extruded mixtures containing buckwheat flour and starch. Starch-protein complexes formed during extrusion played an important role in the stability of the porous structure in the extruded products containing buckwheat starch.

# Therapeutic Effects of Buckwheat

Buckwheat, especially tartary buckwheat, has been regarded as an effective medicinal herb in Chinese medicine for more than 1000 years. Over 80 prescriptions utilizing buckwheat in the treatment of many diseases have been described in Chinese traditional medicine books. In modern times, buckwheat products are popular as functional or therapeutic foods in China and some other countries. Regular consumption of buckwheat foods may reduce the occurrence of hyperlipidemia, obesity, and diabetes. A clinical trial in Beijing Tongren Hospital showed that tartary buckwheat flour had hypolipidemic and hypoglycemic effective in treating hyperlipidemia and diabetes (Table 5). Table 6 shows that health foods made from tartary buckwheat have positive effects in reducing cholesterol, blood lipid, blood sugar, urinary sugar, and other indices. These therapeutic effects should be attributed to unique proteins, resistant starch, and dietary fiber as well as vitamins, minerals, flavonoids, and phytosterols in buckwheat.

Some studies suggest that protein and indigestible carbohydrates (fiber and resistant starch) of buckwheat can be used as potential functional food additives to treat hypertension, obesity, constipation, etc. Buckwheat protein extract could lower blood cholesterol level more efficiently, compared with

Table 5 Clinical effects of tartary buckwheat flour on hyperlipidemia and diabetes (hyperglycemia) after one-month dietary treatment<sup>a</sup>

Testing index <sup>b</sup>	Before treatment	After treatment
Hypolipidemic effect Serum triglyceride (mg dl <sup>-1</sup> ) ( $n$ = 18) Serum cholesterol (mg dl <sup>-1</sup> ) ( $n$ = 13)	310 ± 241 247 ± 57	$233 \pm 173$ $197 \pm 43$
Hypoglycemic effect Blood sugar (mg dl <sup>-1</sup> ) ( $n$ =23) Urinary sugar (g 24 h <sup>-1</sup> ) ( $n$ =27)	202 ± 46 44 ± 43	$157 \pm 40 \\ 20 \pm 22$

<sup>a</sup> Data from Lin RF, Zhou MD, Tao YR, Li JY, and Zhang ZW (eds.) (1992) Proceedings of the 5th International Symposium on Buckwheat (20-26 August, 1992, Taiyuan, China). Beijing: Agricultural Publishing House and Corke H and Lin RF (eds.) (1998) Proceedings of the 1st International Conference on Asian Food Product Development - Focus on Specialty Grains and Grain Products (6-10 September, 1998, Taiyuan, China). Beijing and New York: Science Press. <sup>b</sup> n is number of subjects.

soy protein and casein. Sulfur-containing amino acids and glycine as well as arginine and lysine are also considered to have important influence on this cholesterol-lowering effect. These amino acids can conduct down-regulation of hepatic LDL receptors and improve the removal of cholesterol and its esters in outlying tissues and thus reduce the serum cholesterol level. Recent studies have further indicated that consumption of buckwheat protein can cause suppression in body fat, constipation, mammary carcinogenesis, and colon carcinogensis in rats and in the formation of cholesterol gallstones in hamsters. Some researchers explain that the cholesterol-lowering, obesitytreating, and diabetes-controlling effects are also possibly associated with other components (dietary fiber and resistant starch) in buckwheat. The metabolites, formed during fermentation of resistant starch and other indigestible carbohydrates in the large intestine, contribute to the maintenance of colon health and also have beneficial effects on glucose metabolism.

The flavonoids from buckwheat (especially tartary buckwheat) have been found to be effective in reducing blood cholesterol levels, keeping capillaries and arteries strong and flexible, improving blood microcirculation, and protecting blood vessels from rupturing and forming clots. These flavonoids also demonstrate antioxidant, antimicrobial, and anti-inflammatory activity. Because tartary buckwheat leaves and stems contain high concentrations of rutin and other flavonoids at flowering, extracts of buckwheat plants are helpful for treating keratitis, while dried powdered buckwheat plants are sometimes used for curing external wounds and ulcers.

The vitamins and minerals (Mg, Fe, Se, etc.) in buckwheat can also play a crucial complementary role in the above therapeutic effects. Phytosterols found in buckwheat grain, although at a low level, also showed positive effects on blood cholesterol level. Other functions of buckwheat sterols are waiting for further investigation, because some phytosterols from other plant sources were found to have strong pharmaceutical effects, e.g., antivirus and antitumor activity. Also, there is a report that the

fagopyrins isolated in buckwheat grain are an effective component for the treatment of type II diabetes. However, there is still some dispute on this issue.

# **Buckwheat Food Products**

Buckwheat products are recognized as functional food in some Asian countries. Buckwheat, as a nutritional staple crop and medicinal herb for Chinese consumption, has been widely grown for a long time. A rich array of traditional buckwheat foods are found in various places of China, such as buckwheat wantuo and helao (or hele), buckwheat cat's-ear noodles, buckwheat noodles (vermicelli), pancake, griddlecakes, jelly noodles, and porridge, etc. These foods are famous and popular in China, particular for their long history, rich nutritional profile, special flavor, various processing methods, and "functional food" role in maintaining human health. For example, buckwheat helao (hele), wantuo, and cat's-ear noodles are well-known local foods in northern region of China (Shanxi and Shaanxi provinces).

In Japan, buckwheat noodles called soba and buckwheat roasted-groats have long been very popular, while in Korea a favorite jelly-type food called "mook" is also made from buckwheat. In India, buckwheat flour is used for making "chillare," an unleavened bread fried with ghee and also for a crisp food called "pakora." Honey produced from common buckwheat is prized in both China and India. In Eastern Europe, buckwheat grain forms an important part of the traditional diet. Buckwheat groat porridge and meals as well as soup made from flour are still favorite traditional foods in Poland, Russia, and other some Eastern European countries. In the United States, although buckwheat is mainly used for animal feed, buckwheat cake was once a special breakfast item and buckwheat flour is also an ingredient of some pancake mixes.

Modern buckwheat food products include buckwheat dried noodles or vermicelli, instant noodles, spaghetti or macaroni, biscuits, breads, cakes, pastry, breakfast cereals and snacks, vinegar, drinks and

**Table 6** Therapeutic effects of health foods made from tartary buckwheat<sup>a</sup>

Treatment	Triglyceride	Cholesterol	Lipoprotein	Blood sugar	Urinary sugar
	(mg dl <sup>-1</sup> )	(nmol 24 h <sup>-1</sup> )			
Buckwheat	207.8	148.1	503.2	178.5	284
Control	286.9	202.6	616.0	255.6	363

<sup>&</sup>lt;sup>a</sup> Statistical data from 187 samples of clinical observations by Beijing Grain Science Research Institute, Beijing (Lin RF, Zhou MD, Tao YR, Li JY, and Zhang ZW (eds.) (1992) *Proceedings of the 5th International Symposium on Buckwheat* (20–26 August, 1992, Taiyuan, China). Beijing: Agricultural Publishing House and Corke H and Lin RF (eds.) (1998) *Proceedings of the 1st International Conference on Asian Food Product Development – Focus on Specialty Grains and Grain Products* (6–10 September, 1998, Taiyuan, China). Beijing and New York: Science Press).

beverages (tartary buckwheat tea, buckwheat beer, and liquor). Most of these products, particularly made from tartary buckwheat, are popular healthy foods in China. Tender buckwheat shoots are used as fresh vegetables, and are also processed into canned vegetables.

# **Buckwheat Processing and Development**

Buckwheat grains are de-hulled and milled into flour for food processing. Buckwheat flour milling is similar to wheat dry milling. Buckwheat flour yield ranges from 58% to 75%, mainly depending on variety and milling techniques. Buckwheat flour contains similar levels of starch and protein to wheat flour. However, the behavior of buckwheat flour is different from wheat flour and it is difficult to make wheat flour-like foods because there is no gluten. Therefore, buckwheat flour is usually mixed with wheat flour and other flours before use.

Chinese researchers and food factories have mixed buckwheat and wheat flour along with other flours to successfully make buckwheat pastry, sandwich-cake, dried noodles, instant noodles, and spaghetti (Tables 7 and 8). The processing steps for dried noodles are shown in Figure 1. Suitable levels of buckwheat flour are  $\sim 10\%$  for crisp pastry and cakes, 20-40% for noodles, and 30-50% for palatable spaghetti. The cooking quality of spaghettis mixed from different ratios of buckwheat and wheat flours are shown in Table 8. Buckwheat/wheat spaghettis generally have higher water absorption, higher cooking loss, and higher protein loss rate than pure wheat spaghetti, because buckwheat flour contains much more albumins (water soluble) and globulins (soluble in salt water) than wheat flour. These commercial foods are produced by modern food-processing equipment. However, most traditional buckwheat foods are still hand-made in the countryside and even cities in China. An example of the basic handpreparation method for buckwheat helao is shown in Figure 2.

In Japan, there are many noodle-making factories producing soba noodles. Buckwheat flour used for making soba is commonly mixed with 10-50% wheat flour. These noodles are sold in precooked, boiled, dried, or instant stages, as well as being made for direct sale to buckwheat noodle restaurants, small shops, and stands. In Europe and the United States, buckwheat flour is mixed with other cereal flours to make pancakes, flakes, biscuits, bread, noodles, spaghetti or macaroni, and ready-to-eat breakfast cereals. The processing of these foods can be

**Table 7** Recipes for three selected buckwheat baked foods<sup>a</sup>

Materials (g)	Chinese crisp pastry	Sandwich cake	Mixing bread
Buckwheat flour	1.0	1.0	25 <sup>b</sup>
Wheat flour	8.5	8.5	225
Soybean flour	0.5	0.5	
Egg		10	
Shortening oil	3		10
Salt	0.01		3.75
Sugar		8.0	10
Refined maltose	1.0	1.6	
Skimmed milk			5
Dried yeast			3
Flavor (prickly ash)	0.1 - 0.15		
Jam		3	
Buckwheat flour paste		0.5	

aln these selected foods, buckwheat flour accounts for 10% of total composite flour in the baking foods: data from Lin RF, Zhou MD, Tao YR, Li JY, and Zhang ZW (eds.) (1992) Proceedings of the 5th International Symposium on Buckwheat (20-26 August, 1992, Taiyuan, China). Beijing: Agricultural Publishing House and Cheng CJ, Nie RF, and Zhang ZX (1993) Primary study on buckwheat addition and nutritive preservation in healthy food products of buckwheat. *Buckwheat Trend* 1: 105–108. <sup>b</sup>Tartary buckwheat flour.

Table 8 Cooking quality of spaghetti made from common buckwheat and wheat flour at different ratiosa

Buckwheat:	Water	Cooking loss	Loss of protein (%)		
wheat flour ratio	absorption (%)	of dry materials (%)	Cooking in water	Cooking in salt water	
0:100	218	6.4	1.4	6.6	
25:75	235	6.6	2.0	7.5	
50:50	251	8.2	3.7	13.9	
75:25	278	8.5	2.7	9.4	

<sup>&</sup>lt;sup>a</sup>Data from Wei YM and Zhang GQ (1993) Study on processing of buckwheat spaghetti. Acta of Grain and Oil Science and Technology 3: 18-21 and Wei YM (1995) Quality and Processing of Buckwheat Grain (in Chinese). Xi'an, China: World Publishing Corporation.

conducted using ordinary baking, extruding, or expansion methods. The amount of buckwheat flour added to other cereal flours is generally less than 50%. For instance, acceptable bread contained 25% buckwheat flour with a formulation that included vital gluten, whey, or sour milk. A recipe for bread with 10% tartary buckwheat flour is shown in Table 7. It was also reported that the buckwheat-corn flakes were produced by extrusion processing. The formulations with an addition of 18%, 22%, and 33% buckwheat flour were used. The addition of 30% buckwheat flour gave the best organoleptic properties.

Although buckwheat is still cultivated as a minor crop in the world, buckwheat product development

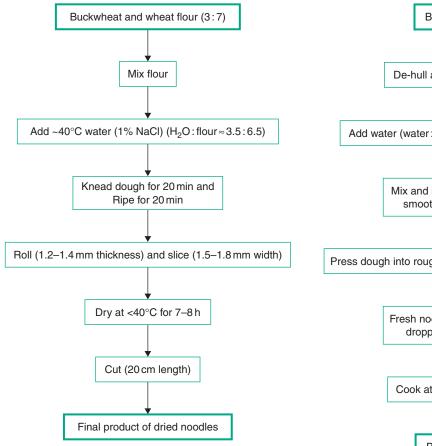


Figure 1 Processing procedure for dried noodles of common or tartary buckwheat.

has quickened since the 1990s, particularly in China. To date there has been an emerging trend in the research and development of high value-added buckwheat products. Tartary buckwheat has high potential in the health food market. Since tartary buckwheat contains a high level of flavonoids and other therapeutic components, some cosmetics and medicine products have successfully been developed and marketed using buckwheat materials in China, such as cream, shampoo, lotion, toothpaste, flavonoid capsules, etc. However, many such new products have not been accepted well in the marketplace, because of their high cost and erratic quality control.

Nowadays buckwheat functional foods and related products have attracted widespread interest and have become more popular. Much more attention should be paid to the biological functions, palatability, and acceptability of buckwheat products. It is necessary to enhance development and commercialization of traditional buckwheat foods and to improve modern buckwheat food-processing techniques.

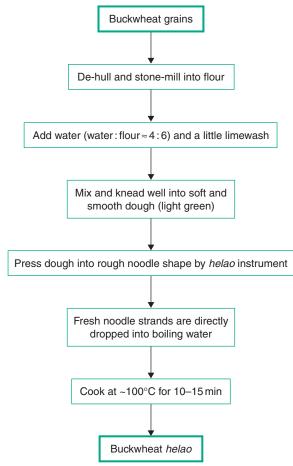


Figure 2 Traditional method of buckwheat helao preparation.

See also: Amaranth. Celiac Disease. Cereals: Protein Chemistry. Pseudocereals, Overview. Starch: Chemistry. Taxonomic Classification of Grain Species.

# **Further Reading**

Bejosano FP and Corke H (1998) Amaranthus and buckwheat protein concentrate effects on an emulsiontype meat product. *Meat Science* 50: 343–353.

Cheng CJ, Nie RF, and Zhang ZX (1993) Primary study on buckwheat addition and nutritive preservation in healthy food products of buckwheat. Buckwheat Trend 1: 105-108.

Corke H and Lin RF (eds.) (1998) Proceedings of the 1st International Conference on Asian Food Product Development - Focus on Specialty Grains and Grain Products (6-10 September, 1998, Taiyuan, China). Beijing and New York: Science Press.

Joshi BD and Rana RS (1995) Buckwheat (Fagopyrum esculentum). In: Williams JT (ed.) Cereals and Pseudocereals, pp. 85-127. London: Chapman and Hall.

Kayashita J, Shimaoka I, and Nakajoh M (1995) Hypocholesterolemic effect of buckwheat protein extract in

- rats fed cholesterol-enriched diets. *Nutrition Research* 15: 691–698.
- Li SQ and Zhang QH (2001) Advances in the development of functional foods from buckwheat. *Critical Reviews in Food Science and Nutrition* 41: 451–464.
- Li WD, Lin RF, and Corke H (1997) Physicochemical properties of common and tartary buckwheat starch. *Cereal Chemistry* 74: 79–82.
- Lin R, Li W, and Corke H (1998) Spotlight on Shanxi province China: its minor crops and specialty foods. *Cereal Foods World* 43: 189–192.
- Lin RF (1994) *Buckwheat in China* (in Chinese). Beijing: Chinese Agriculture Publishing House.
- Lin RF, Zhou MD, Tao YR, Li JY, and Zhang ZW (eds.) (1992) *Proceedings of the 5th International Symposium on Buckwheat* (20–26 August, 1992, Taiyuan, China). Beijing: Agricultural Publishing House.
- Tomotake H, Shimaoka I, Nakajoh M, and Kato N (2002) Physicochemical and functional properties of buckwheat protein product. *Journal of Agricultural and Food Chemistry* 50: 2125–2129.
- Wang LM, Zhong SH, and Cai SL (1991) Contents of vitamin PP and vitamin E in Chinese (common and tartary) buckwheat. *Crop Genetic Resources* 2: 23–25.

- Wei YM (1995) Quality and Processing of Buckwheat Grain (in Chinese). Xi'an, China: World Publishing Corporation.
- Wei YM and Zhang GQ (1993) Study on processing of buckwheat spaghetti. *Acta of Grain and Oil Science and Technology* 3: 18–21.

# **Relevant Websites**

http://www.corn.agronomy.wisc.edu — Description of buckwheat history, uses, and cultivation. http://www.buckwheatgrowers.com — Information on buckwheat growers, products and services. http://www.sarep.ucdavis.edu — Introduction on buckwheat biological aspects and cultivation. http://www.hort.purdue.edu — Website of the New Crop Online Program (http://www.hort.purdue.edu/new crop) of the Center for New Crops and Plant Products at Purdue University. New CROP provides windows to new and specialty crop profiles, including buckwheat.



# CAKES, CHEMISTRY OF MANUFACTURE

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# **Principal Ingredients in Cake Batter**

Flour is the most important ingredient in cakes, functioning primarily to establish crumb structure. Cake flour is milled from soft white or red wheat with low protein and ash (mineral) levels and has a fine particle size. In the USA, cake flour is commonly treated with chlorine gas, causing hydrolytic depolymerization of the starch molecules, which increases the waterholding capacity of the flour. During mixing, proper gluten development is critical, to ensure a fine foam structure without excessive toughening. Protein level and type and mixing procedure are key factors for producing cakes with desirable crumb texture. The optimal specifications for a typical cake flour are: protein content  $8.5\% \pm 0.5\%$ , ash content  $0.36\% \pm$ 0.04%, pH 4.7  $\pm$  0.2, and average particle size  $10 \, \mathrm{mm} \pm 0.5 \, \mathrm{mm}$ .

Shortening performs three basic roles in cakes. First, it aids in aeration or leavening of the batter and finished cake by entrapping air during the creaming process. These minute air cells provide the nucleus for bubble expansion via steam and carbon dioxide during baking. Second, shortening coats the protein and starch particles, preventing hydration and formation of a continuous gluten—starch network. Third, it emulsifies liquids in the batter, which increases the moisture of the crumb. The last two functions contribute to a soft, tender crumb texture.

Eggs perform a variety of functions in cake production, providing structure, volume, tenderness, and nutritional quality to the product. They act as a binding agent due to their high protein content and ability to form a complex network with gluten. They contribute to cake volume and structure by their ability to be whipped into a relatively stable foam. Upon heating, the proteins are denatured, thus setting and stabilizing

the crumb structure. The yolk portion of the egg imparts an emulsifying and tenderizing effect because of the high lipid and lecithin content. Eggs help stabilize the emulsion, retain gas generated by the leaveners, and prevent air cell coalescence in the batter, resulting in a uniform crumb grain and desirable texture. Eggs also add a mild but distinctive flavor and color (from the yolk) and enrich cake's nutritive value.

Chemical leavening agents are added to aerate the batter and produce a light, tender, porous product. The porosity of the batter directly translates into good volume, uniform cell structure, bright crumb color, tender texture, and overall eating quality of the finished cake. In lieu of yeast, chemically leavened cakes utilize sodium bicarbonate (baking soda) plus an acidic agent to generate carbon dioxide (CO<sub>2</sub>) gas when combined in the presence of water.

Baking powders are a combination of sodium bicarbonate and the salt of a weak acid. Baking powder should yield no less than 12% carbon dioxide based on the weight of the product. Starch or flour may be added as a diluent to standardize the baking powder's strength.

Baking powders are classified by their reaction rates as fast-, slow-, or double-acting. The fast-acting types release most of their available carbon dioxide within the first few moments of contact with liquid. If these batters or doughs are not processed very quickly, the gas will be lost before the structure is set and the volume will decrease significantly.

Slow-acting baking powders do not react at low temperatures and therefore require oven heat to release gas. Double-acting types react partially at low temperature, but need higher temperatures to complete the reaction. The double-reacting powders are most commonly used in commercial cake production.

The neutralization value (NV) is used to give the proper balance of the alkaline and acidic agents. The NV indicates the amount of sodium bicarbonate required to release all the available carbon dioxide from 100 units of the acid leavener. NVs enable the formulator to achieve the desired batter pH, which is important to crumb structure and grain, color, and flavor.

Sugar acts primarily as a sweetener in cakes and aids in air incorporation during creaming. In the mid-1900s, emulsified shortenings enabled bakers to produce richer cakes with higher sugar and liquid levels. These high-ratio cakes generally contain 120% sugar, based on flour weight, and have an extended shelf life and tender texture. The type and form of sugar used are also important; liquid sugar or syrup acts as a moistener, whereas crystalline or granular sugar functions as a drier. Granulation affects how quickly the sugar dissolves and generally increases cake volume as granulation becomes finer. Invert or reducing sugars, e.g., high-fructose corn syrup, honey, or molasses, can affect the crust and crumb color, and texture.

Milk, either in fluid or dry powdered form, is a source of protein and lactose, which aid in crust development and browning reactions. Milk also stabilizes the foam and contributes to cake structure. Finally, salt is added as a flavor enhancer.

Formula balance of the major cake ingredients – plus or minus flour, shortening, sugar, and eggs – is important to produce a cake with good volume, grain, texture, and eating properties. Some general guidelines for high-ratio cakes are as follows:

• the weight of the sugar should be greater than the flour weight;

- the weight of the eggs should exceed the weight of the shortening; and
- the weight of the total liquids (milk, eggs, and/or water) should be slightly greater than the sugar weight.

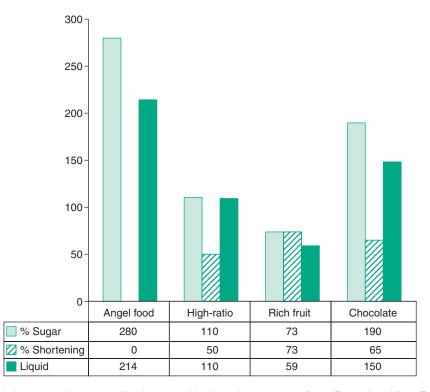
Some exceptions to these rules include foam-type cakes, i.e., angel food and sponge, which contain little or no shortening. In these cakes, whipped egg whites, sugar, and flour are the primary ingredients, along with some minor ingredients. In a typical angelfood cake formulation (Table 1), the weight of the sugar is usually equal to the weight of the egg whites, and the flour is approximately one-third the weight of the sugar. Figure 1 shows the relationship between basic ingredient composition and cake type.

Table 1 Angel food cake formula

Ingredient	Baker's %ª
Flour	100
Sugar	280
Egg white	280
Salt	4
Cream of tartar	4
Vanilla	5

<sup>&</sup>lt;sup>a</sup>Baker's % based on 100 parts flour.

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**Figure 1** Relationship between cake type and basic composition based on 100 parts flour. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 762, Elsevier Ltd.)

Sponge cakes use whole egg instead of only egg whites. To minimize the toughening effect of the eggs, an equal or slightly higher amount of sugar is added. The liquid and flour levels are balanced around the eggs and sugar. In general, the total liquids should be 25% greater than the weight of the sugar.

Flour level should be less than the weight of either the sugar or eggs. Combined, the weight of eggs plus flour should exceed the weight of sugar plus nonegg liquids (milk or water). A typical sponge cake formula is listed in Table 2.

Pound cake is one of the oldest cake types, deriving its name from the original recipe, which suggested one-pound (0.45 kg) increments each of flour, butter,

Table 2 Sponge cake formula

Ingredient	Baker's %ª
Flour	100
Sugar	95
Corn syrup	12
Eggs	105
Water	12
Vanilla	3
Baking powder	1.5
Salt	0.75

<sup>&</sup>lt;sup>a</sup>Baker's % based on 100 parts flour.

Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 272, Elsevier Ltd.

Table 3 Commercial pound cake formula

Ingredient	Baker's %
Flour Sugar Shortening Whole eggs Milk Vanilla Salt	100 100 50 50 50 2 1.5

<sup>&</sup>lt;sup>a</sup>Baker's % based on 100 parts flour.

Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 272, Elsevier Ltd.

Table 4 Ordinary yellow cake formula

Ingredient	Baker's %ª
Flour Sugar Shortening Whole eggs Milk Baking powder Salt	100 85 <sup>b</sup> 45 50 50 2.5
Flavor	1.5

<sup>&</sup>lt;sup>a</sup>Baker's % based on 100 parts flour.

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eggs, and sugar. The expense of butter and eggs prompted commercial bakers to modify the formula, producing a lighter cake with improved volume and eating quality (Table 3).

The modified pound cake formula led to the basic yellow or white layer cake (Table 4). Unlike yellow layer cake, no yolks are used for white layer cake. These layer cakes adapt readily to many formula variations by the addition of fruits, nuts, spices, cocoa, etc. In chocolate layer cakes, the sugar level appears higher, which is due to a reduction in the flour level when cocoa is added. Functionally, low-fat cocoa acts much like flour. High-ratio cakes evolved from layer cakes through the use of emulsified shortenings and may contain as much as 140% sugar (based on flour weight).

# Chemical and Physical Changes during Mixing

Mixing plays an important role in the production of quality of batter-type cakes. The objectives of mixing are to disperse the various ingredients uniformly, and to incorporate air into the batter while minimizing gluten development. There are four different basic methods of mixing: creaming, blending, single-stage, and foam-type methods.

In the creaming, or conventional method, fat and sugar are mixed at low-to-medium speeds until thoroughly blended and aerated. Large volumes of air are incorporated into the fat phase in the form of small air cells and the sugar crystals are encased in the shortening. Next, the eggs are added with continuous beating until the mixture is fluffy and well aerated. Last, the flour and milk are added. The main advantages of this method are: (1) the large number of small air cells in the batter; and (2) gluten development is limited due to delayed hydration and solubilization of the fat-coated flour and sugar particles.

The blending method consists of two separate mixing steps. The shortening and flour are beaten until fluffy in one bowl and the eggs and sugar are whipped separately in a second bowl. These two mixtures are blended together followed by slow addition of the milk. This method produces a cake with a very fine grain and uniform texture. Compared with the creaming method, aeration is lessened, thus reducing cake volume, but higher sugar and liquid levels are possible.

The single-stage mixing method was devised to reduce the number of steps and shorten the mixing-time requirements. All the major ingredients are mixed together at once. Although the procedure is simple, the texture and stability are sacrificed somewhat. This method is generally used for premixed box cakes, but not by the wholesale trade.

<sup>&</sup>lt;sup>b</sup>In the USA, sugar would run to 120 or more.

Unlike shortening-based cakes, foam cake mixing depends on air incorporation in an egg-protein matrix for volume and structure development. Egg whites plus sugar are whipped into a stiff foam, into which the flour and other dry ingredients are gently folded. In angel-food cake batters, the egg whites must be fat free to achieve maximum volume; however, in sponge cakes where whole eggs are used, a lower-volume, emulsified foam is formed.

The batter specific gravity is a measure of how much air has been incorporated into the batter during mixing. It is defined as the ratio of the weight of a known volume of batter to the weight of the same volume of water, at a given temperature. Batter specific gravity is directly related to the volume, texture, and grain of the finished cake. In general, the lower the specific gravity, the higher the finished cake volume, but optimal ranges for different types of cake batters have been established.

# Chemical and Physical Changes during Baking

Air bubbles, creamed in the fat, are released into the aqueous phase as the fat is melted at ~40°C. Carbon dioxide is generated by the baking powder and collects in these air bubbles. As the batter heats, the batter begins to flow, due to natural convection currents, because the batter temperature next to the sides and bottom of the pan increases first and that in the center heats last.

The batter viscosity decreases initially upon heating as the fat melts and before the starch gelatinizes. Between 60°C and 70°C, the starch granules absorb several times their weight in water, increasing the batter viscosity considerably. Major swelling of the gelatinized starch granules insures that the cake structure will not collapse. The amount of sugar and some emulsifiers in the formula control the temperature at which the starch gelatinizes. The cake normally

sets into a solid system well below the boiling point of water (100°C).

As the batter temperature reaches ~80°C, the air bubbles enlarge rapidly, causing the cake to rise. The liberation of carbon dioxide, the expansion of air cells, and the formation of steam — all contribute to the leavening effect. Heat causes the gas to expand and the pressure inside the gas cells to increase. Resistance to expansion results from protein coagulation and starch gelatinization. Timing is critical for the protein film to enlarge with the expanding gases, prior to protein denaturation and starch gelatinization, which set the structure. Emulsifiers, whether occurring naturally in eggs or added, improve the elasticity of the protein film surrounding the gas bubbles, enabling them to increase without rupturing.

Moisture evaporates from the cake surface during baking, keeping it cool. However, as most of the water is removed, the surface gets hot enough to brown. Lower baking temperatures should be used for richer (high sugar and fat content) cakes, as the sugars can cause excessive browning of the crust before the interior is set. The baking time for all cakes should be kept as short as possible to avoid too much color development and formation of a thick crust.

A summary of common cake faults and possible ingredient, mixing, or baking-related causes is given in Table 5.

#### **Role of Additives**

One of the main additives in cake production is added to the flour itself. In the USA, cake flour is made by adding chlorine gas to soft wheat flour at a rate of 0.3-1.5 g per kg (0.5-2.5 oz per 100 lb) of flour. This lowers the pH and improves overall baking performance by increasing the volume, and improving grain, texture, and symmetry. The optimal pH range is between 4.5 and 4.8. The mechanism of chlorine on flour

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Possible causes	Common cake faults						
	Low volume	Toughness	Lacking resilience	Poor crust apprearance	Irregular grain	Peaked center	Undesirable color
Improper chemical leavening	×				×		×
Low batter viscosity	×				×	×	
Excessive oven temperature	×	×		×	×	×	×
Insufficient oven temperature					×		×
Egg/milk protein level incorrect		×	×				
Incorrect sugar level or type		×		×			×
Overmixed batter		×		×	×		
Undermixed batter			×		×		

is not completely understood: various researchers have shown that it affects the gluten, starch, and/or lipid components of wheat flour.

Over-chlorinated cake flour will cause the batter to set too quickly around the sides of the pan before full expansion has been reached. The center continues to rise, and the result is a cake with a strong peak. If the flour is under-chlorinated, the structure sets too slowly, allowing the leavening gases to escape, and the center of the cake collapses upon cooling.

Emulsifiers promote air incorporation in the form of fine bubbles and disperse the shortening into small-sized particles. Emulsifiers' unique behavior is due to their ability to bridge the inseparable oil and water phases at the interface. When their concentration exceeds the solubility limit, they form an interfacial membrane whose hydrophilic portions extend into the aqueous phase. The membrane surrounds the dispersed oil and prevents the emulsion from breaking.

Hydrogenated shortenings typically contain 3% emulsifiers, with glycerol mono- and di-stearate being the most common, although many others, including blends, are also used.

Antioxidants are sometimes added to cake mixes to retard the development of oxidative rancidity during storage. All fats are subject to oxidative and hydrolytic rancidity, which causes objectionable odors and flavors, but antioxidants delay these reactions from occurring within the products' shelf life. Four compounds commonly used as antioxidants are butylated hydroxyanisole, butylated hydroxytoluene, t-butyl hydroquinone, and propyl gallate. Citric and phosphoric acids have a synergistic effect when combined with the antioxidants. The levels are limited by law, economics, and functionality, and vary with the additive and product application, but generally fall between 0.005% and 0.1% of the product weight. Natural antioxidants, e.g., tocopherols, offer a desirable alternative to synthetic varieties, but have other usage issues such as heat lability.

Color additives are used in many baked products, including cakes and their icings. Added color can help the product fulfill consumer perceptions and expectations regarding quality, richness, and overall visual appeal. There are two types of color additives — certified and uncertified. The certified colors are synthetic and strictly regulated, whereas the uncertified usually come from natural sources, and usage level varies greatly. The certified colors used in the USA include FD&C blue no. 1, FD&C red no. 3, FD&C yellow no. 5, and FD&C red no. 40. Uncertified color additives include annatto extract,  $\beta$ -carotene, beet powder,  $\beta$ -apo-8-carotenal, xanthins, caramel, carmine, carrot oil, cochineal extract, toasted partially defatted cottonseed flour, fruit and vegetable juices or

concentrates, paprika and paprika oleoresin, riboflavin, saffron, titanium dioxide, tumeric, and tumeric oleoresin.

Many flavoring agents are used in cake batters, icings, and/or fillings. Spices are processed from different parts of aromatic plants, including fruits, barks, or seeds. Some spices commonly used in cakes include allspice, anise, caraway seed, cardamom, cinnamon, cloves, coriander, ginger, mace, nutmeg, poppy seed, saffron, and sesame seed. Some of these act as both flavoring and coloring agents.

Alcohol extracts can also be used to enhance the flavor of cakes. The sapid and odorous volatile components are extracted from aromatic plants or parts of the plant and solubilized in ethanol or propylene glycol. For example, vanilla extract is a ubiquitous flavoring in cakes, derived from the vanilla bean.

Chocolate and cocoa from the cacao tree bean are also popular flavoring agents. However, defatted cocoa powder also adds bulk to the cake, often replacing up to 10% of the flour weight. Often in chocolate cakes, the sugar level and leavening system must be adjusted to compensate for the cocoa.

See also: Bakeries. Breads. Cakes, Pastries, Muffins, and Bagels. Cereals: Grain – Quality Attributes. Cookies, Biscuits, and Crackers: The Diversity of Products. Consumer Trends in Consumption. Cultural Differences in Processing and Consumption. Food Safety through the Production Chain. Milling and Baking, History. Snack Foods, Processing. Starch: Analysis of Quality; Chemistry; Modification. Wheat: Dry Milling; Dough Rheology; Grain Proteins and Flour Quality. Appendix: Test Methods for Grain and Grain-Based Products.

# **Further Reading**

Bennion M (1980) *The Science of Food.* San Fransisco: Harper and Row.

Bennion EB and Bamford GST (1997) In: Bent AJ (ed.) *The Technology of Cake Making*, 6th edn. London: Blackie Academic.

Blanshard JMV, Frazier PJ, and Galliard T (1986) Chemistry and Physics of Baking. Special publication no. 56. London: Royal Society of Chemistry.

Charley H (1982) Food Science, 2nd edn. New York: Macmillan.

Hoseney RC (1986) *Principles of Cereal Science and Technology*. St. Paul, MN: American Association of Cereal Chemists.

Matz SA (1992) Bakery Technology and Engineering, 3rd edn. New York: Van Nostrand.

Pyler EJ (1988) Baking Science and Technology, 3rd edn. Kansas City, MO: Sosland.

Stauffer CE (1990) Functional Additives for Bakery, 5th edn. New York: Van Nostrand.

# CAKES, PASTRIES, MUFFINS, AND BAGELS

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#### Introduction

Cakes, pastries, muffins, and bagels constitute a rich and diverse topic. It is probably easiest to think of cakes and muffins in one group of chemically "leavened," batter-based products, and pastries and bagels as a separate group of dough products, some yeast leavened and some not. All of these products are sold at local grocery stores and bakeries, and have also been taken to high art forms by pastry chefs and artisan bakers. These products have also been successfully scaled up to production lines churning out thousands of pieces an hour. Many of these large production lines produce elegant versions of these products with very little handwork.

# **Cakes**

The variety and diversity of cake products and formulations within the United States are quite large. Also, cake flour and cake formulation vary substantially across the globe. Putting it simply, in the United States, most industrially produced cakes are baked using a high-ratio formula (more sugar than flour) and chlorine-treated cake flour. Most European cake formulas, however, use a lower level of sugar and soft pastry flour.

Bakery items classified as cakes are generally batter products that are relatively high in sugar, fats, eggs, dairy ingredients, and flavorings. Baking powder and air incorporation are common leavenings. Although there are a few savory products that could be labeled as cakes, in general cakes are sweet flavored and have a tender bite. In the United States, there is a subclass of pastries called "coffee cakes" or Danish pastries. These products will be addressed in the section on pastries, as most of these products are yeast leavened instead of chemically leavened.

Cakes are generally categorized by their ingredients and mixing procedure. The most common cake is the "butter" cake that is mixed by creaming the shortening together with the sugar to incorporate air. Air is further entrapped with the addition of eggs to the creamed mixture. Foam-type cakes such as angel food or sponge cakes rely heavily on the eggs in the system to incorporate the air cells. In the past, these products were made by whipping eggs or egg whites separately from the rest of the batter ingredients and then carefully folding the whipped eggs into the system. With the advent of current emulsifying systems, sponge cakes can be made as a single-stage mix by adding hydrated, blended emulsifiers.

In general, batters contain more water or fluid ingredients than doughs. The distinction between doughs and batters is not clear, but usually batters are pourable and flowable in a short period of time (of the order of seconds). Doughs often require cutting to separate a portion of the dough from the mass. Although doughs flow over time, they cannot be easily poured and their flow is generally measured over a time of the order of minutes.

One of the earliest recorded recipes for cakes was a household recipe for pound cake that contained a pound each of flour, butter, sugar, and eggs. Many early bakeries essentially followed this outline for years with individual bakers modifying the formula to vary the butter and egg level and adding milk. After the advent of finely milled patent flour that was chlorine treated in the 1930s' America, formulas were more dramatically altered to have higher levels of sugar than flour. This was the advent of the "high-ratio" layer cakes. These high-ratio cakes contain between 100% and 140% sugar on a flour weight basis (fwb) as compared to a sponge-type cake with 30-45% sugar on flour weight basis (Table 1).

Table 1 Sample high-ratio cake formulas (percentages on fwb)

Ingredient	Yellow	Sponge	Devil's food
Flour, chlorine treated cake	100.0	100.0	100.0
Sugar	135.0	45.0	135.0
Shortening	55.0		50.0
Milk, nonfat dry	4.0	3.0	4.0
Eggs, dry whole	12.0		12.0
Eggs, dry whites	3.0		3.0
Eggs, liquid whole		100.0	
Baking soda		1.0	2.0
Baking powder, double acting	0.6		2.0
Salt	1.0	1.5	1.5
Cocoa, alkalized red			16.0
Water	130.0		180.0
Hydrated sorbitan monosterate		4.0	
Invert sugar			15.0

## Flour for Cakes

Cake flour in the United States is generally milled from soft red wheat grown in the Ohio River valley states (Ohio, Indiana, Illinois, and Michigan). This type of wheat is also grown in some more southern states such as Missouri, Arkansas, Tennessee, and Texas. The millstreams collected for cake flour are typically those lowest in ash content with an extraction rate of 50-75%. This flour has a small particle size compared to a bread-type flour and is often treated with gaseous chlorine. Most importantly for cakes, the gluten must be of a quality that aids in the forming of films for trapping gas in small air bubbles, but not the quality that lends the toughness/chewiness commonly found in bread flours.

Gaseous chlorine is used as an improver of soft wheat flour for cakes in the United States alone. Gaseous chlorine reacts with nearly all of the components in flour and has profound effects on many of them. Some of the effects of chlorine on flour include bleaching of pigments by addition across conjugated double bonds, lowering pH of flour slurry by freeing of hydrogen ions from pigments, lipids, proteins, and starch, and oxidizing and depolymerizing starch. Most of the chlorine gas reacts first with the lipid and water-soluble fraction. The end result of the addition of gaseous chlorine is that the cake batters are better able to expand during baking and collapse less upon removal from the oven.

In the European Union, law bans the use of chlorine gas to treat flour. Alternative treatments such as heat, light, and ozone treatments have been investigated and currently some cake flour is heat-treated to "improve" the baking quality of the flour. This improvement gives small changes in the flour quality to improve the cake baking quality but not the dramatic changes seen with chlorine gas.

## **Shortenings**

The other major technology improvement was the advent of "emulsifiers" and new emulsified shortenings which were much more consistent than their simpler nonemulsified counterparts. Air cells cannot be added to the mass by any means other than mechanical agitation. The gases created by chemical leavening or by steam "add" to the volume of the air cell created during mixing, but cells are "created" during mixing. The industry has found several methods for aiding in the formation of these cells and protecting them, once formed, by changing the surface tension of the material at the cell/mass interface. Emulsification is one method that protects air cells by changing the surface properties of the interface. Common emulsifiers for cakes such as lecithin or mono- or diglycerides

have a "hydrophilic" and "hydrophobic" region on the molecules. This allows the materials in the batter to interact more closely and in thinner sheets. This results in better dispersion of the emulsified shortening throughout the batter, forming smaller, more uniform air cells, which in turn produce a tender cake with a fine texture.

#### **Sweeteners**

In most parts of the world where granulated sugar prices are low, granulated sugar is the exclusive sweetener found in cakes. Granulated sugar imparts sweetness and tenderness to the texture of the finished cake. The crystalline structure and granulation size of the sugar helps in formation of air cells in the batter, and it contributes to the viscosity of the batter. In the United States, where sugar prices are higher, it is common to substitute part or all of the sugar with high fructose corn syrup (HFCS). When this change is made, there are many formulation changes that are required to make a similar cake. Because liquid sugars introduce more liquid to the cake batter, as well as reducing sugars, the cake formula must be altered. These changes include varying the fat level and/or type, changing the emulsifier level and/or type, and changing the liquid levels. The risk one takes when making these changes is that the high level of "reducing sugars" in the presence of protein in eggs and milk common to most cakes results in a product which is darker in both crust and crumb.

Low-in-sugar or "no sugar" cakes can also be made in several ways. The first is by adding one of several high-intensity sweeteners coupled with a bulking agent such as polydextrose. Another method is to substitute sugar alcohols such as sorbitol or xylitol for the sugar in the formula. Lastly, a new variety of sweeteners that are based on sugar (i.e., sucralose) can be substituted one-for-one for sugar. All of these methods will likely require some modification of the formula ingredients and process to compensate for the changes in viscosity and eating character of the cake when sugar (sucrose) is removed.

# Leavening

Leavenings are a critical factor in aiding the formation of the aerated structure one expects to find in cakes and cake batters. Chemical leavenings commonly found in cake formulations are blends of leavening acids and sodium bicarbonate. The action of the acid portion of the leaveners is largely dependent on the temperature at which the acid reacts with the sodium bicarbonate in the solution, as well as on the level of bicarbonate present. Some acids that are quick to solubilize are leaveners like monocalcium phosphate

(MCP) monohydrate and some of the fast acting sodium acid pyrophosphates (SAPPs). These largely react in the mixing bowl to add gas to the batter, thus adding volume and viscosity to the batter while it is being mixed. Other acids require the higher temperatures of the oven to solubilize. These include the most common second acid in double acting baking powders, sodium aluminum phosphate (SALP), some slow acting SAPPs, and sodium aluminum sulfate (SAS). Double acting baking powders are designed to include both primary acids that solubilize at room temperature during mixing and secondary acids that solubilize in the oven at elevated temperatures.

When designing leavening systems for cakes or any other chemically leavened food products, it is important to balance the alkaline component (sodium bicarbonate) with the acid component or components to achieve complete neutralization in the finished food. Leavening blends may be formulated by mathematical calculation using an equation called the "neutralizing value" (NV). NV is defined as the weight of sodium bicarbonate required to completely react with 100 parts by weight of a leavening acid. This is captured by the following equation:

$$NV = \frac{g \text{ of sodium bicarbonate} \times 100}{100 \, g \text{ of acidic salt}}$$

Neutralizing values for many common leavening acids have been included in Table 2.

#### **Other Cake Ingredients**

There are a number of minor ingredients and flavoring ingredients that are used in modern cake production. It is difficult to find a cake produced in the United States without a modified starch or a gum. Modified starches and gums help to add viscosity during processing. This helps in stabilizing the air cells and thus in producing a fine textured, soft cake. These ingredients also act as a tenderizer by diluting the flour and

Table 2 Types of leavening acids and their neutralizing value

Leavening acid	Common abbreviation	Neutralizing value
Dicalcium phosphate dihydrate Monocalcium phosphate Monopotassium tartrate Sodium acid pyrophosphate Sodium aluminum phosphate Sodium aluminum sulfate	DCP MCP Cream of tartar SAPP SALP SAS	33 80 45 72 100

help maintain moistness in the cake throughout storage. There are also very few cakes made without vanilla or vanillin or other flavoring ingredients. One very important additive is cocoa or chocolate.

Melted chocolate can be added to many cakes during the creaming stage of mixing along with the shortening and sugar. These cakes can be labeled in the US as chocolate cake. More often, cocoa is used to impart the "chocolate" flavor and color as desired by consumers. These cakes cannot be labeled as "chocolate." However, there are several common names that are used for these cakes such as "devil's food" or "fudge." Cocoa is commonly added to cakes either in the creaming stage or in the last stage of mixing along with the flour. In the latter method, the cocoa is usually sifted with the flour to prevent clumping of the cocoa, as it is a rather hydrophobic ingredient. The cocoa used in cakes is usually treated with alkali to produce dutched cocoa; therefore, controlling the pH of the finished cakes is very important because it affects the color and flavor of the finished product. Natural, or untreated, cocoa is often used in frostings, icings, and fudge. Control of the pH in all "chocolate" cakes has a large impact on the flavor and color of these cakes. As a result, many of them are baked with sodium bicarbonate only to ensure that the pH stays in the alkaline region.

# **Pastries**

Pastries are defined as any products made from any of several different doughs that include fats, flour, and water. Examples of pastry include puff pastry, pie dough, or pate sucree (a sweet short pastry). Pastries can also be a general term for sweet baked goods such as Danish pastries or coffee cakes. This is a broad and diverse category of bakery products. It is easiest to divide pastries into dough products that are laminated and those that are not (Table 3).

Table 3 Sample pastry formula (percentages on fwb)

Ingredient	Puff	Danish	Pate sucree
Flour, pastry	100.0	25.0	100.0
Flour, bread		75.0	
Sugar, granulated		20.0	
Sugar, powdered			38.0
Eggs, liquid whole		20.0	0.5
Egg yolks, liquid			32.0
Water, cold	50.0	50.0	
Salt	2.0	2.0	1.5
Shortening or butter	75.0	20.0	45.0
Yeast		7.0	
Milk, nonfat dry		6.0	
Vanilla extract			0.1

# **Laminated Doughs**

Laminated dough products can be further subdivided into those that contain yeast and those that do not. The latter are commonly referred to as "puff pastry." The products that do contain yeast include the broad category known as Danish pastries as well as croissants. In both of these types of products, development of gluten structure is important to the finished product quality. The dough for all of these products must be able to tolerate the stresses in multiple passes through reduction rollers without ripping. In general, the yeasted laminated products are made with slightly lower protein content flour than the puff pastry products. But for all of these products, flour quality and laminating shortening quality are equally important. Processing conditions and resting of the dough between times of stress also play an important role in manufacture of great laminated dough.

Flours used for puff pastry vary from region to region. In the United States, it is not uncommon for pastry chefs to use "bread" flour with a protein level of 11–12% for their puff pastry. This high-quality flour with a relatively high protein level allows the dough to sheet into very thin layers between the layers of fat. In the United Kingdom, where flours tend to have less gluten strength, it is not uncommon for the flour to be supplemented with additional vital wheat gluten to give the final dough the strength that is needed to form the products. This practice may not be appreciated by traditional pastry chefs who roll their laminated doughs by hand, but with the rigors of mechanical sheeting, doughs have different levels of stress placed on them and fortification with gluten may be required to handle the added stress. Some processors of laminated doughs mix the flour, water, and other minor ingredients into a well-developed gluten structure prior to mixing fat to the dough. They believe that this helps shield the proteins from abuse during sheeting. More likely, it just shortens the mix time by allowing the gluten protein matrix to develop before the shortening is added. Shortening lubricates the flour and protects it from absorbing water as quickly, slowing hydration of the gluten proteins; therefore, it usually requires longer mix times to develop the gluten.

Fats or shortenings are used in two ways in laminated doughs. "Dough fat" is the shortening that is added to the flour and water and other ingredients that comprise the dough. "Laminating fat" is the shortening or butter that is formed into thin layers between the thin dough layers during the sheeting and folding of the dough. This laminating fat is also often referred to as "roll-in" shortening. Danishes and puff pastries use butter, margarine, or vegetable

shortening as laminating fats. In any case, the laminating shortening must be relatively plastic at the cool temperatures used to make most of the doughs  $(10-20^{\circ}\text{C})$ . The laminating shortenings must also have a controlled melt point of 39-44°C so that they do not taste waxy upon consumption. Some roll-in margarines and butter contain a significant amount of water but many commercial bakers prefer the control and crisper texture that they get with butter, lard, or shortenings that are devoid of water.

Water is very important in laminated products for the formation of a dough as well as to aerate the products through steam expansion. As steam leaves the dough or the laminating fat during the heat of baking, it pushes against a solid barrier layer of the laminating shortening, pushing the layers of dough apart to create the height and flakiness desired in the product. Increase of water level in the dough of laminated products has little effect on the finished product, but can affect the sheeting and handling properties of the dough. If too little water is added to the dough, this can restrict the dough during proofing and baking and give a final product that is low in volume.

Sugar, egg, and milk added to the dough of laminated products can greatly influence the final finished product. Sugar not only increases the sweetness of the dough and finished product, but also can decrease finished height while increasing shrinkage when added at levels up to 20% (fwb). In general, adding sugar to dough tends to soften the dough, increasing the dough stickiness, and difficulty in sheeting. Sugar also contributes to the color of the finished product. One important consideration when using high levels of sugar in yeasted pastry doughs is that the high sugar levels inhibit the yeast fermentation by elevating the osmotic pressure. On the other hand, levels of sugar below 12% (fwb) have not been reported to inhibit fermentation for most common yeasts used in baking.

Small levels of egg added to pastry doughs can provide some additional protein support to the products as well as the emulsifying benefits of indigenous lecithin. Pastry products with up to 3% egg yolk tend to bake taller and are more tender. At higher levels of egg, the color and flavor of the egg become evident and can contribute to inappropriate browning and off-flavor development.

Much of what makes the laminated doughs so popular with consumers is the shaping and forming of the doughs with added fillings and toppings. It is not uncommon in local bakeries and in full-scale production facilities to see croissants made not just plain but with fillings such as chocolate, apple, sweet almond paste, raspberry, and cream cheese, or a full-scale breakfast such as scrambled eggs and cheese. Coffee cakes are

often braids of these sweet, laminated doughs with apple filling and streusel topping. Many of the dough formulas vary little, but the shapes, fillings, and toppings are endless.

#### Short Doughs, Pie Doughs, and Pate Sucree

Short doughs and pie doughs are slightly sweet and very rich doughs containing high levels of fats. Pate sucree is also a very rich dough, but it is also high in sugar, similar to a shortbread cookie. Pate sucree can contain up to 50% butter (fwb) and 30% sugar (fwb). Pate sucree and pate sablee are both short doughs with similar formulas. The two doughs differ only in their production methods.

Many short doughs and pate sucree are made in similar fashion. The first step is creaming of the short-ening or butter with the sugar. The second step is creating an emulsion with the liquid ingredients, including eggs. The last step is the addition of dry ingredients, including flour and leavenings.

Pie doughs and "pate sable" are made differently. With this product, the shortening is cut into the dry ingredients including the flour and sugar until fine, and the liquid ingredients are added last. The two different mixing procedures give different finished products with the short doughs and pate sucree having a less tender bite and the pie doughs and the pate sablee being more tender, and in the case of pie doughs, flaky texture.

#### **Muffins**

The history of the bakery item that we now call "muffins" is not clear. There are several distinct foods that bear the muffin moniker. "English" muffins are toast-able rounds that are split in the center and generally served as a toast replacement. The "English" muffin is generally baked in a round form on an oven band and is a simple formula with little additional sugar and fat. The bakery muffins that are addressed in this article are readily identifiable by their shape. The muffin shape comes from baking in a "cupcake" or "muffin" pan. They are recognized by their stout "mushroom-like" shape. Muffins can also be identified by formulation. Most muffins are baked from a chemically leavened batter into a moist, coarsegrained mini-cake. Muffins range in formula from those that are rather low in fat, sugar, eggs, and milk to those that are richer in fat and sugar than many cakes.

Ingredients commonly found in muffins include flour, sweeteners, fats, eggs, milk, chemical leavening, salt, and characterizing ingredients such as bran, fruit, corn meal, chocolate chips, or chopped vegetables. The flours commonly used in muffins have to withstand the high levels of sweeteners and particulates. Although there are some muffin formulations that use the chlorine-treated cake flour used in high-ratio layer cakes, most muffin formulas are better suited to a blended flour of pastry flour and bread flour otherwise known as "all-purpose" flour. Some formulas even specify higher protein bread flour. The addition of the higher-protein flour helps create a product that is slightly chewier and coarser in crumb than a cake. Most US muffin consumers expect this chewier, coarser texture (Table 4).

There are some other carbohydrate-based ingredients that are added to some muffin formulas. Some modified starches and gums are added to lengthen shelf life by maintaining a higher-moisture product. In some muffin batters with large particulates or significant quantities of particulates, the batter viscosity can be increased by adding small amounts (0.05–0.2% fwb) of gums, like xanthan or guar. This increase in viscosity helps the particulates stay evenly dispersed throughout the batter during processing.

The most commonly used sweetener for muffins produced in a small-scale bakery is granulated sucrose. Other sugars may be used in these bakeries for specific uses such as honey or molasses to contribute a specific flavor profile or color. Corn syrup may also be used to help contribute color. In large-scale bakery operations, corn syrup or HFCS may be used to lengthen the shelf life of the muffin products. When adding these reducing sugars, care must be taken not to over-brown the products.

As with cakes, nearly every muffin formula has a substantial quantity of eggs and milk. In bakery mixes, these ingredients may be added as dry, powdered ingredients to the mix, thus reducing the likely

Table 4 Sample muffin formulas (percentages on fwb)

Ingredient	Simple	Blueberry	Bran
Flour, all purpose <sup>a</sup>	100.0	100.0	75.0 <sup>b</sup>
Sugar, granulated	80.0	115.0	50.0
Shortening	30.0	85.0	35.0
Baking powder	5.0	2.0	2.0
Baking soda		1.5	3.0
Nonfat dry milk	4.0	4.0	10.0
Whole egg powder	10.0	7.5	5.0
Salt	1.5	2.0	1.5
Bran			25.0 <sup>b</sup>
Molasses, light brown sugar			25.0
Honey		10.0	25.0
Water	65.0	70.0	100.0
Blueberries, fresh whole		0.08	

<sup>&</sup>lt;sup>a</sup> All flour is all-purpose type except for the blueberry muffin which would require a chlorine treated cake flour.

<sup>&</sup>lt;sup>b</sup> For the bran muffin, wheat-based ingredients (flour plus bran) equal 100%.

mistakes in baking and guaranteeing a more consistent product. Eggs are added to muffins to contribute their flexible proteins to the structure of the muffin. Also, eggs contain a natural lecithin that aids in mixing of the batters. Milk is added in large part because lactose and milk proteins contribute to the browning of the finished product.

Most muffins are deceptively high in fats. The average consumer is likely not aware that muffins can have more than 40% fat (fwb). Many muffins are made with vegetable oil (healthier for heart) rather than with a hydrogenated shortening, but muffins still contain a substantial amount of fat. Most muffin formulas do not require an added emulsifier unless very little egg is used in the formula. When emulsifiers are added, they are likely to be lecithin or mono- and diglycerides found in emulsified shortening.

Wide ranges of chemical leavenings are used in muffins – from the addition of simple sodium bicarbonate to a complex double-acting baking powder. Most muffins are rather simple in their mixing, with little air incorporation by whipping. Most of the air is trapped in large air cells, thus adding to the coarse texture of a muffin. There are some muffins made using a more cake-like formula and production method that will give a smaller air cell and a finer texture. Often the muffin formulas rely on a late acting acidulant such as SALP to aid in the formation of cell structure.

Leavening is also one of the major influences on the finished shape of the muffin. Most muffins have either a mushroom top or a simple peak top. Mushroom-topped muffins have a leavening balanced to force the batter to expand and flow before a crust is formed on the top surface of the muffin pan. Additional leavening, in the form of a late-acting acidulant, remains to push the center of the muffin up, thus forming the mushroom cap effect. These mushroom-topped muffins also are frequently richer in formula and have more batter in the cup than a peak-topped muffin. The peak-topped muffins are usually baked at a higher temperature (above 400°F) and the leavening is designed to act early in the baking process so as not to crack the top after the top crust is formed.

One of the characteristics of muffins is the addition of fruits, nuts, chips, wheat or oat bran, or other strongly flavored particulates. These additives can be added as fresh fruit (blue berries, peach, or apple chunks), whole or chopped nuts, chocolate or other flavored chips, or fabricated bits of flavored material. The addition of these ingredients adds to the perception of quality and overall wholesomeness of the muffin. Also, the particulates act to break the structure of the muffin and change the texture. Toppings are also common with muffins and can be as

simple as a cinnamon/sugar blend or as complex as a nut streusel. Toppings are also often materials meant to "sink in" to the muffin creating a "filling" such as the addition of a sweet cream and cheese mixture. Again, this topping adds to the complex nature of the muffin, creating changes in texture and flavor as the consumer eats the product.

## **Bagels**

Bagels have a unique history and formulation. The word bagel comes from the Yiddish word, *beygel*. Bagels are thought to have originated in Poland, where Polish bakers, in an effort to honor their victorious horse-riding king, made a stirrup-shaped roll and boiled it before baking. This boiling helped keep the shape of the roll during subsequent baking.

Jewish immigrants who moved to the Northeastern United States brought bagels to America. Bagels were made ubiquitous in the United States by a successful Polish-American baker, Harry Lender, and his sons, who used humor in advertising to reach out to middle America with a traditionally ethnic, northeastern food.

In general, bagels have a very simple formulation. The formula is very similar to simple bread or roll formulas: flour, salt, yeast, and water. What separates the bagel from the rest of the rolls is the flour quality and the processing. Traditional bagels were made with very high protein, spring wheat flour. The flour for bagels has often 13–16% protein content. Spring wheat grown in the northern plains of the United States and southern Canada produces most of the flour used in baking traditional bagels made today. Many major manufacturers of bagels use less expensive flour in the 12.4-13% protein range but still are forced to pay a premium for the higher protein content. The high protein content of the flour and the mixing of that flour to full development helps give bagels their characteristic chew (Table 5).

The other significant distinction for bagels is the processing. Traditional bagels develop much of their flavor

Table 5 Sample bagel formula (percentages on fwb)

Ingredient	Plain	Egg	Onion
Flour, high protein <sup>a</sup>	100.0	100.0	100.0
Yeast, dry	0.5	0.5	0.5
Yeast food	1.5	1.5	1.5
Salt	3.0	3.0	3.0
Sugar, granulated	5.0	5.0	5.0
Water <sup>b</sup>	65.0	70.0	70.0
Egg, whole powdered		4.0	
Onion, dry toasted			2.0

<sup>&</sup>lt;sup>a</sup> Flour should have added malted barley flour.

<sup>&</sup>lt;sup>b</sup>Water varies by flour requirements and by levels of dry ingredients requiring some hydration.

and distinct crust characteristics from a long, cold fermentation step called retardation. Among large-scale bagel makers, this retardation step has been either a bulk retardation of the whole dough mass or an individual fermentation of preformed bagels. The bulk retardation has more effect on flavor and internal crumb character than on crust characteristics. Piece retardation also affects flavor and crumb characteristics, but also has a significant effect on the crust characteristic. The air cells on the surface of the bagel collapse and the surface dries somewhat giving a thick, chewy crust after baking. This collapse and coalescence of cells is also seen in the crumb. Bagels that have been through a retardation process tend to have a more coarse, thick-walled cell structure than a nonretarded bagel.

The dough for bagels in high-speed production is divided in one of the many typical methods found in roll production. The formation of the ring structure is rather unusual and there are very few manufacturers of the equipment necessary to make the rings. One method deposits a dough ball on the end of a belt that moves the ball under a plate to form the dough into a cylinder and then around a mandrel to form the ring. These are called horizontal formers. The second method in a vertical former where the dough ball is dropped into a series of hinged cups that form the dough around a mandrel.

The last processing distinction for traditional bagels is the boiling process. The setting of the structure of the dough ring can be achieved through an application of moisture to the dough surface either through steam injection into the first zone of a tunnel oven or by moving the dough rings through a boiling water bath. This bath water can contain malt or sometimes a sugar but often it is just simple water that becomes saturated with starch and dextrins. There are advantages to both methods of water application, but the finished food is different. Boiled bagels tend to have a more distinct hole and a thicker, chewier, shiny crust. Steamed bagels tend to have a softer crust.

Bagels may, and often do, contain other ingredients. They can be made with a variety of added grains, fruit and vegetable pieces, nuts, spices, eggs, garlic, cheeses, etc. Bagels often contain dough modifiers such as fats, emulsifiers, malt, enzymes, and other processing aides similar to those typically found in roll or bread formulations. Shelf stable bagels frequently contain the typical preservative systems of breads (i.e., Ca or Na proprionate) and crumb softening systems. The crumb softening systems are usually a combination of starch-modifying enzymes and moisture-holding ingredients such as gums or modified starches. In general, bagels are lower in fat than some other baked goods, but sometimes bakers make

up for this by addition of fat-containing ingredients such as cheeses.

Bagels are also often baked with toppings on them such as seeds, finely chopped onion, or salt. These toppings help contribute some of the characterizing flavor and appearance of flavored bagels. The toppings are often coated on the top of the bagels after boiling and before baking. If the bagels are baked using a steam-injected oven, the toppings may be adhered to the surface of the dough using a wash-like modified starch or a gum that acts as an adherent to keep the toppings on the bagel dough.

See also: Cakes, Chemistry of Manufacture.

## **Further Reading**

Atwell WA (2001) Wheat Flour. St. Paul, MN: Eagen Press.

Benson RC (1988) *Muffin Technology*, Technical Bulletin, vol. X(6). Manhattan, KS: American Institute of Baking. Cauvain S (2002) Laminated bakery products: influencing the outcome. March/April, pp. 48–53. European Baker. Daniel AR (1963) *Bakery Materials and Methods*, 4th edn.

England: Elsevier Applied Science.

Hoseney RC (1995) *Principles of Cereal Science and* 

Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Knight JB and Kotschevar LH (2000) Bakeshop production. In: Knight JB and Kotschevar LH (eds.) *Quantity Food Production, Planning and Management*, 3rd edn. New York: Wiley.

Manley D (2000) *Technology of Biscuits*, *Crackers and Cookies*, 3rd edn. Boca Raton, FL: Woodhead Publishing Ltd. and CRC Press.

McWilliams M (2001) Baking applications. In: McWilliams M (ed.) Foods: Experimental Perspectives. Upper Saddle River: Prentice-Hall.

Pyler EJ (1988) Baking Science and Technology, 3rd edn. Merriam, KS: Sosland.

Townsend GM (2001) Cookies, cakes and other flour confectionary. In: Dendy DAV and Dobraszczyk BJ (eds.) Cereals and Cereal Products: Chemistry and Technology. Gaithersburg, MD: Aspen Publishers.

Willyard M (2000) *Muffin Technology (Update)*, Technical Bulletin, vol. XXII(10). Manhattan, KS: American Institute of Baking.

#### **Relevant Websites**

http://www.aibonline.org. http://www.asbe.org. http://www.bsb.org.uk. http://www.bakeryandsnacks.com. http://www.foodnavigator.com. http://www.kingarthurflour.com. http://www.bakingall.com.

## **CANOLA**

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## **Genetics and Breeding**

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There have been dramatic advances in the improvement of oilseeds over the past couple of decades. The improved quality has brought recognition to rapeseed oil as a nutritionally superior edible oil since the erucic acid content was decreased to zero, and to meal as an important source of protein for animal feeds after the glucosinolate content was decreased to  $10-12\,\mu\mathrm{mol}\,\mathrm{g}^{-1}$ . To emphasize these improvements, the term "canola" has been introduced to refer to seed and seed products of "double zero" cultivars in Canada, and then extended to the world.

### Germplasm in Brassica

The botanical relationship between the *Brassica* oilseed species was established as a result of taxonomic

studies carried out in the 1930s (see Figure 1, U's triangle). There were three primary species with a single cycle:  $B.\ rapa\ (A,\ n=10),\ B.\ oleracea\ (C,\ n=9),\ and\ B.\ nigra\ (B,\ n=8).$  The secondary species with double cycles  $-B.\ napus\ (AC,\ n=19),\ B.\ juncea\ (AB,\ n=18),\ and\ B.\ carinata\ (BC,\ n=17)\ -$  were amphidiploids derived from the primary species. These relationships have been confirmed by the artificial synthesis of  $B.\ napus$ . Recent studies using biotechnology also indicate that some revision of these species and related genera may be necessary.

There are totally ~43 000 genetic germplasm accessions in the world (see Table 1). The main cultivated species — B. napus, B. rapa, and B. juncea — account for three-quarters of all Brassica germplasm. B. rapa and B. juncea are the major parts, 34.09% and 27.2%, respectively. B. napus is widely grown, but accounts only for 14.32% of the germplasm. These data mean that B. napus lacks in genetic diversity. Breeders have resynthesized new B. napus by crossing or protoplasm fusion between B. rapa and B. oleracea according to U's triangle.

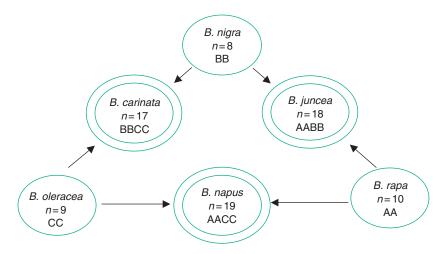


Figure 1 Genomic relationship of the *Brassica* species (U's triangle).

Table 1 Germplasm accessions of oil crops in Brassicaceae

Species	Number
B. rapa	14 631
B. juncea	11 705
B. napus	6 147
B. nigra	1 047
B. species	759
B. carinata	735
Sinapis alba	353
Eruca sativa	339
Crambe spp.	78
B. tournefortii	23
Camelina sativa	17
Total	42 919

## **Genetics and Breeding**

#### **Yield**

Improvement for yield is a major goal in plant breeding. It is also very difficult to achieve. The measurement of yield is usually done in small plots. The yield of rapeseed is composed of three factors: pods per area (square meter), seeds per pod, and weight per one thousand seeds. All of them have shown a positive correlation with the yield, but the relationships between the factors are negative. The influence of environment on the yield of rapeseed is much more than that of cereals. The contribution of each factor to yield is different in different areas, so breeders select the different factors to improve the yield. For example, breeders in Sweden need to increase the weight per pod, but those in Poland must increase the weight per one thousand seeds. The number of pods in the main inflorescence is widely used. Since the yield is affected by many characteristics and the environment, breeders have proposed another approach to select for an ideotype. This ideotype is a list of desirable traits for a population that is expected to give higher yields because of greater efficiency in plant structure and in allocation of resources for plants.

Heterosis can also be utilized to increase the yield. Generally, hybrid yield is 20% more than that of the highest-yielding parent. There are three useable systems to produce hybrids in rapeseed. The first system is cytoplasmic male sterility (CMS), such as polima, ogura (kosena), nap, msl (male sterility Lembke). Hybrid seed production based on this system must have three lines: CMS line (A), maintainer (B), and restorer (R). An interval sowing of A and B is to maintain A line supplies. Another interval sowing of A and R is to produce hybrid seed. This method is very simple, and the cost is very cheap. The second system is genic male sterility (GMS). This way is not usually an economic method for hybrid seed

production because of the need to remove 50% male fertile plants from female lines, but some sources of GMS are being developed as a temporary maintainer to overcome this necessity. The third system is self-incompatibility (SI). One of the major problems of this system is the effort and cost involved in increasing the SI lines by, for example, bud pollination, controlled CO<sub>2</sub>, spraying salt liquid.

#### **Oil Content**

Oil content in most cultivars of rapeseed is  $\sim 38-44\%$ . It is controlled by multiple genes, also strongly influenced by the environment, particularly temperature, moisture stress, and soil nitrogen. To increase the oil content is a long breeding program. The general strategy for this goal is to employ a recurrent selection or selected progenies derived from higher oil content combinations. However, there is a negative correlation between oil content and protein content (which affects meal quality). Breeders must often choose a suitable index (oil + protein) to increase both of them.

Yellow seedcoat has been shown to be associated with low fiber content and therefore higher oil and protein content. The trait is more common in *B. rapa* and *B. juncea*, but is less common in *B. napus*. The inheritance of the trait depends on the species and sources of genes, but there is no general agreement. Recent work, using genes from other species in U's triangle, seems more useful.

#### **Oil Quality**

Oil quality is determined by its fatty acid composition. The nutritional and economic values are dependent on the content of each component in the oil. The goal in improving the oil quality is to reduce the terminal products in the fatty acid synthesis pathway, such as erucic and linolenic acids. Old rapeseed cultivars have a higher erucic acid content. The first lowerucic-acid genes were found in Liho, Germany. This foundation has led to the first low-erucic-acid rapeseed (LEAR) cultivars. Erucic acid content is determined by the embryo genotype, and is controlled by one gene in B. rapa and two in B. napus and B. juncea. Selection for low-erucic-acid content is always by transferring from an LEAR. On the other hand, high-erucic-acid rapeseed cultivars have some markets to supply. Linolenic acid reduces the oil storage time because of its three unsaturated bonds in the carbon chain. The selection for low linolenic acid content has not been easy, as the content does not vary much in the germplasm and it is a necessary product of photosynthesis. Even breeder can select low linolenic content lines from the mutations

induced by some chemicals and irradiations, and the cultivars do not have a higher yield potential than the conventional cultivars.

The fatty acid composition can be changed by reducing the content of saturated fatty acid and by increasing the content of polyunsaturated fatty acid, to regulate the ratio of linoleic and linolenic acids to be 2:1.

#### **Meal Quality**

Canola meal for feed use is recognized for its consistent quality and cost-effectiveness. The meal quality is defined by the glucosinolate content. The glucosinolate is a series of compounds that have a core structure (-S-C=N). The compounds hydrolyze (when the seed is crushed) to produce isothiocyanates, which reduce the amounts of meal that can be incorporated in livestock feed. Bronowski, a Polish cultivar, has been identified as having low glucosinolate content. The genetic analysis has shown that inheritance of the trait involves three pairs of recessive genes at least. The mother-plant genotype determines the glucosinolate level in the seed.

Breeding for low glucosinolate content is usually to transfer the trait by crossing between high and low glucosinolate level. The frequency of the plant with low glucosinolate is less than 2% in an F<sub>2</sub> population. There are a series of methods to analyze its content in seeds, such as the glucose test tape which is used for urine sugar analysis, near-infrared reflectance (NIR), high-performance liquid chromatograply (HPLC), etc. Recent research has indicated that the glucosinolate may be related to some diseases and pest resistances.

There are a series of unhealthy components in the meal, such as tannins, phenolic compounds, phytic acid, etc., which should be reduced.

#### **Diseases and Pest Resistance**

Stem canker (blackleg) is caused by *Leptosphaeria maculans*. It is an important disease of *B. napus* in most countries, except China. Different areas have different pathogen groups. Sources of the resistant genes have been found within the *B. napus* species from Europe and Australia, *B. juncea* and *B. sylvestris* (a wild form of *B. rapa*). The inheritance has different modes depending on the gene sources. Methods of screening genotypes of resistance have been developed using natural and artificial inoculations. The molecular markers related to some loci have been identified and applied to breeding programs.

Stem rot (*Sclerotinia*), the most important disease of rapeseed in central China, is also a major cause of crop loss in France, Germany, and other parts of

Europe. The main agent is ascomycete *Sclerotinia sclerotiorum*. The wide range of the fungus hosts and failure to find gene sources of resistance in many species pose a difficult challenge for breeders. Infection mainly occurs because of the fungus entering the plant by senescing petals that have dropped onto leaves or in leaf axils as a medium for penetration by the ascospores. When the fungus invades the host, oxalic acid is released quickly, which causes necrosis. The breeders have two ways to control the disease: one is to develop apetalous cultivars to block this invaded route and another is to introduce a gene for oxalate oxidase by transformation.

There are many other diseases in rapeseed, such as white rust in *B. juncea* and *B. rapa* caused by *Albugo candida*, alternaria leaf and pod spot caused by *Alternaria brassicae*, light leaf spot caused by *Pryenopeziza brassicae*, white leaf spot caused by the ascomycete *Mycosphaerella capsellae*, club root caused by *Plasmodiophora brassicae*, and virus diseases. For some diseases, intraspecific crossing or genetic transformation may be the only recourse to incorporate resistance into susceptible species.

For the time being, insecticide will remain the basis of insect pest control in *Brassica* oilseed. The breeding for insect pest resistance has not advanced much. Recent work in biotechnogical methods of plant breeding may allow entomologists to design specific oilseed plants for resistance.

#### **Lodging and Shattering Resistances**

There are clear differences of lodging and shattering resistances between cultivars, but the inheritances of the traits are unknown. Breeders select the resistances by evaluating plot trials in the field. The sorts of the traits depend on the eye of the breeders and cannot be defined easily. The lodging resistance may be correlated to the flexibility of the stem and the height of the plant. Seed loss through shattering at harvest time is a problem in *B. napus* but less so in *B. rapa* and *B. juncea*. Attempts have been made to introduce resistant genes from other species but the inheritances are complex.

## **Application of Biotechnology**

Biotechnology has a major impact in three areas. First, it is the application to tissue culture. In microspore culture, one derives a whole plant from the numerous immature microspores in anther by doubling the chromosome number with colchicine. The genotypes of all loci are completely homozygous. This method shortens the breeding time. Protoplasm fusion is applied to introduce some available genes

from other species related to *Brassicaceae*, such as restorer gene for ogura cytoplasm, disease-resistant genes, etc.

Second, it is genetic modification by plant transformation. There have been many great advances in *Brassica* species compared with most other major food crops, such as herbicide resistance, changes in fatty acid composition, pollination control genes (TA29 – barnase, TA29 + barstar), and disease and insect pest resistances.

Finally, it is molecular-assisted selection. Molecular markers can help breeders to select the genotype rather than the phenotype. The selection could be more effective.

Here, it is necessary to present that the model plant, *Arabidopsis*, is very close to the *Brassica* species in genome. All of the information out of *Arabidopsis* researches may be applied to canola improvement.

#### Conclusion

Breeding for the *Brassica* species has played an important role in the improvement of yield, quality, and resistance, and will continue to do so in the future. Breeders need to pay attention to the negative correlations between traits. For example, reducing the glucosinolate content might cause a decrease of the resistances to *Sclerotinia* and pest; increasing the oil content might decrease the yield; increasing the number per pod might decrease the seed weight and the number of pods. Breeders would use all available technologies to regulate all the traits and engineer them into a plant in order to enhance the crop productivity.

See also: Canola: Agronomy. Cereals: Grain — Quality Attributes. Genetically Modified Grains and the Consumer. Genome Mapping. Genomics. Oilseeds, Overview. Plants: Diseases and Pests.

#### **Further Reading**

Downey RK and Röbbelen G (1989) *Brassica* species. In: Röbbelen G, Downey RK, and Ashri A (eds.) *Oil Crops of the World*, pp. 339–362. NY: McGraw-Hill.

Fu TD (1981) Production and research of rapeseed in the People's Republic of China. *Cruciferae Newsletter* 6: 6–7.

Kimber D and McGregor DI (1995) Brassica Oilseeds Production and Utilization. Wallingford: CAB International.

Rashid A, Rakow G, and Downey RK (1994) Development of yellow seeded *Brassica napus* through interspecific crosses. *Plant Breeding* 112: 127–134.

Rimmer SR and van den Berg CGJ (1992) Resistance of oilseed *Brassica* spp. to blackleg caused by

Leptosphaeria maculans. Canadian Journal of Plant Pathology 14: 56-66.

Scarth R and McVetty P (1999) Designer Oil Canola – A Review of New Food-Grade Brassica Oils with Focus on High Oleic, Low Linolenic Types. The 10th International Rapeseed Congress, Canberra, Australia.

Song K and Osborn TC (1991) Origins of Brassica napus: New Evidence Based on Nuclear and Cytoplasmic DNAs. *Proceedings of the 8th International Rapeseed* Congress, pp. 324–327. Saskatoon, Canada.

Stefansson BR (1983) The development of improved rapeseed cultivars. In: Kramer JKG, Souer FD, and Pigden WJ (eds.) High and Low Erucic Acid Rapeseed Oils: Production, Usage, Chemistry, and Toxicological Evaluation, pp. 144–159. New York: Academic Press.

Thurling N (1991) Application of ideotype concept in breeding for higher yield in the oilseed brassicas. *Field Crops Research* 26: 201–219.

U N (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Japanese Journal of Botany* 7: 387–452.

## **Relevant Websites**

http://www.canola-council.org. http://www.statcom-online.com. http://growcanola.com. http://www.canola.com. http://www.cetiom.fr.

# **Agronomy**

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# Introduction: The Three Species and Their Agro-Ecological Niches

This article introduces the three main *Brassica* oilseed species, and analyses the factors affecting their development and growth. A model approach is used to synthesize the information into a coherent whole, based on the way crops use solar radiation and other resources. This is then used as a framework to examine how the crop can be managed to efficiently produce oil and protein, by manipulating cultivar, sowing time, plant population, nutrition, water supply, and other inputs. Finally, the crop is considered in a wider farming-systems context.

Brassica oilseeds have been used as cooking oils in India, China, Japan, and less developed parts of Europe for centuries. Rapeseed oil has also been used as a lighting oil and as a lubricant in western Europe since about the fifteenth Century, but only as an edible oil there and in North America since the Second World War. By the 1960s, it was shown that erucic acid, the long-chain fatty acid characteristically found in *Brassica* sp., which gives the oil excellent lubrication properties, was implicated in heart disease in mammals as it could lead to hardening of the arteries. Pioneering work in Canada showed that erucic acid could be virtually eliminated by breeding, leading to the first low-erucic-acid varieties, Oro and Span (B. napus and B. rapa, respectively) by the late 1960s. At this time it was also shown that the glucosinolates, the mustard-type compounds found in the seed which restricted feeding of the meal (the seed residue after oil was extracted) to livestock, could also be reduced to a low level by breeding, initially using a Polish variety Bronowski.

"Canola" is a name coined in Canada for "double low" varieties containing specific seed quality characteristics: low erucic acid (less than 2%) and low glucosinolate levels (less than  $30\,\mu\mathrm{mol\,g^{-1}}$  of meal). These quality traits are now available in all three species. The development of canola, with new food and industrial applications and a new "image," allowed the rapeseed industry to expand rapidly in western Canada, where it rivaled the wheat industry in size and complemented it in crop rotations by the 1990s.

The three main oilseed *Brassica* species are listed below, with the main growing environments. The first two species are available as either "winter" cultivars, with varying requirements for chilling over winter (vernalization) before they will flower, or as "spring" cultivars, which need little or no vernalization. Winter cultivars are normally sown in autumn or early winter, whereas spring-type cultivars are sown in spring in colder areas, or autumn/winter in milder climates, where their growth over winter and early flowering is not a disadvantage.

## Brassica napus L. (Oilseed rape, or Rapeseed)

This species has the highest yield potential under favorable growing conditions, and has the widest adaptation. Winter cultivars form the main crop in Europe, where winters are mild enough to allow survival and slow growth. Spring-type cultivars are grown in western Canada from spring sowing, where winters are usually too cold to allow crop survival, and in Australia from autumn/winter sowing. In China and Japan, the crop is grown in winter, often

in rotation with rice over summer, so early maturing cultivars are required. These usually have a moderate vernalization response as winters can be cold. Virtually all cultivars, however, will show some response to vernalization, although it may not be necessary to proceed to flower and may be substituted for by the effect of long days in spring/early summer.

# Brassica rapa L. (Turnip Rape, Previously Also Known as B. campestris)

The most cold-hardy of the species, which tends to be cultivated on the northern fringes of the cropping belt in Europe, for example, in Scandinavia (winter and spring cultivars) and Canada (mainly spring cultivars), particularly in the Peace River district and in northern Alberta. The release of earlier-maturing, higher-yielding *B. napus* cultivars has resulted in contraction of *B. rapa* from about half the Canadian area to a much smaller proportion. Three ecotypes are also grown in India, brown sarson (the major area), yellow sarson, and toria.

# Brassica juncea L. Czern and Coss. (Indian or Oriental Mustard)

Mustard is the species with the greatest tolerance of heat and drought. It has been grown extensively as an oilseed and condiment in India, and to a lesser extent in Canada, mainly as a condiment (brown mustard) in low rainfall areas in southern Alberta. The development of canola quality cultivars in Australia and Canada opens up the drier parts of the grain belt in both countries to larger-scale production as an oilseed, and allows diversification away from the existing very limited cereal/fallow systems.

The rapeseed/canola crop needs to be seen as one component of a farming system, being normally grown in rotation with other crops such as cereals and legumes. It is also often included as part of a mixed farming system, with livestock such as sheep and cattle grazing crop residues and sown pasture leys, or pigs and poultry being fed by-products such as canola meal once the oil is extracted from the seed. There are particular benefits to be gained from inclusion of rapeseed in these systems, to be covered below as "biological fumigation," as well as the general break crop effect on diseases, pests, and weeds of other crops.

# **Crop Development and Growth: A Model Framework for Crop Management**

To understand how the crop responds to management of agronomic inputs such as choice of cultivar, seed rate, fertilizer, and sowing time, it is necessary to have

**Figure 1** Diagrammatic representation of development and growth of canola (rapeseed), as used in APSIM model. Tt = thermal time, PP = photoperiod, V = vernalization. (Based in part on: Weir AH, Bragg PL, Porter JR, and Rayner JH (1984) A winter wheat crop simulation model without water or nutrient limitations. *Journal of Agricultural Science, Cambridge* 102: 371–382.)

a clear picture of how it develops through its life cycle and grows with respect to photosynthetic organs and weight of all components (i.e., grows). These two processes, development and growth, proceed in parallel to result in the final yield, but should be clearly distinguished because environmental factors affect them differently. An overview is shown as **Figure 1**.

Much research effort in Europe (particularly France and the Netherlands) and Australia has been devoted over recent years to modeling the development and growth of the rapeseed crop. The agricultural production systems simulator (APSIM) family of models in Australia has been extended to include rapeseed/canola (Figure 1), and will be used to show how the modeling approach has aided thinking about crop management in a farming-systems context. In developing a model, the characteristics of the crop have to be described quantitatively, posing questions that may or may not have been answered by previous research.

#### Crop Development in Relation to Environmental Factors

The timing of length of life cycle stages is critical in fitting cultivars to their intended environment. This includes adapting to overall length of growing season,

minimizing the effect of stress times such as frost and drought, and synchronizing sensitive stages such as flowering and seed set with favorable conditions.

Development rate is largely governed by temperature, so time from sowing to seed germination and seedling emergence, and the rate of leaf production are proportional to temperature, up to  $\sim 20^{\circ}$ C. They can be described by units of "thermal time," or daydegrees (°Cd) above a base, which can be assumed as 0°C for most purposes. Seed germination and plant emergence may take ~120°C d if soil moisture is adequate. Leaves on the mainstem are then produced about every 75°C d until the beginning of stem elongation (i.e., about every 4 days at an average 20°C), then every 20°C d until flowering. The number of leaves produced on the mainstem of a plant ranges from a minimum of  $\sim$ 6 on early maturing cultivars in the long days of the Canadian summer to over 30 in winter cultivars in Europe. Temperature also controls the rate of seed development, so different cultivars may require from 500°Cd to 800°Cd (Canada and Europe, respectively) from flowering to maturity. In Australia, most cultivars require 600–700°C d. This means that, for example, a single European winter cultivar may require 60-100 days from flowering to harvest, depending on whether it is a warm or a cool summer.

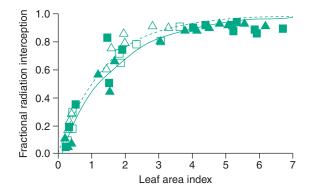
In addition to the basic positive effect of temperature on development rate, the progression towards flowering is also controlled by the vernalization response, as noted earlier on winter cultivars but also to a lesser extent on most others. Here, a period of time at low temperature ( $\sim 2-7^{\circ}$ C is most effective, with temperatures over 15°C having no effect) promotes initiation of the inflorescence at the growing apex, and cessation of production of leaf primordia. The apparently opposite effects of temperature on general development rate and on vernalization have been successfully included in the models. Photoperiod is the other major environmental control, with rapeseed cultivars usually responding to longer days from as soon as they have emerged. As photoperiod increases from ~11 to 16 h, duration of the period from plant emergence to floral initiation is reduced. Following initiation, there is a period of ~250°C d prior to flowering which is unaffected by daylength or vernalization.

### Crop Growth in Relation to Environmental Factors

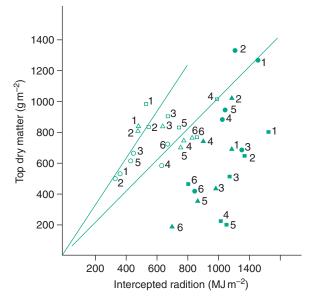
Good crop management can be thought of as a twostage process: (1) the production of an efficient leaf canopy by flowering time, and (2) the efficient conversion of photosynthate into oil and protein during seed development. Agronomic management to achieve this will be the focus of a later section.

Leaf expansion as well as production is proportional to temperature, with favorable conditions during leaf initiation and cell division leading to potentially larger leaves. Final leaf size is also a function of position on the plant, progressively increasing in size to about fifth true "rosette" leaf, then declining during stem elongation until becoming quite small on the upper mainstem. Leaves then remain active on the plant for  $\sim 400-800^{\circ}$ Cd, so the size of the leaf canopy is determined by factors affecting production, expansion, and senescence of leaves. The leaf area index (LAI), or area of leaf per unit ground area, is a key to efficient interception of solar radiation and hence photosynthesis. Figure 2 shows that a canopy of LAI 3 will intercept  $\sim 85\%$  of incoming radiation, with an extinction coefficient (k) for radiation of  $\sim 0.6$  (the slope of the relationship between LAI and the log of the fraction of radiation intercepted, which also varies with leaf angle and shape). If LAI greatly exceeds 3, little further radiation is intercepted, resources are wasted, and the dense canopy may encourage diseases and pests. A target for managers could therefore be the achievement of 85% radiation interception (equivalent to near-full ground cover) by flowering time.

The leaf canopy will convert intercepted total solar radiation into aboveground biomass at  $1-1.5 \text{ g MJ}^{-1}$  (average  $\sim 1.2 \text{ g MJ}^{-1}$ ) radiation use efficiency (RUE), as in the example in Figure 3 for three cultivars



**Figure 2** Fraction of solar radiation intercepted by green leaf area of canola crops at different stages of growth and levels of irrigation. Conventional cultivar indicated by square symbols, triazine tolerant cultivar by triangles, with fitted curves for extinction coefficient *k* of 0.58 and 0.72, respectively. Closed symbols indicate fully irrigated, open symbols partly irrigated. (Reproduced with permission from Robertson MJ, Holland JF, Cawley S, *et al.* (2002a) Growth and yield differences between triazine-tolerant and non-triazine-tolerant cultivars of canola. *Australian Journal of Agricultural Research* 53: 643–651. CSIRO Melbourne.)



**Figure 3** Relationship between the top dry matter (biomass) produced and amount of solar radiation intercepted by rapeseed crops between sowing and flowering (open symbols) and between flowering and harvest (closed symbols). Different symbols denote different cultivars, and numbers next to each symbol indicate order of sowing. Lines drawn indicate RUE of 1.0 and 1.5 g MJ<sup>-1</sup>. (Reproduced with permission from Mendham NJ, Russell J, and Jarosz NK (1990) Response to sowing time of three contrasting Australian cultivars of oilseed rape (*Brassica napus*). *Journal of Agricultural Science, Cambridge* 114: 275–283. Cambridge University Press.)

sown on six dates from autumn until spring. RUE will vary, depending on exposure to stress factors such as drought, high temperature, and disease. In cultivars with tolerance to the triazine group of herbicides, altered photosynthetic metabolism leads to ~20% lower RUE. During flowering, the efficiency of all crops drops, due to reflection of radiation and shading of leaves and earlier-formed pods by the mass of yellow flowers at the top of the crop. Subsequently, during pod development a new green canopy is established, consisting mainly of green pods which, compared to leaves, have ~10% lower efficiency per unit green surface area. While the pods have an extinction coefficient similar to that of leaves, they have fewer stomata able to exchange carbon dioxide and water. They also have a lower nitrogen concentration than leaves, which may reduce efficiency. Green area index (GAI, total green surface area) is therefore more relevant than just LAI in estimating crop radiation interception and efficiency of its use. Figure 3 shows that the crops after flowering grew at RUE mainly below 1 g MJ<sup>-1</sup>, and in the case of some later sowings affected by higher temperatures, water stress, and pest damage, as low as  $0.2\,\mathrm{g\,MJ^{-1}}$ . A lower RUE during seed fill will also be a result of the biosynthetic cost of oil production compared to carbohydrates and proteins earlier in the growing cycle. Oils contain  $\sim 2.5$ times as much energy as carbohydrates. This figure is discussed further below, in the section on sowing time.

# Yield Components and the Efficient Conversion of Dry Matter to Oil Yield

Final oil yield can be thought of as a product of the yield components:

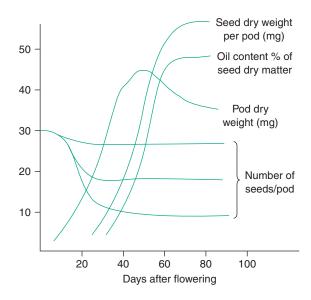
Oil yield = plants 
$$m^{-2} \times pods plant^{-1}$$
  
  $\times seeds pod^{-1} \times mean seed weight$   
  $\times oil seed content (\%)$ 

This numerical approach on its own is of little value, but is useful if combined with a knowledge of when the different components are formed, the external factors affecting them, and internal competition for photosynthetic assimilate, nutrients, and water. Plant establishment (numbers and spatial distribution) and survival over winter form the first component. Flowering and pollination determines the potential number of both pods and seeds, with pollination not normally a limiting factor in the largely self-fertile *B. napus* and *B. juncea*. Seed set in cross-pollinated *B. rapa*, or in hybrid seed production in *B. napus*,

may be dependent on good weather and the presence of bees or other pollinating insects.

The survival of a particular pod is largely determined during the first 2-3 weeks after pollination, when competitive pressures build up as the canopy of pods forms at the top of the crop. Numbers of pods per square meter are thus determined shortly after the end of flowering of the crop as a whole. For the remaining components, Figure 4 shows an example for a surviving pod. Abortion of seeds also occurs over the first 3 weeks, and can result in substantial losses of potential, from  $\sim$ 30 seeds per pod down to nil in an extreme case. This may be due to internal competition for resources if too many pods are formed, or external stress due to drought, low radiation levels, or frost. Significant seed growth does not begin until after  $\sim$ 3 weeks from seed set, once pod walls have reached near their full length and about half their final weight. Seed growth continues at an approximately linear rate until near-full size is reached. Buildup of oil content closely follows that of seed growth. There can be substantial compensation between yield components, for example, if seed abortion is particularly severe, remaining seeds can grow to a larger-than-normal size.

An illustration of an efficient crop canopy compared to an inefficient one after flowering is shown



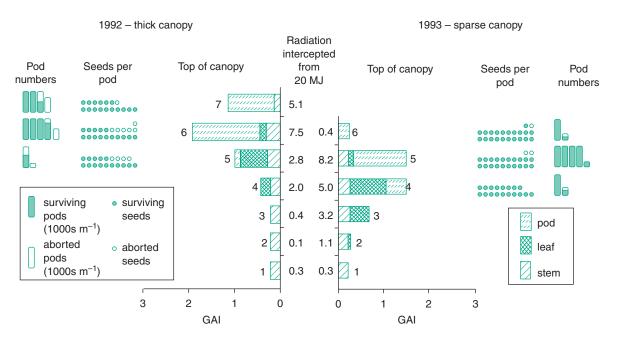
**Figure 4** Example of formation of the yield components within a single pod, as a function of time after flowering. Three hypothetical levels of number of seeds per pod indicated, corresponding to high, medium, and low seed survival. (Based in part on Hocking PJ and Mason L (1993) Accumulation, distribution and redistribution of dry matter and mineral nutrients in fruits of canola (oilseed rape) and the effects of nitrogen fertilizer and windrowing. *Australian Journal of Agricultural Research* 44: 1377–1388. CSIRO Melbourne, with permission.)

as Figure 5. This is a "snapshot" of two canopies produced in different seasons, but with the same cultivar and similar management. One crop had 9500 pods m<sup>-2</sup>, and the other 6000 pods m<sup>-2</sup>. The figure shows the crop in 20 cm thick layers, on a typical day with 20 MJ m<sup>-2</sup> incident solar radiation. In the "thick" canopy, radiation was mainly attenuated within the upper pod canopy, where there were many more aborted pods and seeds than within the "sparse" canopy. In the latter, radiation was spread more uniformly over the pod layer, with about half penetrating to the leaf layer below, allowing continued functioning. This is likely to have been a major contributor to the better seed survival.

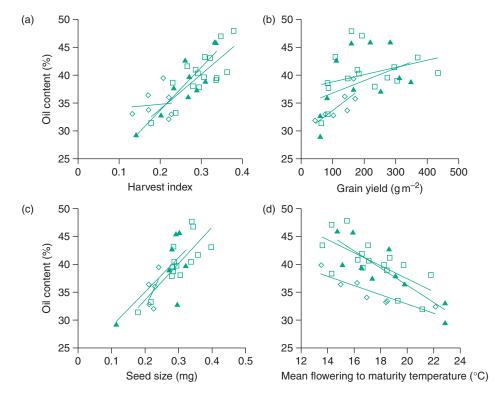
Harvest index measured at maturity (seed yield as a proportion of total aboveground biomass) is a consequence of the balances between biomass accumulation before and during grain-filling, as well as the extent of translocation of stored dry matter from vegetative organs to grain. These processes will be affected by the timing and severity of stresses with respect to the stage of crop development. Harvest index in canola typically varies between 0.25 and 0.35 (lower than for cereals due to the energy cost of oil biosynthesis), although severe stresses at certain stages of development can lead to values lower than this range.

Seed oil content in canola is affected by cultivar attributes and the environmental factors of temperature, water stress, and N supply during seed development. As the last component formed, it is broadly correlated with harvest index (Figure 6a) and seed size (Figure 6c) as similar factors will affect each. It may not be strongly correlated with seed yield (Figure 6b), as the main factors affecting yield may be determined well before oil is being formed.

As in other oilseed crops, high temperature has been shown to lower oil content and alter composition of fatty acids. Oil content declines by  $\sim 1.2\%$  for each rise in mean air temperature of 1°C during grain filling (Figure 6d). Water stress after flowering also can have a severe effect on oil content. Increasing the N supply to the crop almost always reduces the oil content of the seed, because a better supply of N increases the formation of N-containing protein precursors so that protein formation competes more strongly for photosynthates. As a result, less photosynthates are available for fat synthesis. In a review of winter rapeseed experiments the depression of oil content was in the range of 0.8-2% of oil content per  $100 \,\mathrm{kg} \,\mathrm{ha}^{-1}$ of N applied. In the spring rapeseed experiments surveyed the depression was less, in the range of 0.6-1.2% of oil per  $100 \text{ kgN ha}^{-1}$  applied. There was little evidence that late N application, which might be expected to enhance N supply to the seed rather than the vegetative components of the crop, has any particularly depressive effect on oil content. In the field, cultivar maturity will tend to be confounded



**Figure 5** Diagrammatic representation of the canopies of two rapeseed crops in 20 cm layers, showing the distribution and nature of the amount of radiation intercepted, the intercepting green surface area, and the fate of pods and seeds. (Reproduced with permission from McWilliam SC, Stafford JA, Scott RK, Norton G, Stokes DT, and Sylvester-Bradley R (1995) The relationship between canopy structure and yield in oilseed rape. In: Murphy D (ed.) *Proceedings of the 9th International Rapeseed Congress*, pp. 491–493. Cambridge, UK: GCIRC Paris.)



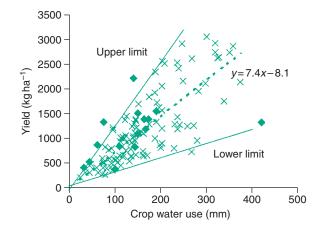
**Figure 6** The relationship between seed oil content and (a) harvest index, (b) grain yield, (c) mean seed size, and (d) mean temperature from flowering to maturity. Crops of two cultivars of canola indicated by square and triangle symbols, and one of Indian mustard (*B. juncea*) indicated by diamond symbols, with regression lines fitted to each. (Unpublished data MJ Robertson.)

with temperature and end-of-season drought. However, even after allowing for flowering time, there are still cultivar differences in oil content. The few studies exploring the issue of genotype—environment interaction for oil content in canola have shown only small effects, indicating that oil content is largely a characteristic of the genotype.

End-of-season drought is likely to limit oil content as well as yield, particularly in regions where autumn or winter sowing is normal. Spring sowing, however, as practiced in short-season cool environments like western Canada can lead to different problems as low autumn temperatures, and particularly frosts, curtail oil buildup and crop ripening. This may also lead to poorer-quality oil as unsaturated fatty acid synthesis may be halted, and high levels of chlorophyll may remain in the seed, particularly on later flowering branches. This leads to downgrading of the seed and greater oil-refining costs.

#### **Water-Use Efficiency**

An alternative approach to the radiation and temperature driven model (as outlined in Figure 1, with example in Figure 3) is a model widely used in Australia based on water-use efficiency (Figure 7). This is based on the principle of the known strong correlation



**Figure 7** Water-use efficiency of measured (diamond symbols) and simulated (crosses) crops. Upper and lower limits of 13 kg ha mm<sup>-1</sup> and 3 kg ha mm<sup>-1</sup> indicated by solid lines, with the fitted average line showing efficiency of 7.4 kg ha mm<sup>-1</sup>. (From Cocks B, Robertson MJ, and Cawley S (2001) Water extraction and water-use efficiency of canola in the north. In: Marcroft SJ (ed.) *Proceedings of the 12th Australian Research Assembly on Brassicas*, pp. 203–207. Australia: Geelong.)

between photosynthesis and transpiration, and hence between biomass production and water use. In water-limited environments, healthy crops will produce seed at up to  $\sim 13 \text{ kg ha}^{-1} \text{ mm}^{-1}$  of available

water, including stored soil water before sowing, and effective rainfall during crop growth. Lower efficiency figures (down to 3 kg ha<sup>-1</sup> mm<sup>-1</sup>) may indicate that other factors are limiting, e.g., nutrition, drainage, or frost. The efficiency will, however, also vary with evaporative demand — a hot or windy season will result in lower efficiency. It will also depend on whether the rainfall was received at a critical time, such as just after flowering, or too late in seed growth to affect yield. In spite of these constraints, this model has been useful for researchers and farmers to compare their crops, and as an extension tool.

## **Crop Management**

The main goal in early crop management is to produce a canopy with near-full ground cover of healthy leaves by flowering time, with efficient use of resources. There is usually no advantage in excessive leaf or stem production.

## **Plant Population and Arrangement**

The crop generally shows very little response to seed rate over a range of plant populations from ~30 to 100 plants m<sup>-2</sup>. Seed rates to produce these populations may vary from 3 to 12 kg ha<sup>-1</sup>, with resulting seed yields within 10% of the maximum. This is because the plants are "plastic," able (within limits) to keep producing leaves, and later branches and flowers, to occupy the site fully. Lower densities may therefore give prolonged leaf persistence, extended flowering and delayed maturity. While narrow (15 cm or less) row spacings will give quicker ground cover, hence radiation interception and weed suppression, wide (20 cm or more) spacings produce similar results, and may be desirable in direct-drilling situations for trash clearance.

Where crop establishment has been poor and crops are patchy, worthwhile yields (over 50% of that at optimum density) may still be obtained down to 10–20 plants m<sup>-2</sup>, provided the season is favorable to allow the compensatory growth. Very low density crops may be subject to more pest damage and weed invasion.

#### **Crop Establishment**

While grower guides such as the "Canadian Canola Growers' Manual" may suggest that "seed must be placed into a firm, moist, warm, aerated, well-structured seed bed for rapid germination and seedling growth," one or more of these requirements are often compromised in practice. Even though seeds are small (2–5 mg), their high oil content does give substantial energy reserves and hence ability to overcome

some constraints. The crop tolerates a wide range of pH ( $\sim 5.5-8$ , measured in water), and will emerge well from sowing depths of 5-30 mm. Sowing up to 50 mm may be required in some seasons to reach moisture, but emergence and early vigor may be reduced. Residues from previous crops on the soil surface may also impede establishment, particularly in direct-drilled situations, so burning, baling, or incorporation may be desirable. The trade-off with the benefits of trash retention needs to be assessed in each situation. Very low soil temperatures (5°C or less) may reduce establishment as well as delay it, and there may be an advantage from using B. rapa or other more cold-tolerant types in this situation. The insulating effect of stubble and trash may also exacerbate frost damage on young seedlings, by preventing heat flow from the soil.

Differences in seed germination rate and vigor have been found between different seed lots of the same cultivar, and use of high quality seed will assist in overcoming the above constraints. Large seed size on its own does not necessarily mean greater vigor, but will usually give initially larger seedlings. Greater seed weight for the same size (i.e., density) may be a better indicator of vigor.

#### **Sowing Time**

The guiding principle is to sow an adapted variety in time for it to reach near-full radiation interception by flowering, which needs to be late enough in spring to avoid frost damage and other unfavorable weather during pollination and seed set. There also needs to be enough growing season left after that to achieve grain fill and an economic yield before constraints of drought (e.g., in Australia) or frost (e.g., in Canada) intervene. The use of a phenology model as part of an overall model such as APSIM will assist prediction of optimum sowing dates. Delayed sowing, whether in autumn or spring, will in most environments result in progressively lower yield (e.g., 4% loss in yield for each week's delayed sowing in widely separated areas of Australia). Delays of a month in sowing may result in only a few days delay in flowering (driven more by responses to photoperiod and vernalization), hence shortening the period for leaf expansion and growth. While most crop species respond in a similar way, the specific morphological change in rapeseed from the leaf canopy to the mass of flowers (reflecting and absorbing radiation) and then pods at the top of the canopy puts a further restriction on vegetative growth, as leaves are largely shaded out during flowering and early pod growth.

For the experiment summarized in Figure 3 earlier, where crops were sown from mid-autumn through

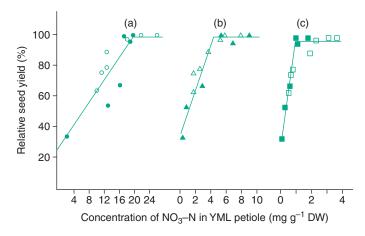
Earlier sowing than for the optimum timing as described above may be practiced for other reasons, e.g., in Europe to ensure good growth before winter so that crops can resist freezing, waterlogging, or bird damage. This may then lead to excessive growth in spring, very large numbers of flowers and pods, and an inefficient canopy, as in Figure 5. A low harvest index may result, with only moderate seed yields on a large biomass. Later sowings may give a higher yield, on sparser but more efficient canopies of pods. Breeding varieties with more restrained flowering, absence of flower petals (apetalous) and more efficient pod arrangement has been suggested to help widen the "sowing window."

#### **Nutrition**

Nitrogen is usually the most important nutrient to consider, due to its role in protein synthesis. This is

of particular relevance for leaf expansion and chlorophyll content, hence radiation interception and efficiency of its use. Requirements are site- and seasonspecific, but  $\sim 50-70$  kg of N are required per ton of seed produced, to be met from soil or applied N. Guidelines are available for soil and plant analysis to achieve optimum yield without wasting resources, as shown in Figure 8 for leaf petioles, one of the most sensitive plant parts. The concentration of N required to achieve high seed yield falls as the crop proceeds through development. Most of the required N is taken up by the crop before flowering, with later distribution from leaves and stems to pods and seeds. In Europe, with a cold wet winter, there is no advantage in large autumn applications. These may be either leached into groundwater, or be taken up to produce excessive soft growth, resulting in winter kill. Application at the late vegetative to early stem extension stages has generally been shown to be of greatest benefit. In Australia or Canada, it may be more practical to include some either at sowing, e.g., with P in diammonium phosphate, or incorporated before sowing as anhydrous ammonia. Excessive N may result in too much leaf and stem growth, lodging, and high protein/low oil levels in the seeds as noted above.

Phosphorus and potassium requirements of rapeseed are similar to that of other crops, with nearly 12 and 20 kg, respectively, being removed per ton of seed. Sulfur is of particular interest, with deficiencies showing up in many countries as industrial air pollution is reduced (particularly in Europe), and high analysis fertilizers without sulfur are increasingly



**Figure 8** Relationships between nitrate—N in dried petioles of the youngest mature leaf (YML) of canola at three stages of development: (a) 5–6 leaf rosette, (b) flower buds visible, and (c) start of flowering, and relative seed yield expressed as a percentage of the best treatment at each of two sites. Note change of scale from (a) to (c) on *x*-axis, in mg g<sup>-1</sup> dry weight. (Reproduced with permission from Hocking PJ, Randall PJ, DeMarco D, and Bamforth I (1997) Assessment of the nitrogen status of field-grown canola (*Brassica napus*) by plant analysis. *Australian Journal of Experimental Agriculture* 37: 83–92. CSIRO Melbourne.)

being used. With  $\sim \! 10\,\mathrm{kg}$  required per ton of seed, plant tests can be used up to the beginning of stem elongation to decide whether to apply more. A deficiency shows up as characteristic pale, stunted, cup-shaped leaves (it is essential in chlorophyll formation and photosynthesis), creamy-colored flowers, and very poor pod development. Excessive S may contribute to high glucosinolate levels, as it is required for their formation. With the micronutrients, rapeseed (like other Brassicas) may benefit from molybdenum, boron, manganese, and zinc, although manganese toxicity may be a problem on acid soils.

## **Water Supply**

While there is usually a good relationship between biomass production and transpiration, yields of seed, as in Figure 7, may be more influenced by water supply at critical stages. In most cropping areas and seasons, water supply through winter and early spring is adequate to allow crops to reach nearfull ground cover. Assuming this, the next critical stage is at and just after flowering, when pod and seed numbers are being determined, and abortion most likely. Management of stored soil water from before sowing onwards can optimize the water supply at this critical time, by reducing losses through previous land management, soil cultivation, weeds, or excess crop growth. Irrigation, if available, is likely to have the greatest effect at or just before the critical time. Subsequently, water supply for grain fill and oil buildup is important, but unless there is a premium for large seeds and high oil content, mild water stress may not affect yield and profitability greatly. Irrigation or rainfall from flowering onward may also encourage disease, e.g., Sclerotinia, which thrives in flower petals shed and then lodged in leaf or stem axils lower in the crop. This has been a particular problem in Canada, but is also now becoming apparent elsewhere.

The greater tolerance of *B. juncea* to water stress appears to be due to osmoregulation maintaining turgor during the critical time for seed abortion, and hence resulting in up to 50% greater water-use efficiency than *B. napus*.

#### **Crop Height and Plant Growth Regulators**

Management should generally be geared to achieving modest crop height as part of an efficient crop canopy, through choice of cultivar, sowing time, N nutrition, water supply etc. as discussed above. Where height is likely to be excessive and leads to lodging, disease, and harvest difficulties, plant growth regulators have been tried, as in cereals, at least as an interim measure.

Yields are not generally improved in the absence of lodging, and may be decreased by growth regulator application, so the economics of use are doubtful.

#### **Harvesting**

With an indeterminate inflorescence structure and lengthy flowering, pods and seeds on each plant ripen over an extended period, and in a field there may be considerable variation between plants in maturity time. The absence of good resistance to pod shattering in the main canola/rapeseed types therefore means that substantial losses can occur. Swathing at near physiological maturity (seeds turning black, moisture content  $\sim 15\%$ ) is therefore carried out on all but the lowest yield potential crops (less than  $\sim 0.8 \, \text{t ha}^{-1}$ ). If swathed too early, lower yield and oil content may follow, and chlorophyll content of seeds may be too high. Dessication using herbicides is an alternative but is not widely used. *B. juncea* does not need to be swathed as it has better resistance to pod shatter.

## Canola/Rapeseed in Farming Systems

#### **Weed Management**

The crop generally fits well into farming systems, as a vigorous broad-leaved break crop. This gives the opportunity to control weeds and crop volunteers built up in previous cereal crops, by a combination of cultivation, competition, and selective herbicide use. If livestock are available on the farm, they can have an important role in cleaning up crop residues and weeds, and grazing pasture leys in rotation. While weed control is not normally a major problem in canola, the use of such integrated weed management systems is supplementing and extending the value of the commonly used herbicides. Resistance in the weed populations has been found to most of these chemicals, and the brassicaceous weeds such as wild radish (Raphanus raphanistrum), wild mustard (Sinapis arvensis), and wild turnip (B. tournefortii) present a particular challenge, being closely related to the crop. In Australia and Canada, triazine-tolerant canola cultivars have been extensively used, but their lower photosynthetic efficiency reduces competitive ability against weeds, as well as growth and yield. Newer herbicide-tolerant cultivars, whether produced by genetic engineering or conventional breeding, offer some advantages. The risks of herbicide-tolerant weeds, developing either naturally or by introgression from the tolerant crop, need to be assessed and suitable strategies implemented. While introgression into the above brassicaceous weeds is not likely, wild B. rapa may be more susceptible.

### **Biofumigation and Other Benefits**

There are specific advantages of the Brassica oilseeds as break crops, supplementing their value as nonhosts, to control some soil-borne diseases such as take-all of cereals (Gaeumannomyces graminis), and some invertebrate pests such as nematodes. This is due to the content of glucosinolates in Brassica crop residues including roots that break down in the soil to form biocidal compounds, in particular isothiocyanates. Yields of cereal crops following canola are therefore often increased more than the non-host benefit of a break crop would suggest. There are opportunities to increase the level of the more highly biocidal compounds in roots by breeding, and also to maximize the effects through crop management. There are, however, concerns about the biocidal effects on beneficial organisms, such as Rhizobia bacteria for future legume inoculation, and vesiculararbuscular mycorrhizal fungi, which aid some crops (but not Brassicas) in uptake of phosphorus and other nutrients.

Canola in Australia has also been observed to have beneficial effects on soil structure, with the long taproot helping to break up subsoil compaction and hard pans resulting from cultivation. Direct-drilled cereals may therefore be more successful following canola than other crops.

See also: Canola: Genetics and Breeding; Harvest, Transport, and Storage; Processing. Grain Production and Consumption: Overview; Africa; Asia; Europe; Cereal Grains in North America; Oceania; South America. Oilseeds, Overview, Teff.

#### **Further Reading**

- Cocks B, Robertson MJ, and Cawley S (2001) Water extraction and water-use efficiency of canola in the north. In: Marcroft SJ (ed.) *Proceedings of the 12th Australian Research Assembly on Brassicas*, pp. 203–207. Australia: Geelong.
- Downey RK, Stringham GR, McGregor DI, and Stefansson BR (1975) Breeding rapeseed and mustard crops. In: Harapiak JT (ed.) Oilseed and Pulse Crops in Western Canada, pp. 157–183. Calgary, Alberta, Canada: Western Cooperative Fertilizers.
- Gabrielle B, Denoroy P, Gosse G, Justes E, and Andersen MN (1998a) Development and evaluation of a CERES-type model for winter oilseed rape. *Field Crops Research* 57: 95–111.
- Gabrielle B, Denoroy P, Gosse G, Justes E, and Andersen MN (1998b) A model of leaf area development and senescence for winter oilseed rape. *Field Crops Research* 57: 209–222.
- Habekotte B (1997) Description, parameterisation and user guide of Lintul-Brasnap 1.1, A crop growth model

- of winter oilseed rape (*Brassica napus* L.). Quantitative approaches in systems analysis No. 9, AB DLO, Wageningen, Netherlands.
- Hocking PJ and Mason L (1993) Accumulation, distribution and redistribution of dry matter and mineral nutrients in fruits of canola (oilseed rape) and the effects of nitrogen fertilizer and windrowing. Australian Journal of Agricultural Research 44: 1377–1388.
- Hocking PJ, Randall PJ, DeMarco D, and Bamforth I (1997) Assessment of the nitrogen status of field-grown canola (Brassica napus) by plant analysis. Australian Journal of Experimental Agriculture 37: 83–92.
- Holmes MJR (1980) Nutrition of the Oilseed Rape Crop. Barking, UK: Applied Science Publishers.
- Kimber DS and McGregor DI (1995) The species and their origin, cultivation and world production. In: Kimber DS and McGregor DI (eds.) *Brassica Oilseeds: Production and Utilization*, pp. 1–7. Wallingford, UK: CAB International.
- McWilliam SC, Stafford JA, Scott RK, Norton G, Stokes DT, and Sylvester-Bradley R (1995) The relationship between canopy structure and yield in oilseed rape. In: Murphy D (ed.) *Proceedings of the 9th International Rapeseed Congress*, pp. 491–493. Cambridge, UK: GCIRC Paris.
- Mendham NJ and Salisbury PA (1995) Physiology: crop development, growth and yield. In: Kimber DS and McGregor DI (eds.) *Brassica Oilseeds: Production and Utilization*, pp. 11–64. Wallingford, UK: CAB International.
- Mendham NJ, Shipway PA, and Scott RK (1981) The effects of delayed sowing and weather on growth, development and yield of winter oilseed rape (*Brassica napus*). *Journal of Agricultural Science*, *Cambridge* 96: 389–416.
- Mendham NJ, Russell J, and Jarosz NK (1990) Response to sowing time of three contrasting Australian cultivars of oilseed rape (*Brassica napus*). *Journal of Agricultural Science*, *Cambridge* 114: 275–283.
- Pouzet A (1995) Agronomy. In: Kimber DS and McGregor DI (eds.) *Brassica Oilseeds: Production and Utilization*, pp. 65–92. Wallingford, UK: CAB International.
- Robertson MJ, Holland JF, Kirkegaard JA, and Smith CJ (1999) Simulating growth and development of canola in Australia. In: Wratten N and Salisbury PA (eds.) *Proceedings of the 10th International Rapeseed Congress.* Sydney: The Regional Institute Ltd. (Available on CD-ROM and on-line at www.regional.org.au.)
- Robertson MJ, Holland JF, Cawley S, *et al.* (2002a) Growth and yield differences between triazine-tolerant and non-triazine-tolerant cultivars of canola. *Australian Journal of Agricultural Research* 53: 643–651.
- Robertson MJ, Watkinson AR, Kirkegaard JA, et al. (2002b) Environmental and genotypic control of time to flowering in canola and Indian Mustard. Australian Journal of Agricultural Research 53: 793–809.
- Salisbury PA, Potter TD, McDonald G, and Green AG (eds.) (1999) Canola in Australia: the First 30 years Organising Committee, 10th International Rapeseed

Congress, Canberra Australia. Chapters 3 (phenology, physiology and agronomy), 4 (Crop nutrition) and 5 (Canola in rotations). (Also available on-line at www.regional.org.au.)

Scarisbrick DH and Daniels RW (eds.) (1986) Oilseed Rape. London: Collins.

Thomas P (1984) Canola Growers' Manual: Grow with Canola. Lacombe, Alberta, Canada: Canola Council of Canada.

Weir AH, Bragg PL, Porter JR, and Rayner JH (1984) A winter wheat crop simulation model without water or nutrient limitations. Journal of Agricultural Science, Cambridge 102: 371-382.

### **Relevant Website**

http://www.regional.org.au - contains the "Proceedings of the 10th International Rapeseed Congress, Canberra 1999 (eds. Wrattan N and Salisbury, P.A)." The "Crop growth and farming systems" section includes papers on most of the topics covered, including APSIM and other models. Also contains "Proceedings of the Australian Agronomy Conferences 8-11. Also contains Canola in Australia: the first 30 years" (as above).

# Harvest, Transport, and Storage

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#### Introduction

Canola is a small-seeded crop, but the principles used in the harvest, transport, and storage of this crop are similar to those used for many other small-seeded grain and oilseed crops. Various factors during harvest and handling process may have to be altered or modified to accommodate this crop. This article summarizes many of these factors and discusses methods used in the successful handling of this crop.

### **Harvesting Methods**

As a high-yield canola crop approaches maturity, it may ripen very quickly. Gauging the correct time to harvest calls for more care and adjustments than with cereals. Canola can be harvested with the same swather and combine equipment as cereal crops,

although the crops are quite different. Canola is often tall and branchy, with thick spongy stems. Swathers and combines must be properly operated and adjusted to facilitate a smooth flow of crop material through the machines while minimizing seed losses. With a few basic adjustments to most modern small-grain harvesting equipment, harvesting of canola is successful.

Canola can be harvested either by direct combining or by swathing and combining, depending upon the species and the growing conditions. The decision on which harvesting method is appropriate is dependent on a number of factors, including which species of canola is grown. Brassica rapa varieties are much more tolerant to pod shatter and drop than Brassica napus varieties and can therefore usually be left for straight cutting, if preferred. Brassica napus varieties can be direct cut as long as crop conditions are such that the crop is well knitted; that is where the crop canopy is thick enough that the branches are well knitted together, and therefore are much less prone to damage from pod shatter or drop from windy conditions.

## When to Swath

Swathing at the optimum stage of ripening reduces green seed problems and seed losses, and ensures the quality required for top grades. To determine when a field of canola is ready to swath, plants from different parts of the field must be examined. The stage of maturity in an evenly maturing field will vary from plant to plant and from area to area within the field. Inspections every 2-3 days should begin when there is some seed color change in the first formed pods on the bottom of the main stem.

The color of the seed is more important than the overall color of the field in determining the stage of maturity. The best time to swath for optimum seed yield and quality is when all the seeds contain  $\sim 30$ 35% moisture. Examine only those pods on the main stem, except in conditions where the plant density is sparse, and more secondary branching has taken place. Seeds in pods on the bottom third of the main stem were formed earlier and will turn color much sooner than seeds in the pods of the top third of the plant. When the overall moisture content of seed from the total plant averages 30-35%,  $\sim 30-$ 40% of the seeds in pods on the main stem only will have changed color or have started to change color. Seeds with only small patches of color should be counted as color changed. Most of the seeds that have changed color will be from the bottom third of the stem in B. napus varieties, whereas in B. rapa varieties some seed color change will also have occurred in the middle and upper pods. When seeds in

the bottom pods turn color, seeds in the top, lastformed pods are filled or nearly filled.

Most of the seeds will be firm, and roll, as opposed to break, when pressed between the forefinger and thumb. At this point, seed yield and quality are usually optimized.

Although the optimum time to swath canola is when 30-40% of the seed on the main stem has changed color, swathing should usually begin before the crop reaches the optimum color change stage. This is particularly important if one is growing large acreages of similar maturing canola. If the crop is left until the optimum stage before starting to swath, some of the crop will be too ripe by the time swathing is finished. Swathing earlier rather than later reduces risk for factors such as frost or diseases, e.g., blackleg, alternaria, and sclerotinia, if present. One or two days advantage may be critical in the fall when weather conditions can be unfavorable in a very short space of time. Swathing before 10% seed color change in any situation is not recommended unless the crop was seeded so late that it has not begun to reach seed color change before imminent frost. In that case, the canola can be swathed in order to salvage as much yield as possible before frost damage.

Work conducted in canola production centers across the Canadian prairies over 25 site years, for 1990–94, confirms that earlier swathing of *B. napus* canola (at 20% seed color change) can be an acceptable practice which causes minimal loss in yield and oil content (Table 1). The trials were conducted under field-scale conditions using standard farm equipment.

As *B. napus* canola has a narrower range in development time (due to less branching and podding) than *B. rapa* canola, it is possible to swath *B. napus* canola earlier without yield loss due to immature seed. This range in development time is affected by any factor that influences branching and podding. For example, a dense stand of canola branches less and, therefore, has a narrower range in development and can be swathed earlier. However, a light stand tends to branch more profusely and has a wider range in development. It may be advantageous to delay swathing a light crop to the higher end of the seed color change recommendation.

Swathing late, when seed moisture content is much lower (~80% seed color change), will result in fluffy windrows susceptible to blowing and increased shattering. To reduce shattering losses, over-ripe fields should be swathed when humidity is high, such as after a rain, after heavy dew, or at night.

It is difficult to determine when to swath unevenly maturing fields. Uneven maturity is usually the result of uneven spring germination where two or more

**Table 1** Effect of time of swathing on *B. napus* canola yield, oil, and protein in western Canada

% Seed color change	Location years	Yield (%)	Oil content (%) @ 8.5% moisture	Protein content (%) @ 8.5% moisture
0-10	25	90	40.8	20.7
10-20	25	96	41.5	21.7
20-30	25	100	42.2	21.6
30-40	25	100	42.8	21.6

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flushes of germination have occurred. Determine which of the different stages of maturing plants is the majority. Swathing should occur as soon as some color change is visible on bottom pods of the main stem toward on the end of the major stages on the field.

## **Swathing Operation**

The swather should be run just low enough to get all the seed pods, leaving the maximum amount of stubble in which to anchor the windrow and ensure adequate air circulation through the windrow. Such windrows tend to settle into the stubble and escape wind damage. This also minimizes the amount of material that must be handled by the swather and the combine.

The average crop of canola is handled quite readily with most swathers. However, stands that are exceptionally tall (1.5–2 m), thick, or lodged and tangled, make it difficult to lay an unbunched windrow. The windrow must flow smoothly through the swather without bunching. Bunching leads to uneven drying and combining problems. A large throat opening is important, particularly for wider swathers, to ensure that the swath will be of a size and shape that will dry and cure properly.

The swather should have good reel and table adjustments so that the crop can be cut as high as practical without losing any pods or causing shelling or bunching windrows. The reels should be set as high and as far forward as possible. Reel speed should be set to correspond with the forward speed of the swather. This speed will lay the cut material gently back on the table to avoid shelling. Table dividers, which are long and gently sloping, are generally less prone to blockages than short, abrupt types. When the crop is tall, tangled, and lodged, or laid across the seeded rows, divider blockages are almost inevitable unless special vertical cutter bars or power blades

are fitted on the swather. These can cause minor loss of pods and whole seed tops, but they prevent stops and bunching.

In areas where light fluffy windrows could be lifted and blown by the wind, a light roller pulled behind the swather will help anchor the windrow in the stubble. The roller should be set so that it just anchors the windrow into the stubble without shelling any ripe pods. Excessive rolling will produce a windrow that is too compact to dry quickly and difficult to pick up without shelling the canola.

#### **Chemical Desiccation and Pod Sealants**

The use of a chemical desiccant, which kills the green growth of pods, stems, and leaves, can result in a more uniformly ripened crop. This may allow earlier combining. A crop must be desiccated at the proper stage to achieve the best yield and seed quality. A desiccant should not be applied when the crop is immature or past the recommended stage of maturity. Desiccation may reduce the incidence of pod shattering and seed loss making it a possible alternative to swathing. However, the costs of the desiccant and its application must be taken into consideration.

When seed is mature but before pods become dry and split, pod sealants will slow pod dry-down and prevent the movement of moisture into and out of the pod, reducing shatter losses. Combine timing is crucial for minimizing shatter losses as the sealant efficacy diminishes with time and from repeated rains. Sealants slow crop dry-down and harvest by 5–14 days. This could be a concern in shorter season growing areas.

### **Combining Canola**

Combining should begin when the seed is mature, fully color changed with no green seeds, and of a moisture content of less than 10% unless a drier is being used.

Canola crops which have been desiccated, or that are uniformly mature and free of green weeds, may be straight-combined without swathing. Work at canola production centres in western Canada has shown that most varieties of *B. raga* canola lend themselves well to straight combining. Straight-cutting of *B. napus* canola should only be attempted if crop conditions are favorable where crop growth is relatively heavy, with subsequent lodging, and where the risk of crop canopy movement from wind is low, and where the crop has low levels of disease (Table 2).

One of the main problems in straight combining in tall canola crops is ensuring freedom from blockages at the dividers and other table components so as to

Table 2 Effect of swathing vs. straight combining canola in western Canada on yield, oil, and protein

Treatment	Location years	Yield (%)	Oil content (%) @ 8.5% moisture	Protein content (%) @ 8.5% moisture
B. rapa (S) <sup>a</sup> B. rapa (DC) B. napus (S) B. napus (DC)	24 24 28 28	100 99 100 89	40.4 40.6 42.8 43.5	20.3 20.3 20.1 19.9

<sup>&</sup>lt;sup>a</sup>S = swathing; DC = straight combine.

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maintain a smooth crop flow into and through the combine. Again, long dividers or vertical power dividers can be effective in preventing crop blockages. To minimize shatter losses, the reel should be set well back and as high as possible to act as a precaution against stems falling forward. Reel speed must be matched carefully to forward speed to minimize seed shatter. Shatter losses can be reduced by combining a ripe crop when the pods are still damp from rain or dew, provided the moisture level of the seed does not exceed the safe storage level.

Canola in the windrow is ready to pick up and thresh when the seed temperature and moisture content have dropped to a safe storage level and most seeds are mature with no green color. While it is better to start on the early side for swathing, the same does not necessarily hold for combining. Hot or windy weather at, or after, swathing can cause canola seed to be at the appropriate moisture content for combining before it has cured and cleared the green chlorophyll. This occurs because the plant dries up before sufficient moisture can move into the seed to finish curing it. Canola requires at least 20% moisture in the seed for the maturing process to take place and eliminate the green seed color. It is important to check both moisture content and green seed count before starting to combine. Delaying combining can help clear the green color particularly if the swath sits through several heavy dews or light showers. If there are some green seeds, a few more days in the windrow may be all that is needed. Green seeds may ripen in the windrow but they do not cure significantly once they are combined. Before combining, a crush strip is used to ensure that the seeds are not green inside. A small percentage of seeds with green meat will reduce the grade. If wide swathers are used on tall, heavy crops, the result is very large windrows, which take a longer time to cure, especially at the center of the windrow. Bunching in the windrows also may contribute to a green seed problem as the crop dries out and cures unevenly.

#### **Combine Checkout and Adjustment**

Before combining, the combine should be checked over completely to ensure that it is in a good mechanical condition. Any holes through which the small seed can escape should be covered and plugged. Besides plugging all leaks it is important to keep the rest of the combine in good mechanical condition. Worn or loose components such as feeder conveyors, internal conveyors, and elevators are sources of seed cracking. The first setting of any combine — including threshing cylinder or rotor speeds, cleaning fan speeds, and cleaning sieve settings — should be set to the specifications in the operator's manual.

To minimize front-end seed losses on a combine equipped with a pickup, pickup speed and forward travel speed should be equal so that the windrow will be gently lifted without tearing or pushing.

Threshing cylinder or rotor speed will depend on crop conditions. Speeds set at about one-half to two-thirds of that used for cereals can often be used for canola. Speed reduction is important to prevent over-threshing of the pods and stems and overloading the cleaning sieves. Cracked canola is caused by impact when the cylinder speed is too fast. A speed that is too slow reduces the capacity of the combine. The cylinder speed should be maintained at a level where the amounts of cracked seeds are acceptable.

Once the cylinder speed has been set, concave clearance should be set as wide as possible to give a good threshing the first time through. The stems and pods should be broken no more than necessary and unthreshed pods should be kept to a minimum. This will reduce overloading of the sieves and allow seed separation without excessive dockage or load on the return conveyor.

For efficient threshing and cleaning, the combine should be operated as near as possible to its recommended capacity. Increasing the forward speed of the combine will increase the straw and chaff feed rate to a point where seed losses may increase dramatically. Too high a feed rate will exceed the capacity of the separating and cleaning components.

The proper adjustment of the fan and sieves for cleaning action is important since canola seeds are light and can easily be blown out of the combine or remain mixed with the chaff. Unlike the cleaning action for cereals, the cleaning action for canola should depend more on a shaking separation and less on a wind separation. While it is necessary to reduce the fan speed, enough wind must be used to maintain a "live" sieve. Air should be directed as uniformly as possible under the entire length of the sieve to keep the sieve "alive;" otherwise, stems, pods, and canola

will move over the sieves in a mat and losses will be extremely high. The top sieve or chaffer should be opened enough for good separation (1/4 to 1/3 open, or  $6-10 \,\mathrm{mm}$ ). This will keep the seed from going over the top and out the back. Air should lift the chaff on the sieve with a shaking action conveying the material along. The chaffer extension should be raised slightly  $(5-10^{\circ})$  at the rear and be open sufficiently to allow unthreshed pods through to the return. A chaffer opening that is too narrow, coupled with insufficient wind, will result in high seed losses. If the air blast is too fast (high fan speed), canola will be blown out with the chaff. Starting with a low fan speed and gradually increasing it until separation of chaff and seeds occurs, with no canola being blown over the chaffer sieve, is advisable.

The lower sieve should be adjusted depending on the sample in the hopper. Usually a setting of 3–6 mm will be sufficient for the lower sieve. If too much trash is in the grain hopper, the sieves are likely open too much. If the sample is perfectly clean, it means that canola may be going back to the return conveyor so that sieves should be opened slightly. Excessive returns can result in crackage of the seed. If the returns are too high, there may not be enough wind, the top sieve may be open too much, or the cylinder (rotor)-concave is overthreshing.

Since high losses are possible where combines are incorrectly operated or adjusted, the operator must make frequent checks and readjustments. Changing conditions during the day will change combine performance. Regular stops are suggested to check for losses and make adjustments in the field. A grain loss monitor aids in the detection of losses and a function monitor reports any problems in mechanical operation. Monitors must be installed correctly, calibrated properly, and handled carefully if they are to give reliable service.

### **Handling and Transport of Canola**

Grain handling and transport systems are similar to those of cereals and other small grains and are designed to suit the needs of a particular farm, not for any special requirements associated with handling canola. Therefore, most transport and conveying equipment that is successfully used with small grain cereals can be used with canola. During transportation, all cracks in trucks and other equipment must be sealed with duct tape or caulking to prevent leakage and tightly covered to prevent canola seeds being blown away. Augers should operate at full capacity to prevent the seeds from flowing back down the tube and belt conveyors should be enclosed in a trough to

keep the seeds from running off-line. Kernel damage during handling is usually not a problem unless below 7% moisture content.

## **Storage of Canola**

The main functions of grain storage structures are:

- protect the grain from damage due to moisture, heating, and insects;
- prevent losses due to leakage, rodents, and livestock; and
- provide reasonable convenience for handling or inspecting grain.

The requirements for canola storage facilities differ little from those for cereals. The nature of canola seeds and the fact that canola historically has been more valuable than an equal volume of cereals impose greater demands on storage structures. Canola is very sensitive to heating in storage and, therefore, requires better construction to exclude moisture. The small size and free flowing characteristics of canola mean that high-quality construction is necessary to prevent leakage. Roof and door openings, joints between structural components (roof to wall, wall to floor), and even bolt holes must be sealed carefully to avoid losses. The problems of heating and moisture migration tend to be more severe in large storage structures. Thus, canola should be stored in the smallest bins available, without sacrificing convenience and efficient handling.

## **Construction of Storage Structures**

Some authorities have advocated the use of wooden, rather than steel, granaries for storing canola because they "breathe," thereby allowing heat and moisture to dissipate. However, the porous nature of these structures makes control of leakage difficult and also provides access for the influx of moisture, insects, and rodents. Steel granaries require almost no maintenance and can be more easily sealed against pests and weather. If some conditioning of hot or damp grain is necessary, metal bins are best suited to the controlled movement of air through the grain mass.

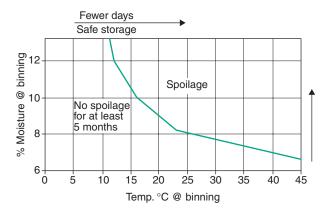
Regardless of the construction material used, storage structures must be as weatherproof as possible, yet still allow easy access to the bin for sampling and monitoring. The weatherproofing process must include the floors of bins set on grade. Concrete floors may resist the movement of water through the slab, but moisture can still enter the bin in the form of vapor. For this reason, a vapor barrier such as polyethylene should be placed between the concrete and the gravel base.

## **Conditions Necessary for Safe Storage**

Conditions for successful storage of canola seed depends upon the moisture and temperature conditions. Grain absorbs moisture from the air or gives up moisture to it, depending upon the relative humidity of the surrounding air and on the moisture content of the seed. The oil fraction of canola seed absorbs less moisture than the starch and fiber fractions, so the equilibrium moisture content for canola is much lower than wheat. Canola seed must be stored at lower moisture content than cereal grains to prevent spoilage. Moisture levels of 8–9% at temperatures below 20°C are necessary for long-term storage. The relationship of canola seed moisture content and temperature to safe storage is shown in Figure 1.

Any combination of canola seed temperature and moisture content to the left of the bar on the chart is acceptable for longer-term storage. Only under conditions of <9% moisture and cooler than 15°C, does canola require only occasional monitoring. The chart does not allow for deterioration due to infestation by insects and mites that can occur in hot, dry seed. High moisture content but low temperature, or high grain temperature even though the grain is very dry, serves as warnings that extra attention is needed.

The optimum temperature for rapid growth of insects is in the range of 30–35°C; their activity is greatly retarded by temperatures below 18°C. If the grain is cool and dry, insects will not thrive. However, seed may go into storage at acceptable levels of moisture content and temperature and at a later date develop pockets of high moisture and temperature, which are favorable for insect activity. Infestation of hot and dry seed by insects and mites will reduce the safe storage time.



**Figure 1** Canola storage time chart. (Reproduced with permission from Thomas PM (1984) Swathing – combining, storage, and conditioning of canola. In: *Canola Growers Manual*, pp. 1101–1215. Winnipeg, Manitoba: Canola Council of Canada.)

Seed moisture content is fairly uniform throughout a bin when canola is first stored. Over time, air movement through convection in the bin may relocate moisture, bringing some canola to a critically high moisture content. Convection currents are caused by temperature differentials between the grain temperature, and ambient temperature.

## **Factors Contributing to Storage Problems**

Even though the seed moisture content and temperature have been measured and determined to be safe, problems may still occur. The severity of the problem may depend on how close the moisture—temperature relationship is to the critical line shown in Figure 1. If all the canola in a storage bin becomes damp and warm, immediate and positive action must be taken to condition the grain.

Quality canola may be stored for 2 or 3 years if its moisture and temperature are properly maintained. To successfully store canola for periods of 6–24 months, special attention must be paid to conditioning and monitoring.

#### **Heating of Stored Canola**

If the stored crop is not sufficiently dry and cool, its own respiration and the respiration of microorganisms and insects will increase the temperature of the grain mass. If left unchecked, this can lead to heating of canola, where damage occurs from microorganism activity and oxidation. Heating affects the viability of the seed. A slight degree of visible heating brings about a drastic reduction in germination. Heating of the seed lowers the quality of the protein in the meal and causes large increases in free fatty acid content. Free fatty acids are a complete loss to the processor and must be refined out of the oil. The oil from heated seed has poor quality. Heating produces a strong tobacco-like odor in the oil and meal that is difficult to remove by processing.

### Conditioning

Many producers must provide storage for significant portions of each crop for one or more years. Frequently, harvesting the canola in this state is difficult, if not impossible. Furthermore, processes that occur in storage facilities can cause deterioration in the quality of the canola, even when it has been harvested under ideal conditions. These factors illustrate some reasons for conditioning canola. Conditioning could refer to all treatment of the canola between the time it is harvested and delivered to a selling point, but the term usually describes those processes that involve the movement of air through the grain.

The advantages of conditioning canola include:

- avoiding spoilage in storage;
- extending the harvest season; and
- reducing field losses.

Canola conditioning systems can be divided on the basis of both the purpose of the operation and the state of the air used in the operation. The simplest conditioning operation involves cooling of tough or dry canola, while some elaborate systems remove a considerable amount of moisture from the grain before cooling it for storage. Natural air systems, as their name implies, use the surrounding or ambient air to condition the grain. In most cases, there is no deliberate modification of the conditioning air. Heated air conditioning systems, on the other hand, use energy to heat the ambient air before its use in conditioning. The heated air has a lower relative humidity and, therefore, increased drying potential. The increased drying ability of heated air, combined with the high airflow rates typically used, give these systems a large capacity for drying canola.

Combinations of these systems, involving two or more of the operations, are also used.

## **Summary**

Although canola is a small-seeded oilseed crop, many of the same principles that are used in the harvest, transport, and storage of other small grain cereals and oilseeds are used in the handling of canola. Specific factors, unique to canola, need to be taken into account, in order to successfully handle this crop relative to other small seeded crops.

See also: Barley: Harvesting, Storage, and Transport. Canola: Genetics and Breeding; Agronomy; Processing. Contaminants of Grain. Food Safety through the Production Chain. Oilseeds, Overview. Stored Grain: Handling from Farm to Storage Terminal; Invertebrate Pests; Pest Management; Physico-Chemical Treatment. Wheat: Harvesting, Transport, and Storage.

### **Further Reading**

Arinze EA, Sokhansanj S, and Schoenau GJ (1993) Development of optimal management schemes for inbin drying of canola grain (rapeseed). Computers and Electronics in Agriculture 9: 159–187.

Auld DL and Mahler KA (1991) *Production of Canola and Rapeseed in the US*, pp. 978–983. 8th International Rapeseed Congress, Saskatoon, Saskatchewan, July 1991. Bailey J (1980) Oilseed rape harvesting losses can be high.

*Arable Farming* May: 59–61.

Bandel VA, Mulford FR, Ritter RL, Kantzes JG, and Hellman JL (1990) Canola Production Guidelines

- Cooperative Extension Service, University of Maryland, System, College Park. Fact Sheet 635.
- Bergland DR and McKay K (1992) Canola Production. North Dakota Extension Service, Fargo, ND. Bulletin A-686 (revised).
- Bergstrom DW (1993) Effect of Seed Moisture Content at Swathing on Yield and Quality of Rapeseed in Northwest Alberta. Alberta Agricultural Research Institute. Project No. 83-0025.
- Canola Council of Canada (1995) Canola Production Centres – Five Year Summary Report. Winnipeg, Manitoba: Canola Council of Canada.
- Canola Council of Canada (2001) *Time of Swathing Guide*. Winnipeg, Manitoba: Canola Council of Canada.
- Cass J (1991) Canola Storage. Tips to help you through Harvest. Canola Farming Fact Sheet 4, 2pp. Intermountain Canola Newsletter, Fall 1991. Idaho Falls, Idaho. pp. 3–4.
- Cenkowski S, Sokhansanj S, and Sosulski FW (1989) Effect of harvest date and swathing on moisture content and chlorophyll content of canola seed. *Canadian Journal of Plant Science* 69: 925–928.
- Daun JK (1987) Chlorophyll in Canadian Canola and Rapeseed and its Role in Grading. 7th International Rapeseed Congress, Poznan, Poland. May 1987.
- Hang AN and Gilliland GC (1988) Winter Rapeseed Research Program in Washington State. Proceedings of the Pacific Northwest Canola Production Conference, Feb. 1988. Pasco, Washington.
- Kephart KD, Rice ME, McCaffrey JP, and Murray GA (1988) *Spring Rapeseed Culture in Idaho*. Idaho Agricultural Experiment Station Bulletin 681.
- McGregor DI (1992) Swathing Canola Stops Seed Filling. Research Letter. Agriculture Canada Research Station, Saskatoon, Saskatchewan.
- McGregor DI (1994) Relationship between chlorophyll clearing and moisture loss. In: An Overview of Canola Agronomic and Varietal Research 1990–1993, 26pp. Winnipeg, Manitoba: Canola Council of Canada.
- Mills JT (1989) Spoilage and Heating of Stored Agricultural Products. Prevention, Detection, and Control. Agriculture Canada, Ottawa, Ontario Publication 1823E.
- Mills JT (1990) Protection of Farm-stored Grains and Oilseeds from Insects, Mites, and Moulds. Agriculture Canada, Ottawa, Ontario Publication 1851E.
- Mills JT and Sinha RN (1980) Safe storage periods for farm-stored rapeseed based on mycological and biochemical assessment. *Phytopathology* 70: 541–547.
- Murray GA, Auld DL, O'Keeffe LE, and Thill DC (1984) Winter Rape Production Practices in Northern Idaho. University of Idaho Agricultural Experiment Station Bulletin no. 634.
- Sylvester R, Makepeace RJ, and Broad H (1984) A code for stages of development in oilseed rape (B. napus L.). *Aspects of Applied Biology* 6: 399–419.
- Szot B and Tys J (1991) The Influence of the Spodnam DC Preparation on the Agrophysical Properties of Rape Siliques and on the Seed Losses at Maturation and

- Harvest, 174pp. 8th International Rapeseed Congress, Saskatoon, Saskatchewan, July 1991.
- Thomas DL, Breve MA, and Raymer PL (1991) Influence of timing and method of harvest on rapeseed yield. *Journal of Production Agriculture* 4(2): 266–272.
- Thomas PM (1984) Swathing combining, storage and conditioning of canola. In: *Canola Growers Manual*, pp. 1101–1215. Winnipeg, Manitoba: Canola Council of Canada.
- Thostenson AA and Hennings CR (1990) Canola (rapeseed) Production Systems in the Pacific Northwest. First International Canola Conference, Atlanta, GA.
- Underwood N (1994) Canola Production Centre Western Canada A Three Year Report, pp. 26–27. Canola Council of Canada, Winnipeg, Manitoba, Canada.

### **Relevant Website**

http://www.canola-council.org.

## **Processing**

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#### Introduction

Canola, also known as oilseed rape, has become one the most important oilseeds in world production, with the annual production of rapeseed reaching  $\sim 33$  million tons (Mt) during the 1990s. Rapeseed oil has accounted for  $\sim 10\%$  of the total world production of fats and oils over the same period and the proportion has been closer to 14% in recent years. Over the past years, rapeseed destined for human consumption has been changing in composition due to plant breeding efforts. These new varieties, low in erucic acid or both erucic acid and glucosinolates, have come to be known as low erucic acid rapeseed. In many parts of the world, rapeseed varieties with low erucic acid and glucosinolates are known as canola.

Canola and low erucic acid rapeseed are the leading varieties of rapeseed grown in the major producing areas of the world except China and India. However, low erucic acid rapeseed production in China is increasing, reaching  $\sim 50\%$  of total production of oilseeds in that country in 1998. While most oilseeds are restricted to a single species, canola and rapeseed may include seed from the species *Brassica napus* L., *Brassica rapa* L., or *Brassica juncea* L. While *B. napus* predominates in most of the world, significant

amounts of spring-type *B. rapa* are grown in Canada and northern Europe while in India, rapeseed grown includes *B. juncea* and a Toria type of *B. rapa*. The multiplicity of species, each with different seed structure and characteristics, has created some challenges for processing. In addition, canola and rapeseed processing has the additional difficulties of dealing with glucosinolates and their hydrolysis and also, especially in *B. napus* grown in short season conditions, with significant levels of chlorophyll pigments in the oil.

Canola and rapeseed are classified as soft oilseeds. They are small, generally round seeds ( $\sim 250-500$  seeds per g). They are dicotyledons and the cotyledons are the major oil storage sites. The seeds possess hulls (testa) that are thin and difficult to remove. Seeds usually contain  $\sim 35-45\%$  oil and  $\sim 18-23\%$  crude protein (leaving an oil-free meal with 35-40% crude protein).

The principal objective of canola and rapeseed processing is to remove the oil from the seed and to process that oil to a final product for use as salad oil, margarine stock or shortening, or other vegetable oil products. At the same time, the solid portion of the seed is processed to provide an edible meal product used as a high-protein supplement in animal feeds. This article describes the most common practices for canola processing and also indicates current

trends towards processing techniques that use alternate technologies which are either friendlier to the environment or produce a higher quality product.

# Primary Processing – Prepress-Solvent Extraction and Alternative Techniques

The most common extraction processes for oilseeds with a high oil content, such as rapeseed and canola, sunflower seed, and flaxseed involves a combination of pressure and solvent extraction processes to remove the oil from the seed (Figure 1).

## **Seed Preparation**

Seed arriving at an extraction plant is first cleaned to remove stones and any impurities that will impair the quality of the product or the efficiency of the extraction process. Seed-cleaning equipment usually operates with a combination of the processes of aspiration, sieving, and indent cleaning. At present, hull removal is not a common option although it is a feature of some "green" processing plants.

In some extraction plants, especially in those where seeds have very low initial temperatures and moisture content (e.g., from the Canadian prairies in winter), there may be adjustments in temperature (up to

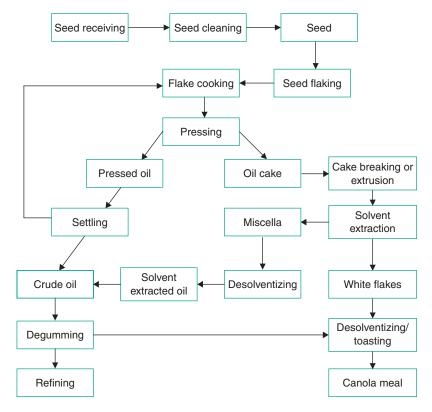


Figure 1 General flowchart for canola prepress solvent extraction technique.

 $30-40^{\circ}$ C) and moisture content ( $\sim$ 8%) to avoid seed-shattering in the flaking process.

After cooking, the seed is passed through flaking rolls that flatten it to a thickness of  $\sim$ 0.25 mm. This process helps rupture cells to free the oil and makes it easier for the solvent to percolate through the seed.

The flaked seed is usually cooked in order to inactivate enzymes that might cause problems during later processing. In canola and rapeseed, the key enzymes are myrosinase, which catalyzes the hydrolysis of glucosinolates releasing oil-soluble sulfur compounds and lipoxygenase. This in turn catalyzes the formation of hydroperoxides that later break down to form compounds with an undesirable flavor. Canola is often cooked in stack cookers with temperatures reaching as high as 90°C during the process. Achieving a balance of cooking temperature and time is important. If temperatures exceed 110°C, problems may be encountered due to the thermal degradation of glucosinolates.

#### Oil Removal

From the cooker, seed flakes are passed through a screw press, also known as an expeller. These consist of rotating screw shafts in a cylindrical barrel. The barrel "staves" consist of steel bars that have small spaces between them to allow the oil to flow out. Pressure is created in the barrel by an adjustable choke at the discharge end. This squeezes out  $\sim 70\%$  of the oil, which is routed to a settling tank. Solid material from the settling tank is rerouted through the conditioning stage.

The material leaving the press has  $\sim 16-20\%$  oil remaining. This material may be broken, or in some plants extruded to form expanded collets that have improved extraction properties. The cake is sent to the solvent extractor, in which a solvent dissolves the oil from the meal. It is important that the material entering the solvent extractor be cooled below the boiling point of the solvent during its conveyance to the extractor. A variety of extractor designs are used. In most, the process takes place in a succession of stages with the cake first being extracted with a solvent that already contains oil. Fresh solvent is used for extraction at the last stage. This process is known as a counter-current extraction process and provides the most efficient means for extraction of the cake. The cake leaving the extractor is known as "white flakes" and has typically less than 1% oil remaining.

The solvent is separated from the oil and the meal by distillation. It is important to reclaim and recycle as much solvent as possible, for both economic and environmental reasons, in particular to avoid the emission of significant amounts of solvent into the atmosphere. A processing plant may loose as much as 21 of hexane for every metric ton of seed processed.

#### **Alternative Solvent Uses**

The solvent most commonly used for extraction is hexane or hydrocarbon mixtures rich in hexane. Aside from being flammable and potentially explosive, there are health concerns about exposure to hexane, particularly related to development of nerve problems after chronic exposure. This has resulted in increasingly stringent environmental controls on plant emissions and concerns about the residues of hexane in oilseed meals. As a result, there has been research into the use of alternative solvents for oil extraction. The solvents investigated have included a large number of volatile hydrocarbons, halogenated hydrocarbons such as trichlormethylene, oxygenated hydrocarbons such as ethers and alcohols, water and supercritical fluids.

#### **Use of Enzymes – Alternative Processes**

In order to reduce the need for high temperature and pressure and the use of solvents, there has been considerable research into the use of enzymes to break down cell walls allowing the seed to be processed, perhaps even using water to assist in separating the oil and meal. A "green" process plant is under operation in Denmark.

## **Direct Pressing - Cold Pressing**

Cold pressing is a traditional method in which the seeds are not heated before, during, or after the pressing process. Seeds are selected, cleaned, and flaked as described above; they are then mechanically pressed at a slow pace to limit friction and to avoid elevating temperatures above 60°C. Retrieval of oil through this process is usually much lower than through prepress-solvent or solvent extraction processes. Therefore, the residual meal has higher levels of oil left over. Its color, taste, and odor are much more pronounced than those of refined oils. The price of cold pressed canola oil tends to be higher because of the lower recovery of oil. Cold pressed canola oil is generally sold in bottled form directly to consumers.

## **Secondary Processing of Oil**

Once the oil has been extracted from the seed, further processing is usually necessary to remove undesirable components and to make the oil suitable for functional uses. Traditional further processing, often referred to as "refining," employs the steps detailed below (Figure 2).

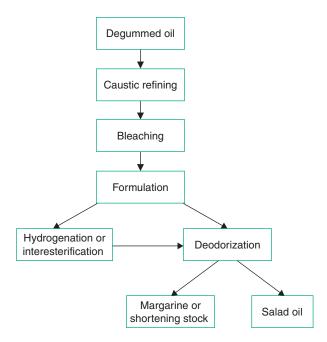


Figure 2 Secondary processing of canola oil.

## **Degumming (or Desliming)**

This process removes the phospholipids (lecithin or gums) from the oil. It involves washing the oil with water (which may contain other ingredients such as phosphoric acid or metal-sequestering agents) to cause the phospholipids to precipitate from the oil. The heavier phospholipid/water component is separated from the oil by centrifugation. Traditional water degumming produces oils with phosphorus levels of  $\sim$ 30 ppm. Most oils today are degummed by processes that involve the use of acidic solutions and careful temperature and mixing regimens. The process is known as super-degumming or acid degumming and produces oil with less than 2 ppm of phosphorus.

Gums, especially from soybean, are often treated to recover lecithin that is used as an emulsifier in the food industry. Because soybean-based lecithin is plentiful and relatively cheap, in Canada, gums removed during processing of canola are usually added back to the meal as a source of energy. Canola and rapeseed lecithin tends to have an undesirable color due to the presence of chlorophyll pigments. Commercial rapeseed lecithin is available in Europe where its production has been promoted by the desire to avoid genetically modified soybean products.

#### Refining

The refining process is designed primarily to remove the free fatty acids from the oil. Free fatty acids are formed by the hydrolysis of the triglycerides in the oil. The fatty acids are undesirable as they impart an unpleasant metallic taste to oils. Two processes, caustic refining and physical refining, are currently used to remove free fatty acids.

In caustic refining, the oils are first treated with  $\sim 0.05 - 0.1\%$  of concentrated phosphoric acid to precipitate phosphatides. The oil mixed with carefully measured amounts of 12% aqueous sodium hydroxide (caustic soda), usually  $\sim 2-3\%$ . The sodium hydroxide reacts with the free fatty acids to form water-soluble soaps. The soaps and phosphatides are removed by centrifugation. Some oils are also refined in the presence of the solvent or miscella. This process involves a combined degumming and refining step and may produce oil with better color.

Physical refining cannot be carried out successfully on oils with high levels of phosphorus and acid degumming is a prerequisite. In the physical refining process, the acid-degummed oil is first subjected to a further phosphoric acid pretreatment followed by a bleaching process with acid-activated clay at  $\sim$ 100°C. The fatty acids are removed by distillation under low pressure in the presence of steam. This process is very similar to the deodorizing process described below. While many initial studies of physical refining demonstrated that it was particularly suitable for canola or rapeseed oil, rapeseed oil today is still largely processed by caustic refining.

The fatty acids removed by refining are referred to as soap stock. This product may be acidulated and sold as an industrial chemical. Soap stock, either with or without acidulation may also be added to the meal.

#### Bleaching

Color bodies, particular chlorophyll, as well as some oxidation compounds and iron are removed from the oil by mixing the oil with 1-3% of an absorbing solid, usually diatomaceous earth or bleaching clay. The clay oil mixture is mixed and dried under vacuum at  $\sim 100^{\circ}$ C for 5-30 min. Occasionally, small amounts of activated charcoal may be added. The color bodies are absorbed onto the solid. After filtration, the oil is tested to ensure it meets color standards specified in contracts for each oil type. The color testing equipment is often based on the Lovibond system that measures oil color by comparing it with standard red, yellow, and blue filters. The high levels of chlorophyll pigments, often found in canola oil produced in areas where the seed does not have time to fully mature, are difficult to remove from the oil. Besides imparting an undesirable color to the oil, these pigments, when present in excessive amounts, have been

associated with the development of off-flavors due to oxidation, even though the chlorophyll levels are reduced to below industry specifications during the processing stage.

## Hydrogenation

Liquid vegetable oils, particularly canola, soybean, and sunflower oil are highly unsaturated making them susceptible to oxidation especially when heated to frying temperature. In addition, the uses of oils in margarines or shortening require that the fat is solid or semisolid to obtain the functionality required. Unsaturated liquid oils can be made more solid using hydrogenation.

Hydrogenation involves treatment of the oil with hydrogen in the presence of a catalyst under controlled pressure (100-300 kPa) and temperature (160-200°C). Two processes take place. In the first process, hydrogen is added to the double bonds, increasing the stability of the oil to oxidation during frying and raising the melting point of the oil. In the second process, some of the double bonds are converted from the naturally occurring cis form to the trans form. Trans fats also have higher melting points than cis fats. When treating liquid oils for reduction of the number of double bonds, the hydrogenation process is made selective to ensure that the hydrogen reacts with the highly unsaturated linolenic acid first. When preparing solid fats, it is more economical to maximize the trans production.

The canola and rapeseed industry has encountered a problem while using these oils for preparing margarines and shortenings. The high proportion of  $C_{18}$  fatty acids in canola oil and low erucic acid rapeseed oil mean that the desirable small  $\beta'$  crystals in the hardened fat tend to be unstable and convert eventually to large  $\beta'$  crystals. This problem has been overcome to a certain extent by mixing small amounts of palm oil into the margarine or by adding a crystal stabilizer.

Health concerns about *trans* fat have resulted in changes in processing. Of late, some solid fats are manufactured using the transesterification process with solid palm oil fractions or with fully saturated oils from the hydrogenation process.

#### Inter-esterification

In order to prepare a solid fat without resorting to hydrogenation, the liquid canola or rapeseed oil may be inter-esterified with a harder fat such as palm oil or a fully hydrogenated vegetable oil. Adding a catalyst to the oil results in the fatty acids rearranging themselves on the glycerol backbone, providing new triglycerides with different properties.

The process involves the addition of  $\sim 0.05\%$  of the catalyst sodium methoxide to the heated (150°C) oil as a powder under agitation. After 30 min, the oil is cooled and the base is neutralized with phosphoric acid before washing and bleaching. This process is in use to prepare nonhydrogenated canola margarines.

#### Winterizing or Dewaxing

Oils may contain small amounts of waxes and fully saturated triglycerides that may precipitate or haze when the oil is stored at refrigerator temperature. While these compounds have no undesirable health or flavor effects, consumers see the haze as unfavorable. Deodorized oils may be treated to a cooling period followed by filtration of any precipitate. This process reduces the amount of haze formed during refrigeration. While canola and rapeseed oils were originally perceived to be free of waxes and hence not require winterization, waxy sediments have been found in canola and rapeseed oils in recent years. As a result, plants now routinely winterize the oil.

#### **Deodorizing**

Refined, bleached, and hydrogenated or interesterified oils usually contain small amounts of volatile flavor components as well as low levels of free fatty acids generated during bleaching. These are removed by a distillation process known as deodorization during which the oil is treated with steam (2-4%) at high temperature  $(260-265^{\circ}\text{C})$  and low pressure (2-4 mmHg). Deodorization also removes some sterols and, more importantly, the antioxidant tocopherols from the oil. Tocopherols, sterols, and fatty acids are valuable by-products used in the pharmaceutical and chemical industries. However, a minimum level of tocopherols is required in the oil to prevent oxidation. The deodorizing process is designed to allow the most economic combination of tocopherol recovery without jeopardizing oil stability.

## **Refining Loss**

The above steps result in a slight loss in the amount of oil originally extracted from the seed. This refining loss is significant and is impacted by the quality of the original seed. Lower quality seeds give oils that are more expensive to refine. They require greater use of chemicals and treatment time and also have a higher refining loss.

Characteristics	Super degummed	Degummed	Crude
Free fatty acids (as oleic acid), % by mass, max	1.0	1.0	1.0
Moisture and impurities, combined, % by mass, max	0.3	0.3	0.5
Phosphorus, ppm, max	50	200	
Chlorophyll, ppm, max	30	30	30
Sulfur, ppm, max	8	10	10
Erucic acid, % by mass, max	2.0	2.0	2.0

Table 1b Canadian General Standards Board requirements for canola oil - Refined, bleached, and deodorized canola oil

Characteristics	Min.	Max.
Free fatty acids (as oleic acid), % by mass		0.05
Moisture and impurities, combined, % by mass		0.05
Lovibond color (133.4 mm cell)		1.5 red, 15 yellow
Peroxide value, milliequivalents per kg		2.0
Cold test, h	12	
Smoke point, °C	232	
Unsaponifiable value, g kg <sup>-1</sup>		15
Saponification value, mg potassium hydroxide per g oil	182	193
Refractive index $(n_D 40^{\circ}C)$	1465	1467
Iodine values $(W_{ii}s)$	110	126
Relative density (20°C/water 20°C)	0.914	0.920
Erucic acid, % by mass		2.0

<sup>&</sup>lt;sup>a</sup> Source: Tables 1a and 1b, Canadian General Standards Board CAN/CGSB-32.300-M87.

#### Formulation

Deodorized oil may be bottled directly or, more often, small amounts of citric acid or antioxidants are added to improve the oil's shelf life.

#### **Standards**

Standards for crude and refined, bleached and deodorized canola oil have been published by the Canadian General Standards Board (Tables 1a and 1b) and also in trading rules published by the National Institute of Oilseed Processors and national trading organizations such as the Canadian Oilseeds Processors Association.

#### Meal

Removal of solvent from the meal is carried out in a desolventizer. This is a vertical tank equipped with heated trays and agitators. The meal enters the top of the tank and is treated with reduced pressure and sometimes live steam to evaporate the hexane. During the process, the temperature may reach as high as 110°C and some glucosinolate breakdown and denaturation of the protein occurs. Newer processes have placed a great emphasis on carefully controlling this

process in order to avoid excessive denaturation of protein in order to obtain the best quality meal possible. In areas where there is no external market for gums (q.v.), the gums may be added back to the meal at this point. Canola meal typically has more than 36% crude protein and usually  $\sim$ 3% neutral lipids. Canola and rapeseed meal are valuable sources for protein supplement in animal feeds, particularly when the deficiencies and strengths of canola meal amino acid composition are combined with other protein sources such as pulses (soybean meal or pea meal).

Oilseed protein, and in particular soybean protein, is finding wide use in food products particularly as a result of the FDA's allowance of a health claim for soy protein in reducing cholesterol. Oilseed proteins (isolates or concentrates) are extracted from oilseed meals using aqueous solvents. The extracted proteins are precipitated using a number of different reagents and the precipitate is collected by filtration. While most protein isolates from oilseeds have been developed with soybean, there has been recent interest in the use of canola protein isolate with a pilot plant being established for this process.

See also: Oilseeds, Overview. Snack Foods, Processing. Soybean: Processing.

## **Further Reading**

Anjou K (1972) Manufacture of rapeseed oil and meal. In: Appelqvist L-A and Ohlson R (eds.) Rapeseed Cultivation, Composition, Processing and Utilization, pp. 198-248. New York: Elsevier.

Beach DHC (1983) Rapeseed crushing and extraction. In: Kramer JKG, Sauer FD, and Pigdent WJ (eds.) High and Low Erucic Acid Rapeseed Oils Production, Usage, Chemistry, and Toxicological Evaluation, pp. 181–230, 563-570. Toronto: Academic Press.

Daun JK and Bushuk W (1983) Rapeseed. In: Wolff, Ivan A. (ed.) CRC Handbook of Processing and Utilization in Agriculture, pp. 257–297. Boca Raton: CRC Press.

Daun JK, Buhr N, Mills JT, Diosady LL, and Mag T (1993) Oilseeds - Processing. In: Bass E (ed.) Grains and Oilseeds, Handling, Marketing, Processing, 4th edn., pp. 883-935. Winnipeg: Canadian International Grains Institute.

Hui YH (ed.) (1996) Bailey's industrial oil and fat products, 5th edn., vol. 4. Edible Oil and Fat Products: Processing Technology. New York: Wiley.

Mag TK (1995) Food industry requirements for fats and oils: functional properties. In: Przybylski R and MacDonald BE (eds.) Development and Processing of Vegetable Oils for Human Nutrition, pp. 36-46. Champaign: AOCS Press.

Roden AD (1983) Some recent innovations in canola processing technology. In: Kramer JKG, Sauer FD, and Pigdent WI (eds.) High and Low Erucic Acid Rapeseed Oils Production, Usage, Chemistry, and Toxicological Evaluation, pp. 181–230, 563–570. Toronto: Academic Press.

Teasedale BF and Mag TK (1983) The commercial processing of low and high erucic acid rapseseed oils. In: Kramer JKG, Sauer FD, and Pigdent WJ (eds.) High and Low Erucic Acid Rapeseed Oils Production, Usage, Chemistry, and Toxicological Evaluation, pp. 181–230, 563–570. Toronto: Academic Press.

Unger EH (1990) Commercial processing of canola and rapeseed: crushing and oil extraction. In: Shahidi F (ed.) Canola and Rapeseed: Production, Chemistry, Nutrition, and Processing Technology, pp. 235-249. New York: Van Nostrand Reinhold.

Wan P and Wakelyn PJ (eds.) (1997) Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils. Champaign, IL: AOCS Press.

Wettström R (1972) Processing of rapeseed oil. In: Appelqvist L-Å and Ohlson R (eds.) Rapeseed

Cultivation, Composition, Processing and Utilization, pp. 198-248. New York: Elsevier.

#### **Relevant Websites**

http://www.agr.gc.ca - A description of Canada's oilseed (and other grains) processing sector can be found at this website under Markets and Trade.

http://www.dhs.ca.gov - A description of the hazardous properties of hexane can be found at this website.

http://www.emcentre.com - A description of the pilot plant process for enzyme-assisted extraction of rapeseed may be here.

http://www.foodtech.uni-kiel.de - A description of alternative processing for canola can be found at this website.

http://www.atma.asn.au - Australian Technical Millers Association (ATMA), with members from Australia, New Zealand, and Papua New Guinea. This website contains news and information on milling and milling training courses, and also provides useful links to other milling associations and industry organizations.

http://www.grainnet.com - Grainnet. This website provides news and information for the grain, milling, feed, and seed industries.

http://www.aomillers.org - International Association of Operative Millers (IAOM). The IAOM is an international organization devoted to advancement of technology in the flour milling and seed processing industries.

http://www.nabim.org.uk - National Association of British and Irish Millers (NABIM). The NABIM website contains useful downloadable information articles, and information on training courses.

http://www.namamillers.org - North American Millers Association (NAMA). This association has members from Canada and the United States. The website provides industry news and background information on industry issues.

http://world-grain.com - A grain and grain processing information site. It contains many useful links to industry, including many flour milling associations worldwide.

## CARBOHYDRATE METABOLISM

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Published by Elsevier Ltd.

## Introduction

Carbohydrates constitute two-thirds to three-quarters of plant dry-matter, and are the major components of plant storage organs. Cellulose, the most-abundant plant carbohydrate provides structural integrity to the plants and is used for fiber and fuel by humans. In seeds, carbohydrates provide energy and substrates for germination and initial growth of a new plant. The major reserve carbohydrate present in grains is starch, which constitutes a major source of calories in the human diet and animal feed. Carbohydrates form the basis of several important industries in the food and feed sectors, and provide renewable and environmentally friendly raw materials for industrial applications such as biodegradable plastics, adhesives, and ethanol-based fuels. With the development of genetic modification technologies, the biosynthesis of carbohydrates can be altered in planta to generate novel products for various food, feed, and nonfood applications. This article will describe the structure, occurrence, and distribution of some of the common storage and structural carbohydrates present in the grains of major crops. The biochemical reactions occurring during the biosynthesis of carbohydrates and their utilization during seed germination will be presented.

## **Carbohydrates**

#### **Definition and Classification**

The term carbohydrate is derived from its basic elemental formula  $[C_x(H_2O)_y]$ , in which carbon is joined to hydrogen and oxygen present in the same ratio as in water. Chemically, carbohydrates are polyhydroxy aldehydes or ketones, their simple derivatives or their polymers. Carbohydrates in grains are classified based on their chemical structures or their digestibility when consumed by humans as food or by livestock as feed. Based on the polymeric nature of carbohydrates, they are classified as monosaccharides, disaccharides, oligosaccharides, and polysaccharides (Figure 1). Monosaccharides are the simplest of all sugars,

contain at least three carbon atoms, and are the building blocks of all carbohydrates. The most common monosaccharides in grains are the hexoses (fructose, glucose, and galactose) and the pentoses (arabinose and xylose). Two sugar moieties joined by a glycosidic bond (an oxygen bridge) form a disaccharide. Upon hydrolysis of disaccharides, the glycosidic bond is split to yield the component monosaccharides. Sucrose and maltose are the most commonly occurring disaccharides in most grains. Oligosaccharides include the sugars that contain 3-20 sugar molecules joined by glycosidic bonds. The most common oligosaccharides are soluble α-galactosides, characterized by galactose moieties joined by  $\alpha(1,6)$  linkages. In legumes, raffinose, stachyose, verbascose, and  $\alpha$ -galactosides are the most common oligosaccharides that constitute 6–18% of the legume seeds dry weight. Polysaccharides are polymers with more than 20 monosaccharides joined together by glycosidic linkages. These are complex molecules because of the diversity in monosaccharide units and type of linkages present in the polysaccharides. Some of the most common polysaccharides present in grains are starch, cellulose, and xylans, of which xylans include pentosans,  $\beta$ -glucans, and arabinoxylans. In relation to human and animal nutrition, carbohydrates are also classified as available and unavailable. The available carbohydrates represented by sugars and starches are broken down and absorbed by the human and animal digestive tract to give energy to the body. Structural cell-wall carbohydrates such as cellulose, hemicellulose, and nonstarch complex carbohydrates, e.g., xylans are categorized as unavailable carbohydrates because they are not assimilated by the digestive tract. The unavailable carbohydrates are also often referred to as dietary fibers.

### **Distribution in Important Grains**

All the above-described carbohydrates are present in most grains, where they form the major constituent of the dry weight (Table 1). However, in oil seed grains, such as *Brassica* spp., lipids and proteins are the major storage grain components and carbohydrates make up only one-quarter of the grain dry-weight. In cereal grains, the total concentration of mono-, di-, and oligosaccharides varies from less than 1–3% (Table 2). Corn kernels contain the highest percentage of glucose and fructose, whereas in barley, sucrose is the major free sugar. Wheat grains have the highest concentration of raffinose among the cereal grains (Table 2).

 $\alpha$ -D-Glc(1 $\rightarrow$ 4) linkage

## Monosaccharides ÇH<sub>2</sub>OH HOH<sub>2</sub>Ç $\alpha\text{-D-Glucose}$ $\beta ext{-}\text{D-Fructose}$ $\alpha$ -D-Xylose ÇH<sub>2</sub>OH ÓН $\alpha$ -D-Galactose $\alpha$ -L-Arabinose **Polysaccharides** ÇH<sub>2</sub>OH ÇH₂OH Branch $\alpha$ -D-Glc(1 $\rightarrow$ 6) branch point Main chain ÇH<sub>2</sub>OH CH<sub>2</sub>OH ÇH₂OH

Amylopectin Amylose ОН ĆH<sub>2</sub> O 0-н CH<sub>2</sub> H O 0

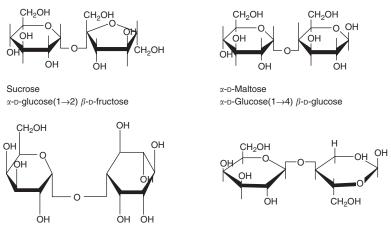
Repeat units of cellulose: two  $(1\rightarrow 4)\beta$ -glucan chains showing inter- and intra-chain H bonds

## Disaccharides

Galactinol

 $\alpha$ -D-Glc(1 $\rightarrow$ 4) linkage

Main chain



Lactose

Figure 1 Classification and chemical structures of representative carbohydrates found in common grain crops.

 $\beta$ -1, 3-xylan

Figure 1 Continued.

Legume crops in general have a higher concentration of di- and oligosaccharides as compared to cereal grains (Table 3). Especially chickpeas have a high concentration of soluble sugars, of which sucrose is the major component. Lentils and peas have higher concentrations of stachyose and verbascose as compared to other legumes. Starch is the major storage

carbohydrate in cereal grains, where it forms twothirds to three-quarters of the dry weight. However, in oat, the starch concentration is slightly less, ranging from 50% to close to 60%. Legume seeds also contain 50% starch, except lupins and soybeans in which only 0.5-1.4% of the dry weight is starch. The structural carbohydrates, which constitute the major contributors of unavailable carbohydrates (dietary fibers), range in distribution from slightly above 5% in rice to almost one-third of the grain dry-weight in barley. In grain legumes, chickpeas have a higher concentration of total nonstarch polysaccharides, whereas the concentration of total dietary fiber corresponds to that of other legumes (Table 3). The major nonstarch complex carbohydrate in grain legumes is cellulose which represents about one-eighth of the grain dryweight in the majority of the grain legumes (Table 3).

**Table 1** Chemical composition of seeds of some important grain crops

Crop	Comp (percentage	oosition e dry weig	ıht)
	Carbohydrate	Protein	Lipia
Cereal crops			
Wheat (Triticum aestivum)	82	14	2
Barley (Hordeum vulgare)	80	9	1
Corn (Zea mays)	84	10	5
Rice (Oryza sativa)	88	8	2
Oat (Avena sativa)	67	28	1
Sorghum (Sorghum vulgare)	82	12	4
Rye (Secale cereale)	82	14	2
Pulse crops			
Lentil (Lens culinaris)	65	27	1
Pea (Pisum sativum)	68	27	2
Chickpea (Cicer arietinum)	65	23	5
Common bean ( <i>Phaseolus</i> vulgaris)	70	24	2
Mung bean (Vigna radiata)	69	26	1
Pigeon pea (Cajanus cajan)	65	21	1
Soybean (Glycine max)	32	38	20
Cowpea (Vigna unguiculata)	60	24	1
Lupin (Lupinus albus)	37	38	20
Oilseed crops			
Rape (Brassica napus)	25	23	48
Sunflower (Helianthus annuus)	48	20	29
Flax (Linum usitatissium)	32	26	38
Peanut (Arachis hypogaea)	25	27	45
Sesame (Sesamum indicum)	19	20	54
Hemp (Cannabis sativa)	27	29	41

#### Metabolism

Plants have the ability to convert simple nutrients such as carbon dioxide, water, and inorganic ions into intermediates leading to the biosynthesis of complex molecules, such as nucleic acids, proteins, lipids, polysaccharides, and secondary metabolites needed for growth and development (Figure 2). Through the process of photosynthesis, light energy is captured to fix reduced carbon dioxide and water into a simple carbohydrate backbone, CH<sub>2</sub>O. The reaction takes place in chloroplasts, in which CO2 entering the Calvin cycle is incorporated into 3-phosphoglycerate (3-PGA) molecules, which are subsequently converted to triose-phosphates. In addition, the absorbed light energy produces reducing equivalents and ATP for several biosynthetic reactions needed for plant growth and development. The reactions associated with light absorption are diurnal, which results in large variations in nutrients available to plants during light and dark periods. However, plants have developed considerable flexibility in their metabolism to cope with fluctuations in nutrient concentration. Plant cells can be envisioned to have a series of metabolic intermediate pools, which are interconnected to each other through enzyme reactions or transport mechanisms that are often reversible (Figure 2). According to the physiological need(s) of a plant, metabolites can be added to or withdrawn from these pools. Two metabolite pools of the reductive pentosephosphate pathway and its intermediates are the triose-phosphate (3-PGA and dihydroxyacetone phosphate, DHAP) pool and the hexose-phosphate (glucose-1-P; glucose-6-P, fructose-1-P, ADP-glucose) pools, which play major roles in carbohydrate metabolism (Figure 2).

The triose-phosphates are either transported by triose-phosphate transporters to the cytosol, or converted to other phosphorylated compounds, such as fructose-6-phosphate, in the plastid. The plastidial fructose-6-phosphate is used both for regenerating ribulose-1,5-bisphosphate and producing

Table 2 Carbohydrate composition of some cereal grains

Crop			Free sugar (	(%)		Star	rch (%)	Nonsta	arch comple	x carbohyo	Irates (%)
	Total	Glucose	Fructose	Sucrose	Raffinose	Total	Amylose	Total dietary fiber	Pentosan	β-Glucan	Cellulose
Wheat	1.4-1.3	0.02-0.03	0.02-0.04	0.57-0.80	0.54-0.70	63-72	23-28	14.6	6.6	1.4	2.0
Barley	2.0 - 3.0	0.1 - 0.2	0.1	1.9-2.2		57-59	22 - 26	19-23	5.9	3-7	4.3
Rice	1-1.1	0.12 - 0.13	0.11-0.13	0.6 - 0.66	0.1 - 0.2	66	16-33	3.9	1.2	0.11	1.7 - 2.0
(brown)											
Corn	1.0-3.0	0.2 - 0.5	0.1 - 0.4	0.9 - 1.9	0.1 - 0.3	64 - 78	24	13.4	5.8 - 6.6	0.1	2.1
Oats	1.4	0.05	0.09	0.64	0.19	43-61	16-27	9.6	7.7	3.9-6.8	6.0-12.9
Rye	3.2	0.08	0.10	1.9	0.4	69	24 - 31	14.6	8.5	1.9 - 2.9	1.5

 Table 3
 Carbohydrate composition of some grain legumes

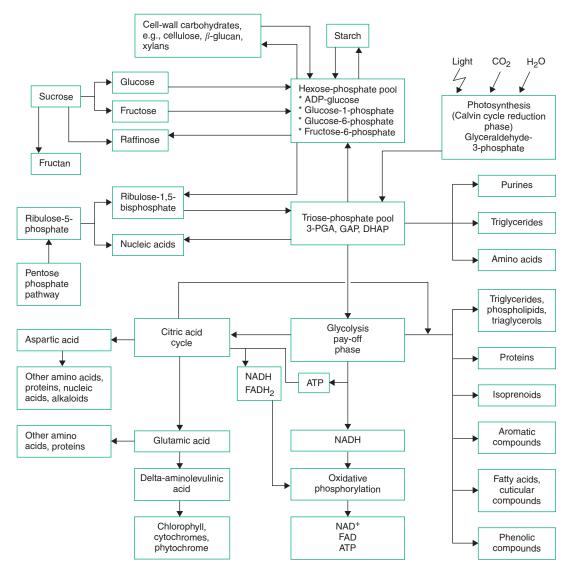
Crop			Soluble car	Soluble carbohydrates (%)	(%		Starc	Starch (%)	Nonsta	arch complex ca	Nonstarch complex carbohydrates (%)	
	Total soluble sugars	Sucrose	Raffinose	Stachyose	otal soluble Sucrose Raffinose Stachyose Verbascose Total galac	Total galactosides	Total	Amylose	Total nonstarch Total dietary Hemicellulose Cellulose polysaccharides fiber	Total dietary fiber	Hemicellulose	Cellulose
Common bean 2.0-9.6	2.0-9.6	1.6–3.9	1.6-3.9 0.2-2.5	0.5–3.9	0.1–1.8	0.4-8.0	51–51.9	51-51.9 22.1-36.0 6.4-20.4	6.4–20.4	11.2–27.5	0.5–5.6	3.2-13.1
Peas	3.5 - 13.8	0.9 - 5.4		0.3 - 4.2	0-4.3	2.3-9.6	24.7-57.4	23.5 - 33.1		16.1–21.6	0.9 - 12.4	0.9 - 13.3
Lentils	3.3 - 9.5	1.1 - 3.0	0.1 - 0.8	1.1-4.0	0-6.4	1.8-7.5	40.1 - 57.4	20.7-45.5	6.9-14.7	11.0-21.4	1.2-15.7	3.5 - 14.8
Chickpeas	4.6 - 14.2	2.8-6.9	0-0.3	0.4 - 2.0	trace-0.4	2.0-7.6	43.0 - 59.0	31.8-45.8	5.5 - 35.4	8.2-24.0	0.6 - 16.0	1.1 - 13.7
Faba beans	2.2-8.5	0.1 - 3.8	0.1-3.8 0.1-1.5	0.2 - 1.6	1.1 - 2.4	1.0 - 4.5	39.2-47.2	39.2-47.2 22.0-35.0 17.5	17.5	17.1-23.8	1.6-8.9	8.3 - 14.3

glucose-1-phosphate. Glucose-1-phosphate, when converted to ADP-glucose by ADP-glucose pyrophosphorylase (AGPase), is the immediate precursor for starch biosynthesis as described below. Starch synthesized in the chloroplast during the day is known as transitory starch, as it is degraded to maltose, glucose, hexose-phosphates, and triose-phosphates at night. Some of the starch degradation products are transported to the cytosol and are converted to sucrose, the major transport sugar. Sucrose through symplastic or apoplastic transport enters the phloem, through which it is mobilized to the storage tissues, where symplastic or apoplastic up-take into sink cells takes place. The transported sucrose is further converted to substrates that give rise to diverse carbohydrates present in grains of different crops as described above. The hexose-phosphate pool also plays an important role in starch and sucrose utilization by the plants. During the glycolytic pathway, which acts in reverse of the triose-phosphate synthetic pathway during photosynthesis, starch and sucrose are oxidized to provide energy and hexose-phosphates that on phosphorylation contribute to the hexose-phosphate pool. In addition, the glycolytic reactions provide several intermediates that contribute to the synthesis of proteins, lipids, and organic acids (Figure 2). These compounds are important grain components, and are described in other articles.

The hexose-phosphate pool is an important converging point for both carbohydrate synthesis and degradation, and thus is considered to play an important role in carbohydrate metabolism in plants. The main components, glucose-1-phosphate, glucose-6phosphate, and fructose-6-phosphate, are readily interconvertible when present in the same system. However, in plants the hexose-phosphates are compartmentalized to the plastids and cytosol. In photosynthetic tissues, the plastidial and cytosolic hexosephosphate pools are independent of each other and their exchange is often restricted. Therefore, the regulation of the carbohydrate metabolism in plants is more complex than in other organisms, where such subcellular compartmentalization of hexose-phosphates is not present.

#### Sucrose

Sucrose is a nonreducing disaccharide, formed by joining the glucose with a fructose molecule. Sucrose accounts for most of the CO<sub>2</sub> fixed during photosynthesis. It is the major form of transported carbon in plants and in some plants it is the major storage carbohydrate. The translocation of sucrose from source to sink tissues is very active during grain development when storage carbohydrates are synthesized in the



**Figure 2** A schematic overview of the carbohydrate metabolism and its inter-relationship with other biochemical pathways to show the production of major plant cell constituents.

grains. A boost in sucrose concentration in seeds is observed during seed germination when stored carbohydrates are broken down to provide energy to the germinating embryo. Sucrose synthesis is most apparent in leaves, but the ability to synthesize sucrose is fairly widespread among plant cells. In photosynthetic cells, the precursor for sucrose synthesis, fructose-6-phosphate, is synthesized in the cytosol from triose-phosphates by the action of an aldolase and fructose-1,6-bisphosphatase (FBPase). In all cell types, cytosol is the site for the synthesis of sucrose, which is derived from the hexose-phosphate pool, through the concerted action of UDP-glucose pyrophosphorylase, sucrose-phosphate synthase (SPS), and sucrose phosphatase as described below. The action of UDP-glucose pyrophosphorylase controlled

by the pyrophosphate concentration plays a central role as it catalyzes the entry and exit of glucose-1-phosphate from the hexose-phosphate pool.

SPS is highly active and plays a key role in regulating sucrose synthesis. Based on changes in free energy, SPS activities and turnover rates of metabolic intermediates during sucrose synthesis, it has been possible to correlate SPS activity to the amount of sucrose produced in green leaves. The SPS is deactivated by protein phosphorylation and is allosterically activated by glucose-6-phosphate and inhibited by inorganic phosphate providing an additional control of the hexose-phosphate pool. Sucrose synthase (SS) is another enzyme, which can catalyze both sucrose synthesis and degradation. This is also a cytosolic enzyme, which participates in sucrose synthesis in tubers,

but its role in sucrose synthesis in other organs such as developing grains is not very clear.

Oligosaccharides Carbohydrates that on hydrolysis give only monosaccharides are termed oligosaccharides. Depending on the number of monosaccharide residues per molecule, the oligosaccharides are classified as trisaccharides, tetrasaccharides, and so forth. Oligosaccharides in grains are synthesized from sucrose or fructose or both.

Raffinose family of oligosaccharides Second to sucrose,  $\alpha$ -galactosides are the most widely distributed soluble carbohydrates in the plant kingdom, comprising 6-18% of the dry weight of the mature legume seed. Galactosyl cyclitols and the raffinose family of oligosaccharides (raffinose, stachyose, and verbascose) are the predominant  $\alpha$ -galactosides and contain α-galactosidoglucose and α-galactosidogalactose bonds and are nonreducing. Raffinose oligosaccharides are nonreducing sugars and constitute an important form of transported carbon in some plants. These oligosaccharides have generally been considered to have low nutritional value, due to their indigestibility and promotion of flatulence in humans and animals. However, recently it has been found that raffinose oligosaccharides have a beneficial effect on the gut microflora and therefore is recommended in diets to prevent cancer development in the digestive tract.

Raffinose oligosaccharides are synthesized from sucrose by a stepwise addition of galactose units provided by galactinol. The reaction is catalyzed by at least four enzymes resulting in the trisaccharide, raffinose that serves as an acceptor for another galactosyl residue from galactinol, to produce the tetrasaccharide, stachyose. These two reactions are catalyzed by raffinose synthase and stachyose synthase, respectively. A specific galactosyltransferase utilizes UDP-galactinose and myoinositol to produce galactinol for raffinose oligosaccharides. The galactinol molecules are formed from UDP-galactanose and myoinositol by the action of a galactosyltransferase.

Fructans Fructose polymers (fructans) serve as soluble storage carbohydrates in ~12–15% of all flowering plants including cereals (e.g., wheat, barley, oat), forage grasses (e.g., Lolium, Festuca), vegetables (e.g., chicory, lettuce, onion), and ornamentals (e.g., dahlia, tulips). In cereals, fructans can be found in young internodes, from which fructan-derived carbohydrates are mobilized to kernels during grain filling. Besides being an energy reserve in plants, fructans have a role in regulation of osmotic pressure, sink strength, and resistance to cold and drought. The sweet taste makes fructans useful as low-calorie

sweeteners and they have also found some use as fat-replacers in foods. Fructans with low degree of polymerization are promoted as a soluble dietary fiber in the human diet as they are poorly digested in the small intestine, but stimulate growth of beneficial microbes in the large intestine.

Sucrose is the primary substrate for fructan biosynthesis, which takes place in the vacuoles by the action of two or more fructosyltransferases. Sucrose: sucrose 1-fructosyltransferase (1-SST) catalyzes addition of a fructosyl residue derived from one sucrose molecule to another sucrose to form the trisaccharide, 1-kestose. 1-kestose can be further elongated by fructan:fructan fructosyltransferase (1-FFT), which reversibly adds a fructosyl residue from one fructan (DP  $\geq$  3) to another fructan or sucrose molecule. The concerted action of 1-SST and 1-FFT results in a mixture of fructans with 10-20 fructose residues. Plant fructans show a variety of branching patterns. Barley produces bifurcose, which is composed of both inulin ( $\beta$ -2,1linked) and levan ( $\beta$ -2,6-linked) -type fructans. The enzyme Suc:fructan6-fructosyltransferase (6-SFT), which only uses sucrose as fructosyl donor, catalyzes the synthesis of bifurcose. The liliaceous species, such as onion and asparagus, synthesize a fructosyltransferase, 6G-FFT, which catalyzes the formation of the trisaccharide neokestose formed by the transfer of a fructose residue from 1-ketose to the C6 of the glucose moiety of sucrose. The 1-FFT catalyzes extension of the fructan chain at either end of the glucose molecule.

# **Sugar Alcohols**

Sugar alcohols are primary photosynthetic products that are accumulated temporarily in leaves during light and are translocated to other plant organs at night. As compared to the corresponding sugar, sugar alcohols have an additional hydroxyl group, and therefore, are designated as polyols, polyalcohols, or polyhydric alcohols. Mannitol, sorbitol, galactitol, and gluctinol are the main sugar alcohols, which have been studied in plants. These compounds are rare in monocots but contribute significantly to transported and stored carbon in some horticultural plants, such as members of the Rosaceae, Rubiaceae, and Plantaginaceae families. Sugar alcohols have been implicated in abiotic stress tolerance. In comparison to sucrose, sugar alcohols are more metabolically sequestered, and this has important implications in their physiological role in translocation and storage of carbohydrates.

Sugar alcohols are synthesized from hexoses or hexose-phosphates, through the consecutive action of reductases and phosphatases. In green celery tissues, three cytosolic enzymes, mannose-6-phosphate isomerase, NADPH-dependent mannose-6-phosphate reductase, and mannitol-1-phosphate phosphatase, convert fructose-6-phosphate to mannose-6-phosphate, mannitol-1-phosphate, and mannitol, respectively. Glucitol is similarly synthesized from glucose-6-phosphate by the action of a glucose-6-phosphate reductase and a sorbitol (glucitol)-6-phosphate phosphatase.

#### Starch

Starch is the most common storage carbohydrate in plants. Besides being an important energy source in the human diet and animal feed, starch is also utilized in many nonfood applications, e.g., paper, pulp, and textile production, because of its excellent gelling, pasting, and adhesive properties. Since starch is a renewable and biodegradable resource, it has received increased interest for production of environmentally friendly biomaterials.

Starch is a complex macromolecule composed of two glucan polymers, an essentially linear chain amylose, and a highly branched amylopectin. Starch is normally made up of one-quarter amylose, with the remaining three-quarters being amylopectin. The two components are arranged in a three-dimensional, semicrystalline structure - the starch granule. Starches from different sources differ in their overall properties due to differences in granule size distribution and shape, amylose and lipid content, distribution of chain length in amylopectin, phosphorylation, and crystallinity. For example, native starches from pulse crops generally have a higher percentage of amylose as compared to cereal starches (Tables 2 and 3). Most of the gelling and pasting behavior of extracted starch is determined by the amylose/amylopectin ratio, amylopectin structure, and starch granule size distribution. Genetically altered starches derived from mutagenesis and breeding programs have already been commercialized.

Biosynthesis of starch in plants has been actively studied over the years but the biochemistry of amylopectin synthesis and the precise mechanism for starch granule initiation is still not clearly understood. Studies of starch mutants have so far produced a general consensus that ADP-glucose pyrophosphorylase (AGPase), soluble starch synthases (SSs), starch branching enzymes (SBEs), starch debranching enzymes (DBE; pullulanase; isoamylase), and possibly also, disproportionating enzyme (D-enzyme) catalyze the final steps leading to amylopectin synthesis. Granule bound starch synthase 1 (GBSSI) is the only enzyme committed to amylose synthesis. Since several of the enzymes exist in different isoforms, some of

which vary in their subcellular distribution, enzyme specificity, temporal activity, and interaction with other enzymes, the starch biosynthesis pathway is complex. The AGPase catalyzes the reaction between Glu-1-P and ATP to produce ADP-glucose, which is the primary building block required for chain elongation to initiate starch synthesis. The priming mechanism for starch glucan polymerization in plants is unclear, but it likely involves short-chain maltodextrins that are extended and branched by the action of GBSSI, SBEI, and SSI to form a molecule with amylopectin-like backbone. The primary glucan polymer is further polymerized through cycles of chain extensions and branching catalyzed mainly by SSI and SBEII. Debranching enzymes have been proposed to play a role in starch biosynthesis by trimming the ends of the water-soluble preamylopectin molecules to produce amylopectin that can be crystallized and packaged into granules. De-branching enzymes are also suggested to degrade water-soluble glucans synthesized by starch synthases and starch branching enzymes to prevent accumulation of phytoglycogen, which cannot form granules. Observations made in Chlamydomonas suggest that the D-enzyme is also involved in starch synthesis and granule formation in addition to starch degradation. Polymerization of amylose is initiated from soluble malto-oligosaccharides that serve as primers for GBSS1.

#### Cellulose

Cellulose is the major component of cell walls and accounts for 28–30% of dry matter in forage grasses and 42-45% of wood. It is the most abundant renewable biomass synthesized on earth and has since the early days of human civilization been used for fuel, timber, fiber, and forage. Today, cellulose has additional uses as raw material for production of paper, pulp, and many chemicals. Cellulose is composed of several dozen glucan chains that are arranged parallel to each other and hydrogen-bonded to form a microfibril with an average thickness of 36 chains. The chains contain a few thousand glucose units giving an individual length of 2-3 µm, but the chains start and end at different points of the microfibril, thus extending the length of the microfibril to hundreds of micrometers.

In higher plants, cellulose synthesis occurs in a plasma membrane-bound complex known as "rosette TC." The structure contains six subunits, each composed of six glucan (cellulose) synthase molecules that are symmetrically arranged into a rosette. The subunits of the complex synthesize simultaneously  $36\,$  1,4- $\beta$ -D-glucan chains that are bundled into a microfibril emerging from the rosette TC.

The basic biochemical understanding of cellulose synthase (Ces A) has mainly been obtained from studies of the bacterium, Acetobacter xylinum. Ces A consists of at least two subunits, one with catalytic and the other with a regulatory role. The catalytic subunit of Ces A is a progressive glycosyltransferase, which binds the substrate, UDP-glucose, derived from sucrose by the action of a membrane-bound sucrose synthase. At the initiation of polymerization, two UDP-glucose molecules are present in the substratebinding site and as the chain elongates, glucose is added to the nonreducing end of the chain. The first plant Ces A genes were characterized in cotton and have been followed by Ces A and cellulose synthase-like (Csl) genes from Arabidopsis thalliana, rice, and poplar. In addition to the Ces A, it has been reported that membrane-anchored endo-1,4-β-glucanases may also play a role in cellulose biosynthesis, based on studies with the Arabidopsis Korrigan mutant and the cellulose-producing bacterium Agrobacterium tumefaciens.

#### **Other Cell-Wall Carbohydrates**

Other important polysaccharide components of the cell wall include cross-linking glycans, which are hydrogen-bonded to cellulose microfibrils. These glycans coat the cellulose microfibrils and link two or more cellulose microfibrils to form a network in the primary cell walls of all plants. The two major classes of the cross-linking glycans are xyloglucans (XyGs) and glucuronoarabinoxylans (GAXs), of which XyGs are present in cell walls of most dicots and almost half of all monocot species. The XyGs are made up of linear chains of 1,4- $\beta$ -D-glucan with numerous  $\alpha$ -D-Xyl units linked to the O-6 position of the glucan units. In some cases, the xylosyl units are further substituted by arabinose or galactosyl units. In addition to the two xyloglucans, some members of the Poaceae family, which includes the majority of cereal crops, such as wheat and barley, contain a third cross-linking glycan,  $\beta$ -glucan. This is a linear polymer that consists of 3-D-glucopyranosyl monomers joined by both  $(1\rightarrow 3)$  and  $(1\rightarrow 4)$  linkages, normally in a 2:1 ratio.

Arabinoxylans and  $\beta$ -glucans affect the quality and utilization of cereal grains due to their presence in the endosperm cell walls. The predominant component of starchy endosperm cell walls in barley, oats, and maize is  $\beta$ -glucan, whereas arabinoxylans are the major component found in rye and wheat. These compounds have a significant impact on the utilization of the grains for both food and animal feed. In wheat flour, pentosans absorb water and significantly affect the quality of baked products. The cell-wall carbohydrates are not completely digested in the stomach or

small intestine of humans or other monogastic animals, but are digested by bacteria in the large intestine. They tend to reduce the absorption of monoand di-saccharides in the gut by raising the viscosity of the gut contents, thus decreasing the levels of blood glucose and insulin.  $\beta$ -Glucans have been shown to significantly reduce blood cholesterol levels, when present in the diet at high levels. In addition, they have also been shown to have antibacterial, antiviral effects and accelerate wound healing. In animal feed, both gluconoarabinoxylans and  $\beta$ -glucans have antinutritional effects, as these may decrease the absorption of nutrients due to their high viscosity and indigestibility.

Cell-wall polysaccharides are synthesized from sucrose through the action of SS, UDP-glucose dehydrogenase, UDP-glucuronate decarboxylase, xylan synthase, and arabinosyl transferase that sequentially give rise to UDP-glucose, UDP-glucuronate, UDP-xylose, 1,4- $\beta$ -xylan, and arabinoxylan, respectively. The action of three epimerases further convert UDP-glucose to UDP-galactose, UDP-glucuronate to UDP-galactouronate, and UDP-xylose to UDP-arabinose, respectively.

# Carbohydrate Breakdown

Germination is initiated when the dry seed imbibes water and the process is completed with the elongation of embryonic axis (Figure 3). One of the first signs of metabolic activity in a germinating seed is the activation of enzymes that hydrolyze storage reserves. This process has been well characterized in cereal grains (Figure 3). A group of plant growth regulators known as the gibberellins (GA) are the first to be synthesized in the scutellum of the embryo. The GAs diffuse into the aleurone layer and induce the production of hydrolytic enzymes such as  $\alpha$ -amylases active on the starch granules in the endosperm. The hydrolysis products are taken up by the embryo to initiate its growth. Carbohydrates stored in the endosperm or cotyledons are the primary source of energy in the developing embryo until it forms leaves and the photosynthetic process takes over to supply the necessary energy and chemical constituents for the developing plantlet.

#### Starch Breakdown

In cereal grains, starch is the major carbohydrate that is broken down during germination. Starch degradation occurs in three distinct phases: (1) reduction of starch granules to soluble maltodextrins; (2) degradation and de-branching of larger maltodextrins to glucose and glucose-1-phosphate; and finally

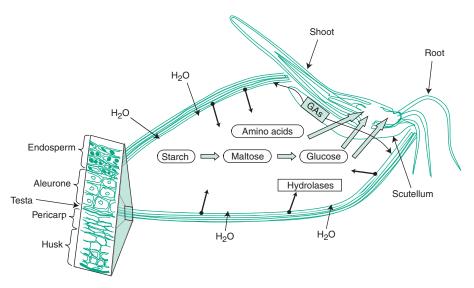


Figure 3 A generalized diagram of a germinating grain to show the various parts of a grain and the major biochemical activities during the time of germination.

(3) transport of the glucose or glucose-1-phosphate from the storage organs to the hexose-phosphate pools of developing plant cells for further metabolism into chemical constituents and energy. In germinating seeds, the initial degradation of starch granules is associated with an increase in the  $\alpha$ -amylase activity. Intact starch granules are attacked by α-amylase resulting in the formation of random pits on the granules. During the  $\alpha$ -amylase attack,  $\alpha(1,4)$  bonds both in amylose and amylopectin are randomly broken to release large glucan polymers. Subsequent degradation occurs with assistance from  $\beta$ -amylases, phosphorylases, maltases, and starch de-branching enzymes. α-Amylase action on amylopectin, which contains both  $\alpha(1,4)$  and  $\alpha(1,6)$  glucan linkages, results in maltose and branched dextrins of short chain length as  $\alpha$ -amylase cannot break the  $\alpha(1,6)$  linkages.  $\beta$ -Amylases first act only on the nonreducing ends of the glucan polymer and hydrolyzes starch into  $\beta$ -maltose, which is rapidly converted into natural mixtures of  $\alpha$ - and  $\beta$ -isomers by mutarotation. Starch phosphorylases are a group of phosphorolytic enzymes, which degrade starch molecules from the nonreducing end of glucan chain. The reaction results in complete breakdown of amylose to glucose-1-P units, but in amylopectin the degradation stops within a few glucose residues of the branch point to give dextrins. Like plant amylases, starch phosphorylases are also widely distributed in plants, and have an important role in starch degradation in leaves and seeds of many plants. However, in germinating cereal grains, starch phosphorylase activity is not detected giving the  $\alpha$ - and  $\beta$ -amylases a dominant role in starch degradation. In seeds of other species and in leaves, starch phosphorylases also contribute towards starch degradation.

The  $\alpha(1,6)$  glucan linkages in the starch molecule are hydrolyzed by starch de-branching enzymes, which have also been implicated in starch synthesis as described above. Depending upon the substrate specificity, the debranching enzymes are classified as isoamylases and pullulanases. This group of enzymes hydrolyze  $\alpha(1,6)$  linkages, thus producing additional end groups for action by amylases and phosphorylases. Concerted action of these enzymes results in the production of maltose, glucose, or glucose-1-phosphate. Maltose rarely accumulates during starch digestion as it is broken down to glucose by maltase.

#### **Raffinose Oligosaccharide**

Stored raffinose oligosaccharides are rapidly utilized during germination. In higher plants, a α-galactosidase hydrolyzes the galactoside bonds of the raffinose oligosaccharides or other  $\alpha$ -D-galactosides to release a galactose moiety. The galactose unit is thereafter phosphorylated by a galactokinase to produce α-Dgalactose-1-P, which is further metabolized through UDP-galactose to produce either UDP-glucose or glucose-1-P, which enter the cellular hexose phosphate pool. Depending on the metabolic activities of the tissues, the carbon may flow into the respiratory pathways as in the case of germinating tissues, or to the synthesis of starch, sucrose, oligosaccharides or other cellular components such as glycolipids or glycoproteins (Figure 2) in young meristematic tissues. Most of the raffinose oligosaccharide degrading enzymes have been purified from seeds of pulse crops and increased activities have been shown to be associated with seed germination. However, strategies to

modulate the activities of raffinose oligosaccharide degrading enzymes are complicated by the presence of two distinct pathways for the metabolism of galactose-1-P to UDP-glucose and glucose-1-P.

#### **Fructans**

Fructans are hydrolyzed by  $\beta$ -fructofuranosidase enzymes having specificity for the  $\beta(2,1)$  or  $\beta(2,6)$  linkages. A  $\beta$ -fructofuranosidase from Jerusalemartichoke tuber successively cleaves fructose units from inulin until a mixture of fructose and the terminal sucrose unit remains. Fructose can enter directly the respiratory pathway, whereas sucrose must be broken down to glucose and fructose first. In the last few years, fructan degrading exohydrolytic enzymes have been purified from many plant species. Two exohydrolytic enzymes, one with a  $\beta$ -(2-6)-linkage-specific fructan- $\beta$ -fructosidase and another with  $\beta$ -(2-1)-linkage-specific activity, from *Lolium perenne* and Jerusalem artichoke, respectively, have been isolated.

#### **Sucrose**

Invertase and SS are the two enzymes capable of cleaving sucrose present in plants. Invertases catalyze the irreversible hydrolysis of sucrose to free glucose and fructose. Invertases are present in the cytosol, vacuole, and in the cell walls. The cytosolic invertase is an alkaline type, active at pH 7.5, whereas the vacuolar and cell-wall invertases are acidic enzymes active at pH 5 or lower. The cell-wall invertases hydrolyze the incoming translocated sucrose into glucose and fructose molecules for conversion into storage carbohydrates.

SS catalyzes the reversible reaction converting sucrose and UDP to fructose and UDP-glucose. Fructose is available for respiration and the UDP-glucose can enter the hexose-phosphate pool for further metabolic processes. SS, compartmentalized in the cytosol, is the main enzyme that degrades sucrose in starch-storage organs such as developing seeds or in rapidly growing tissues that are converting sucrose to cell-wall structural polysaccharides. In slow growing and mature cells, invertase is the major enzyme hydrolyzing sucrose and provides substrates for respiration.

# **Cell-Wall Polysaccharides**

The cell-wall polysaccharides have a physiologically different role in seed germination. The process of germination and seedling development requires the participation of several endo- and exohydrolases that degrade or modify the cell-wall structure. During germination, (1,3) and (1,4)- $\beta$ -D-glucan endohydrolases hydrolyze the (1,4)-linkages adjacent to the (1,3)

 $\beta$ -D-glucosyl residues to release large oligosaccharides. The resulting oligosaccharides are further hydrolyzed by broad-specificity exohydrolases to produce glucose, which can subsequently be used in numerous ways (**Figure 2**). Some of the other cell-wall polysaccharides such as arabinoxylans can be degraded to oligosaccharides which subsequently follows the oligosaccharide degradation pathway as described above.

# **Concluding Remarks**

In most grains, carbohydrates are the major components that provide energy needed during seed germination and early seedling development. Carbohydrates also play a major role in determining the quality of the grain and its subsequent use by human beings. Carbohydrate metabolism in plants differs from most other organisms as it occurs in two distinct cellular compartments, the plastid and the cytosol. Most of the biosynthetic activities take place in the plastids, chloroplasts in leaves and amyloplasts in the storage organs. The carbohydrate metabolism in the cytosol communicates with that of the plastids through metabolic carriers in the plastid envelope. The stationary nature of the plant forces it to withstand changes in the external environment and therefore has developed inherent flexibility in its metabolism to cope with both biotic and abiotic stresses. It is often found that a single reaction in carbohydrate metabolic pathways is catalyzed by multiple isoforms of an enzyme. Differences in temporal and spatial expression of genes encoding these isoforms present several challenges to modify carbohydrate metabolic pathways by genetic engineering. To date, it has been possible to alter the expression of genes encoding starch biosynthetic enzymes to produce crops with modified grain carbohydrates that have health benefits for human beings and/or grain processing advantages. Some advances have also been made in changing the cell compartment and/or tissue in which a specific polysaccharide is stored. As the understanding of the carbohydrate metabolic pathway increases, novel strategies to alter carbohydrate biosynthetic or degradation pathways will emerge, which can be utilized to develop crop cultivars with novel products in the grain. These new crops will diversify the uses of the grain to benefit both the producers and consumers.

#### **Acknowledgments**

Saskatchewan Agricultural Development Fund, Brewing and Malting Barley Research Institute, Natural Sciences and Engineering Research Council, National Research Council Canada, Genome Canada/Genome Prairie, and Canada Saskatchewan Agri-Food Innovation Fund are gratefully acknowledged for supporting research in our laboratories.

See also: Cereals: Chemistry of Nonstarch Polysaccharides. Grains Other than Cereals, Nonstarch Polysaccharides. Protein Synthesis and Deposition. Starch: Uses of Native starch; Analysis of Quality; Chemistry; Modification; Synthesis. Lipid Chemistry.

# **Further Reading**

- Bressani R and Elias LG (1988) Seed quality and nutritional goals in pea, lentil, faba bean and chickpea breeding. In: Summerfieldf RJ (ed.) World Crops: Cool Season Food Legumes, pp. 382-411. Dordrecht, The Netherlands: Kluwer Academic.
- Buchanan BB, Gruissem W, and Jones RL (2000) Biochemistry and Molecular Biology of Plants. Rockville, MD: American Society of Plant Physiologists.
- Chibbar RN and Båga M (2003) Genetic modification of primary metabolism - carbohydrates. In: Thomas B, Murphy DJ, and Murray BJ (eds.) Encyclopedia of Applied Plant Sciences, pp. 449-459. Oxford, UK: Elsevier.
- Hedley CL (2001) Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomic Characteristics. Wallingford: CABI Publishing.
- Hrmova M and Fincher GB (2001) Structure-function relationships of β-D-glucan endo- and exohydrolases from higher plants. Plant Molecular Biology, 47: 73-91.

- Keller F and Pharr DM (1996) Metabolism of carbohydrates in sinks and sources: galactosyl-sucrose oligosaccharides. In: Zamski E and Schaffer AA (eds.) Photoassimilate Distribution in Plants and Crops -Source-Sink Relationships, pp. 157-183. New York: Marcel Dekker.
- Kruger NJ (1997) Carbohydrate synthesis and degradation. In: Dennis DT, Turpin DH, Lefebvre DD, and Layzell DB (eds.) Plant Metabolism, pp. 83-104. Harlow, Essex, England: Addison Wesley Longman Limited.
- Sathe SK, Deshpande SS, and Salunke DK (1984) Dry beans of Phaseolus. A review: Part 2. Chemical composition: carbohydrates, fiber, minerals, vitamins, and lipids. CRC Critical Reviews in Food Science and Nutrition 21: 41-93.
- Shelton DR and Lee WJ (2000) Cereal carbohydrates. In: Kulp K and Ponte JG, Jr (eds.) Handbook of Cereal Science and Technology, 2nd edn., pp. 385-415. New York: Marcel Dekker.

#### **Relevant Websites**

http://www.chem.gmul.ac.uk - International Union of Pure and Applied Chemistry and International Union of Biochemistry and Molecular Biology; IUPAC-IUBMB Joint Commission on Biochemical Nomenclature (JCBN) – Nomenclature Carbohydrates.

http://www.fao.org - Includes classification and chemistry of carbohydrates, carbohydrate metabolism in fish, factors affecting it and energy transformation.

# **CELIAC DISEASE**

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#### Introduction

Celiac (coeliac) disease (CD) is a permanent intestinal intolerance to dietary wheat, rye, and barley proteins (gluten) that produces mucosal lesions and nutrient malabsorption in genetically susceptible individuals. The current essential therapy is a strict lifelong

withdrawal of gluten from the diet. The prevalence of CD was underestimated for a long time; with the development of sensitive serological tests, it is now becoming clear that CD is one of the most frequent food intolerances in many parts of the world. An overview of extensive research on CD is provided.

#### **Definition**

CD also known as celiac sprue and gluten-sensitive enteropathy, may be defined as an inflammatory disease of the upper small intestine (duodenum, jejunum)

#### **Historical Review**

CD was already noted by the Roman physician Aretaeus the Cappadocien as early as the second century AD, describing that "if diarrhea does not proceed from a slight cause of only one or two days duration and if, in addition, the general system be debilitated by atrophy of the body, the celiac sprue of chronic nature is formed." However, it was not until 1888 that Samuel Gee described the classical account of CD features. He recognized that "if the patient can be cured at all it must be by means of the diet." Thereupon the dietary treatment of CD was continued more or less successfully. For example, all sources of carbohydrates such as bread, cereals, and potatoes were excluded or a banana diet was recommended. Such extreme dietary therapy was then used extensively for many years.

In 1950, WK Dicke, a Dutch pediatrician, observed a decline in CD in Holland during the grain-deprived years of the Second World War. A clear association between CD and the ingestion of wheat and, later on, of rye and barley, was established. Fractionation of wheat flour and testing led to the conclusion that gluten was toxic, whereas starch and the water-soluble albumins were not. Since that time, a "gluten-free diet" was the conventional treatment of CD, which excluded products containing wheat, rye, and barley only excepting pure starches produced from these cereals. The celiac toxicity of oats has been judged controversially up to this day.

#### Symptoms and Pathology

A range of symptoms may be associated with CD and these can be divided into intestinal features and those caused by malabsorption (e.g., deficiency of vitamins and minerals). In infants, classical symptoms appear after weaning and introduction of cereals into the

diet: diarrhea, abdominal distension, failure to thrive, vomiting, muscle wasting, and apathy. Older children tend to have more varied symptoms, besides diarrhea or constipation features such as anemia, loss of appetite, and short stature may be predominant. Diarrhea is the main presenting feature of adults, in some patients anemia, osteoporosis, abdominal pain, loss of weight, and weakness may be found. A minor number of patients present psychological or psychiatric symptoms. Frequently, the disease may be clinically silent or masked by associated diseases like diabetes, though the patients exhibit the full mucosa lesion. They are discovered only when intestinal studies are undertaken.

The small intestinal lesion of CD has a highly characteristic morphology, but it may vary from patient to patient depending on the severity and extent of disease. Patients show a wide variation of mucosa appearance from a complete flat to a convoluted pattern. The parameters usually assessed for the measurement of intestinal mucosa are villous height, villous width, villous height to crypt depth ratio, mucosal thickness, epithelial cell height, crypt mitotic activity, and number of intraepithelial lymphocytes. Electron microscopy demonstrates fewer microvillous intramembrane particles and abnormal tight junctions between epithelial cells. In untreated CD, there are raised serologic antibodies to gliadin, reticulin, endomysium, and tissue transglutaminase (tTG).

# **Epidemiology and Genetics**

CD is mostly a disease prevalent in Europe and those countries to which Europeans have emigrated including North and South America and Australia. However, CD is also known to occur in many other parts of the world. In former times, CD was considered a comparatively uncommon disorder with prevalence rates of 1 in 2000–1000. Several recent studies using serologic screening followed by small intestinal biopsy have shown a much higher prevalence, and it is now estimated that CD may affect 1 in 300-100 individuals. The iceberg is a common model to describe the epidemiology of CD. The tip of the iceberg is formed by patients who have been diagnosed by conducting a biopsy demonstrating a flat mucosa. Below the waterline, there is a big group of undiagnosed patients with a flat mucosa, but with no or weak symptoms (silent CD). Just at the bottom of the iceberg there is a small section of patients with a normal mucosa, but with the genetic predisposition and increased levels of celiac-specific antibodies. These subjects may develop clinically overt CD later in life (potential CD).

The occurrence among first-degree relatives of CD patients has been reported to be fairly strongly (10-15% prevalence). Concordance between dizygotic twins has been found to be in a range of 11–20% and that between monozygotic twins 70–86%. CD can occur at any age, but in adults, the peak incidence is in the fifth decade. Females are more commonly affected than males, the ratio has been suggested to be 2:1.

CD is associated with histocompatibility complex class II alleles HLA-DQ2 and HLA-DQ8. HLA-DQ2 was found in 98% of celiac patients from northern and central Europe. However, 25% of the healthy population carry DQ2 and will never develop CD. Thus, genetic factors alone do not explain the development of CD and additional factors such as infection agents and hormonal status may be involved. In southern Europe and Israel, DQ2 is also the major susceptibility genotype (92%), there is another small section who carry DQ8. The only nonHLA region to which association with CD has been convincingly shown until now is the CTLA-4/ CD28 region on chromosome 2q33.

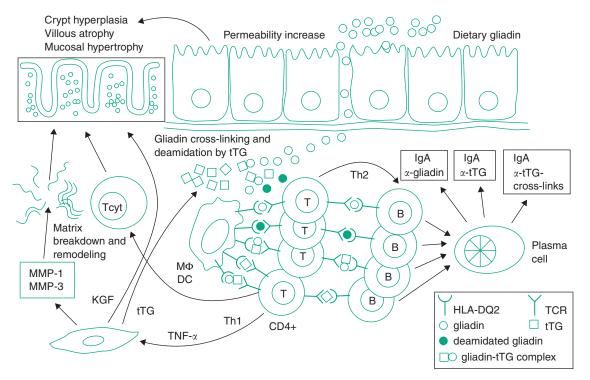
# **Pathogenesis**

The earliest theory on the pathogenesis of CD was based on the missing peptidase hypothesis. The basic defect was considered to be a deficiency of an enzyme in the small intestine which is required for the digestion of celiac toxic proteins. This theory was supported by studies of the celiac-active peptide fraction 9 isolated from an enzymatic digest of gluten. This fraction was only partially digested by homogenates of remission celiac mucosa, whereas it was completely digested by corresponding homogenates from normal people. Other authors, however, have shown that peptidase activities are normal in the mucosa of CD patients on a gluten-free diet. Nevertheless, the enzymatic hypothesis cannot be completely ruled out, because enzymatic activities within the enterocytes have not been studied sufficiently until now. Another theory was based on a nonimmunomediated lectin-like effect of celiac toxic proteins due to binding to glycoproteins on intestinal epithelial cells of CD patients. Carbohydrate side chains of toxic proteins were postulated to be important in activating CD. However, there was little evidence to support this because A-gliadin, a group of aggregative α-gliadins known to be celiac toxic, lacks such carbohydrate side chains. Partial sequence homology between an adenovirus E1B protein and α-type gliadins led to the proposal that human adenovirus type 12 might be an environmental factor involved in the pathogenesis of CD.

The currently accepted theory of pathogenesis is based on a primary immune response to gluten proteins as antigens and to tTG which has been identified as the autoantigen recognized by the endomysial antibodies. The antigens are regarded not to be the intact gluten proteins, but certain peptides which are formed from the proteins as a result of exposure to proteolytic enzymes of the gastrointestinal tract. The peptides are bound to the intestinal mucosa, then pass through the enterocytes by means of endo- and exocytosis and come into contact with the macrophages and antigen-presenting cells of the lamina propria. There are indications that the process is induced by a hyperimmune response in individuals with the histocompatibility antigens DQ2 and DQ8. The gluten peptides are bound specifically to these HLA molecules, processed, e.g., deamidated by tTG and presented to the T cell receptors of gluten-sensitive CD4 helper cells (Figure 1). The T cells then provide help for the production of antibodies to gluten proteins and autoantibodies to tissue transglutaminase. Simultaenously, the stimulation of T cells leads to the secretion of pro-inflammatory T helper (Th-1) cytokines, in particular  $\gamma$ -interferon and nitric oxide which produces premature senescence of small intestinal enterocytes, shedding of these cells and, in turn, the observed small intestinal enteropathy.

#### **Associated Diseases**

Dermatitis herpetiformis, a skin disorder, is associated with CD, because some degree of gluten-sensitive enteropathy is common to both conditions. It appears as patches of itchy red papules and blisters on the extensor surface and pressure areas; healing up results in pigmentation. Histologically, there is infiltration of the dermal papillae with inflammatory cells. The histology of the small intestine is similar to that of CD, but the abnormalities tend to be milder and more patchy. In many cases, a gluten-free diet results in a significant improvement. Recent screening studies have shown an increased prevalence of CD in autoimmune disorder such as type 1 diabetes, thyroid disease, primary biliary cirrhosis, and Sjögren syndrome. Moreover, IgA deficiency, chronic fibrosin alveolitis, and other interstitial lung diseases, concomitant distal ulcerative colitis have been reported in association with CD. In other instances, there are unexpected associations such as epilepsy and various undefined neurological disorders. A small number of patients with CD have exhibited symptoms of various psychiatric disorders, e.g., schizophrenia. A lymphoma of the small intestine is the classical malignancy associated with CD. The prevalence of carcinoma of the small intestine, esophagus, or pharynx



**Figure 1** Immune response in the gut-associated lymphoid tissue and destruction of enterocytes in the pathogenesis of CD (B = B cell, CD4 = helper T cell, DC = dentritic cell, HLA = human leucocyte antigen, Ig = immuno-globulin, KGF = keratinocyte growth factor, M = macrophage, T = T cell, TCR = T cell receptor, TNF = tumor necrosis factor, tTG = tissue transglutaminase). (Courtesy of D Schuppan, Erlangen, Germany (previously unpublished).)

also increases with CD. It has been reported that a strict gluten-free diet helps safeguard against these malignancies.

#### **Diagnosis**

Most important for the diagnosis of CD was the introduction of small intestinal biopsies in 1960. Since that time mucosal biopsy is regarded as the golden standard for the firm diagnosis of CD. The histological examination of biopsy specimens demonstrates a flat mucosa with the absence of normal intestinal villi, a cellular infiltrate of the lamina propria, and an increase in the number of intraepithelial lymphocytes. The European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) recommended three intestinal biopsies in 1970. One should be performed at the time of presentation, another after the patient has been on a gluten-free diet demonstrating improvement and the final biopsy after the patient has been rechallenged with gluten, when villous atrophy is expected to have recurred. The question of whether three biopsies and supervised gluten rechallenge are necessary has been a matter of debate. Recently ESPGAN has suggested that it is not mandatory to proceed to a gluten challenge, if a gluten-free diet has produced a good improvement in symptoms and in the morphology of the biopsy specimen. Noninvasive screening tests would be of considerable aid in diagnosis, but cannot replace histology. In untreated CD, there are enhanced antibodies to gliadins, reticulins, endomysium, and tTG (Figure 1). IgA antigliadin antibodies have a sensitivity of ~80-90% and a specificity ~85-95%. IgG antigliadin antibodies are less sensitive and specific. Until recently the determination of IgA antiendomysial antibodies was the most important test in the diagnosis of CD reading sensitivity and specificity  $\sim 97\%$ . The combination of antigliadin and antiendomysial antibodies has positive and negative predictive values approaching 100%. The recent demonstration that the antigen for endomysium is tTG has allowed the development of an enzyme-linked immuno-sorbent assay (ELISA) for both IgG and IgA tTG. They are similar in sensitivity and specificity like the endomysial antibody test, but they are cheaper and their results are much easier to reproduce.

#### Therapy

After a diagnosis of CD has been established, permanent withdrawal of gluten from the diet is the current essential treatment. Gluten-free diet involves avoiding products containing wheat, rye, and barley. Starches

of these cereals are permitted, when the nitrogen content does not exceed 0.05% (Codex Alimentarius Standard 1981) or the gluten level is lower than 200 mg per kg (Draft Revised Codex Standard 2000). The daily intake of gluten should not exceed 20 mg. The toxicity of oats has been discussed controversially. Recent studies have, however, provided strong evidence that oats do not damage the mucosa of CD patients. Since small amounts of gluten are hidden in many foods, dietary counseling is absolutely necessary. In most countries celiac societies are an invaluable help in providing dietary guidelines and food lists. Keeping a strict gluten-free diet most patients show a rapid clinical response with improvement of symptoms within weeks. Histological improvement is slower and complete recovery can take months or years. The prognosis for patients who are correctly treated is excellent. Failure to implement a strict diet may result in continuing symptoms and in the two major complications of osteoporosis and malignancy.

CD patients consume gluten-free food from two different categories. First, they are allowed to eat a wide range of common products such as meat, fish, milk, fruits, and vegetables. In the case of composite foods, however, it is difficult to recognize whether they are gluten free or not. An informative labeling of gluten as an "allergen" is currently discussed in the framework of Codex Alimentarius and in the European Union. Second, patients consume dietetic food that is gluten free according to the Codex Alimentarius Standard. The new standard draft proposes a gluten level of 20 mg per kg dry mass for naturally gluten-free food and 200 mg per kg for food rendered gluten free such as wheat starch. For gluten analysis, extraction with 60% ethanol and an immuno-chemical method for quantitative determination (ELISA with mono- or polyclonal antibodies) are recommended. Dietetic gluten-free foods are mostly substitutes of products usually containing wheat, rye, and barley such as bread, other baked products, pasta, and beer. Common basic materials used for these products are flours from maize, sorghum, rice, buckwheat, or chestnut, and common thickening agents are locust bean gum or guaran gum.

# **Toxicity Testing**

Most investigators would agree that *in vivo* testing is the "gold standard" for assessing CD toxicity of proteins or peptides. Early workers established toxicity in a series of feeding tests based on the production of symptoms such as steatorrhea or on tests such as malabsorption of fat or xylose. However, an important impediment was that CD patients differed widely in

their sensitivity to gluten and consequently, the optimal amount of gluten equivalent used to challenge patients and the duration of challenge were uncertain. In any case, 10–100 g of gluten equivalent were necessary for the testing of each patient and such large amounts were the most crucial limiting factor for the feeding tests of purified proteins or peptides.

By direct instillation into the small intestine followed by biopsy, the required amounts could be reduced to 1 g equivalent of gluten. Some of the histological changes of the mucosa have been noted as early as 2 h from the beginning of instillation. Similarly, mucosa of CD patients is sensitized to gluten and offers a more convenient approach for investigative and diagnostic purposes. One to two hours after challenge with 2 g gluten equivalent, mucosa showed significant swelling of the lamina propria, a rapid fall in most cells, and a marked rise of intraepithelial lymphocytes. Because in vivo tests require relative large quantities of substances and only a limited number of test patients are available, a series of *in vitro* tests has been developed. The organ culture of human small intestine, which requires only milligram equivalents of gluten, has been proposed as providing the most reliable in vitro approach. The intestinal tissue from patients with active CD is removed as a part of the diagnostic procedure and incubated in a culture medium. The tissue shows improvement of enzyme activity and morphology in the medium alone, but not in the presence of CD toxic substances. Apart from human material tissue cultures with fetal rat and chicken intestine have been used. Assays based on the stimulation of peripheral blood lymphocytes, the production of the leukocyte-migration inhibition factor or macrophage proagulant activity, the agglutination of leukemia K562 cells, and the disruption of rat liver lysosomes have been applied for screening tests. However, in vivo testing ultimately will be necessary to evaluate conclusions on in vitro testing.

#### Toxicity of Cereal Proteins

The relationship between CD and the ingestion of wheat flour was established in 1950. Soon after, a series of investigations led to the conclusion that rye and barley were also harmful, whereas maize, rice, buckwheat, and potatoes were not. There has been disagreement about the toxicity of oats; the reason for that could have been that commercial oat flour is frequently contaminated with small amounts of wheat, rye, or barley. Recent studies, however, indicated that pure oats do not activate CD. Accordingly, CD is closely related to the taxonomy of cereals: only species found in the tribe Triticeae within the grass

family Poaceae are likely to exacerbate CD. All wheat species besides bread wheat such as durum wheat, spelt, emmer, einkorn, and kamut has been proposed to be toxic for CD patients.

Immediately after wheat was established as the CD activating cereal, fractionation of the flour and in vivo testing led to the conclusion that gluten is toxic, whereas starch and albumins are not. Gluten is the rubbery protein mass that remains when wheat dough is washed with water to remove starch granules and other soluble constituents. By its cohesive viscoelastic properties gluten to a large extent determines the unique baking quality of wheat. Gluten proteins can be divided according to their solubility in aqueous alcohols into the soluble gliadins and the insoluble glutenins. Toxicity tests indicated that the gliadin fraction was the most toxic factor, whereas the effect of the glutenin fraction was described either nontoxic, weakly toxic, or as toxic as gliadins, but on a very inadequate evidence. The reason for this disagreement could be that glutenin preparations isolated from wheat flour or gluten have been differently contaminated by gliadin components covalently bound to the glutenin aggregates.

Flour protein fractions of rye and barley have not been tested for celiac toxicity until now, but equivalent to the gliadin fraction of wheat, the corresponding prolamin fractions of rye (secalins) and barley (hordeins) were associated with CD. Although the prolamin fractions of cereals are crude mixtures of different proteins, their amino acid compositions show a close relationship to both taxonomy and celiac toxicity (Table 1). Toxic gliadin, secalin, and hordein fractions are characterized by the highest contents of glutamine (35-37 mol.%) and proline (17-23%). Both amino acids have been considered to be important for CD toxicity. The prolamins of rice, millet, and maize are lower in glutamine and proline, but rich in alanine (9-14 mol.%) and leucine (12-19 mol.%). Oats are in a medium position, the glutamine content being similar to the Triticeae prolamins and the values of proline and leucine in agreement with those of other species.

Among the prolamin fractions, only gliadin from wheat has been investigated in detail. The digestion with pepsin and trypsin alone or followed by pancreatin resulted in the retention of toxicity, and a peptide mixture with a molecular mass less than 1000 was still toxic. Also, the breakdown of the disulfide bonds by oxidation or heating during the baking process did not destroy toxicity. In consequence, the three-dimensional structure of gliadin proteins is not important for the toxic effect. The complete degradation into free amino acids by acid hydrolysis, however, rendered it harmless, as did extensive deamidation of glutamine side chains with a limited cleavage of peptide chains. Among the different gliadin types ( $\alpha$ -,  $\gamma$ -, and  $\omega$ -gliadins), A-gliadin, a well-defined small group of aggregable  $\alpha$ -type gliadins, was shown first to be toxic by instillation into small intestine followed by biopsy. Subsequent in vivo and in vitro studies demonstrated that all gliadin types produce toxic effects. In contrast, the major protein types of the glutenin fraction, high-molecular-weight (HMW) and lowmolecular-weight (LMW) subunits have not been tested in vivo until now. Recent studies demonstrated an intestinal T cell response to TG-deamidated HMW subunits of glutenin.

Only few attempts were made to detoxify gluten, namely by enzymatic treatment; whereas the digestion of gluten by pepsin, trypsin, and pancreatin resulted in the retention of toxicity, further digestion with fresh pig intestinal mucosa rendered the preparation nontoxic. By another study, the toxic action of gluten was eliminated by crude papain, but not after digestion with pure papain. The crude papain used was found to contain a deamidase which liberates free ammonia from gluten. Recently an endopeptidase derived from the bacterium Flavobacterium meningosepticum was demonstrated to cleave the normally resistant proline-rich region of  $\alpha$ -gliadins corresponding to a 33 amino acid fragment. Accordingly, the T cell activating properties of the peptide was rapidly diminished by the enzyme. The authors suggested a strategy for oral peptidase supplement therapy for CD.

Table 1 Partial amino acid composition (mol.%) of prolamins

	Wheat (gliadin)	Rye (secalin)	Barley (hordein)	Oats (avenin)	Rice (oryzin)	Millet (kafirin)	Corn (zein)
Glx	37	35	35	34	20	22	19
Pro	17	18	23	10	5	8	10
Leu	7	6	6	11	12	13	19
Ala	3	3	2	6	9	14	14
Met	1	1	1	2	1	2	1
Lys	1	1	1	1	1	0	0
Trp	0	0	1	0	1	2	0

# **Toxicity of Peptides**

Because gliadin could be partially hydrolyzed by enzymes without loss of toxicity, further investigations were focused on the testing of peptides. The introduction of in vitro test systems, in particular the organ culture test, enabled the detection of toxic compounds in milligram and even microgram amounts. Pure peptides were isolated from enzymatic digests of whole gliadin and A-gliadin by different separation techniques. A dodecapeptide corresponding to residues 75-86 of  $\alpha$ -gliadins and isolated from a peptic tryptic pancreatic digest of gliadin was shown to be effective on fetal chick intestine and on rat liver lysosomes. Organ culture tests with tissues from CD patients revealed that peptides corresponding to the residues 1-30, 3-55, 3-24, 25-55, and 31-55 of  $\alpha$ -gliadins were toxic. Peptides corresponding to the residues 56-68 and 247-266, however, were inactive. Altogether the results indicated that regions from the N-terminal domain of  $\alpha$ -gliadins are involved in the pathogenesis of CD. They are characterized by high contents of glutamine and proline, and the tetrapeptide sequences PSQQ and QQQP common for the toxic peptides have been considered as key sequences for further studies.  $\beta$ -Turn conformations of the peptides has been suggested to contribute to toxicity.

Since 1987, a panel of synthetic peptides containing sequences of gluten proteins has been tested using *in vitro* and *in vivo* systems. In the following the results of instillation (*in vivo*) and organ culture tests (*in vitro*) are summarized (**Table 2**). Five synthetic peptides derived from  $\alpha$ -gliadin sequences were tested both *in vivo* and *in vitro*. Peptides  $\alpha$  31–43,  $\alpha$  31–49,

and  $\alpha$  44–55 were found to be toxic and peptides  $\alpha$  3–21 and  $\alpha$  202–220 were not toxic. Peptides comprising alanine-substituted variants of peptide 31–49 remained toxic in some patients when residues L31 and P36 were substituted, but lost toxicity when residues P38, P39, and P42 were substituted. In further in vivo studies, peptides  $\alpha$  206–217 and  $\alpha$  56–75 were toxic, whereas the shortened peptide α 56-68 turned out to be inactive. Although the investigations described were somewhat unsatisfactory with regard to both number of tests and purity of peptides, it could be concluded that regions of most toxic sequences of α-gliadins occur in the repetitive N-terminal domain and consist mainly of glutamine, proline, and aromatic amino acids. Corresponding repetitive sequences of  $\gamma$ - and  $\omega$ -gliadins fit well into the potentially toxic sequences of  $\alpha$ -gliadins, whereas the repetitive sequences of LMW and HMW subunits of glutenin show significant differences.

Recently, a number of intestinal T cells from CD patients have been studied in order to determine the stimulation capacity of gluten peptides. A selection of such peptides is presented in Table 3. Several stimulatory epitopes were found in  $\alpha$ - and  $\gamma$ -gliadins that are mostly located in the glutamine and proline rich N-terminal domain. With one exception ( $\gamma$  60–79), the peptides were barely stimulatory in an untreated form, but became stimulatory by deamidation after acid-heat or tTG treatment. Studies of peptides from the N-terminal domain of α-gliadins demonstrated that the deamidation of a single glutamine residue at position 65 was critical for T cell recognition. Such negatively charged residues were proposed to be preferred in positions 4 and 6 of the antigen binding grove of HLA-DQ2 molecules. Modification

Table 2 Toxicity of synthetic gliadin peptides

Origin <sup>a</sup>	Sequence <sup>b</sup>	Toxicity (test) <sup>c</sup>
α 3–21	VPVPQLQPQNPSQQQPQEQ	- (OC, IN)
α 31-43	LGQQQPFPPQQPY	+ (OC, IN)
α 31-49	LGQQQPFPPQQPYPQPQPF	+ (OC, IN)
α 31–49, A 31	AGQQQPFPPQQPYPQPQPF	(+) (OC)
α 31–49, A 36	LGQQQAFPPQQPYPQPQPF	(+) (OC)
α 31–49, A 38	LGQQQPFAPQQPYPQPQPF	– (OC)
α 31–49, A 39	LGQQQPFPAQQPYPQPQPF	- (OC)
α 31–49, A 42	LGQQQPFPPQQAYPQPQPF	– (OC)
α 44-55	PQPQPFPSQQPY	+ (OC, IN)
α 56-68	LQLQPFPQPQLPY	– (IN)
α 56-75	LQLQPFPQPQLPYPQPQLPY	+ (IN)
α 202-220	QQYPLGQGSFRPSQQNPQA	– (OĆ, IN)
α 206–217	LGQGSFRPSQQN	+ (IN)

<sup>&</sup>lt;sup>a</sup>Positions within A-gliadin.

<sup>&</sup>lt;sup>b</sup>One-letter code for amino acids

 $<sup>^{</sup>c}OC = organ culture$ ; IN = instillation; (+) toxic in some patients; (-) negative.

Table 3 Amino acid sequences of selected peptides tested for intestinal T cell stimulation of CD patients

Origin (positions)	Sequence <sup>a</sup>	Activity		
γ5 (60–79)	LQPQQPFPQQPQQPYPQQPQ	+/+b		
γ5 (66–78)	FPQQPQQPYPQQP	$-/+^b$		
γ5 (102–113)	FSQPQQQFPQPQ	$-/+^b$		
γ36 (140–150)	QQPQQSFPQQQ	$-/+^c$		
γ36 (140–150, E 148)	QQPQQSFPEQQ	+/+		
γ5 (228–236, E 232)	IIQPEQPAQ	+		
α2 (57–68)	QLQPFPQPQLPY	_		
α2 (57–68, E 65)	QLQPFPQPELPY	+		
α2 (62–75)	PQPQLPYPQPQLPY	_		
α2 (62-75, E 65)	PQPELPYPQPQLPY	+		
α2 (62-75, A 63, E 65)	PAPELPYPQPQLPY	_		
α2 (62–75, E 65, A 71)	PQPELPYPQAQLPY	_		
α2 (62-75, E 65, A 72)	PQPELPYPQPALPY	+		
α2 (226–237)	PSGQGSFQPSQQ	+		
α2 (226–235)	PSGQGSFQPS	_		
α2 (227–238)	SGQGSFQPSQQN	+		
α2 (229–238)	QGSFQPSQQN	_		
LMW 156 (40-59)	QQQQPPFSQQQQSPFSQQQQ	+		
LMW 17 (46-60)	QQPPFSQQQQPLPQ	+		
HMW2 (722-734)	GQQGYYPTSPQQS	+		
HMW2 (722-731)	GQQGYYPTSP	_		
HMW2 (724-735)	QGYYPTSPQQSG	+		
HMW2 (726-735)	YYPTSPQQSG	_		

<sup>&</sup>lt;sup>a</sup>One-letter code for amino acids.

of single positions with an alanine residue indicated that the sequence region 63–71 was essential for the stimulatory effect. T cells of CD patients recognized also natural peptic fragment of the C-terminal domain of  $\alpha$ -gliadins. The smallest active peptides included the residues 226-237 and 227-238, respectively. Deamidation of glutamine residues was not necessary for the stimulatory effect. By combining highperformance liquid chromatography (HPLC) and mass spectrometry peptides from digested gluten derived from LMW and HMW subunits of glutenin were also identified as activating T cells from CD patients. The effect of deamidation by tTG has been shown to be heterogeneous and can be positive, neutral, and negative. Altogether, numerous immunodominant peptides from gluten have been identified and much more epitopes are thought to exist. These in vitro experiments with isolated T cells pose a lot of questions, regarding the relations between the amino acid sequences and the stimulatory effects, the extent to which the stimulatory peptides reach the small intestinal mucosa after exposure to gastric and duodenal proteases and if these peptides are responsible for toxicity leading to enteropathy. Up to now in vivo toxicity of peptides able to stimulate T cells has been proven in only one case (peptide  $\alpha$  56-75, Table 2).

# **Future Prospects**

Research work will continue on full characterization of all epitopes within proteins from wheat and related cereals that exacerbate CD. Recombinant proteins produced by transgenic microorganisms will aid these investigations. Analytical systems for the detection of such epitopes in foods for CD patients will be advanced in terms of their specificity and sensitivity. Scientific knowledge about the molecular mechanism of CD will increase step by step and facilitate the development of therapies, for example, based on immuno-modulation or the application of specific enzymes for detoxification of proteins. Wheat, rye, and barley proteins might be modified by gene engineering to CD inactive epitopes without loss of their wished functionality as bread-making properties of wheat.

See also: Cereals: Overview; Protein Chemistry. Gluten and Modified Gluten. Nutrition: Effects of Food Processing. Taxonomic Classification of Grain Species. Appendix: Foods for Celiac Diets.

# **Further Reading**

Auricchio S, Greco L, Maiuri L, and Troncone R (eds.) (1999) Proceedings of the Eighth International Symposium on Coeliac Disease. Naples, Italy: JGC Editions.

<sup>&</sup>lt;sup>b</sup>Native/tTG treated.

<sup>&</sup>lt;sup>c</sup>Native/acid-heat treated.

Ciclitira PJ (2001) AGA technical review on celiac sprue. *Gastroenterology* 120: 1526–1540.

Cornell HJ and Wills-Johnson G (2001) Structure activity relationship in celiac-toxic gliadin peptides. *Amino Acids* 21: 243–253.

Feighery C (1999) Coeliac disease. *British Medical Journal* 319: 236–239.

Howdle PD (ed.) (1995) *Coeliac Disease*. London: Balliére's Clinical Gastroenterology.

Mäki M and Tossavainen M (eds.) (2001) Proceedings of the Workshop on Transglutaminases, Protein Crosslinking and Coeliac Disease. Tampere, Finland: Coeliac Disease Working Group. Marsh MN (ed.) (1992) Coeliac Disease. Oxford: Blackwell Scientific Publications.

Marsh MN (ed.) (2000) *Methods in Molecular Biology*. Totowa, NJ: Humana Press.

Schuppan D (2000) Current concepts of celiac disease pathogenesis. *Gastroenterology* 119: 234–242.

Sollid LM (2000) Molecular basis of celiac disease. *Annual Review of Immunology* 18: 53–81.

Troncone R and Auricchio S (1991) Gluten-sensitive enteropathy (celiac disease). Food Reviews International 7: 205–231.

Wieser H (1996) Relation between gliadin structure and celiac toxicity. *Acta Paediatrica Supplement* 412: 3–9.

# **CEREALS**

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# **Overview**

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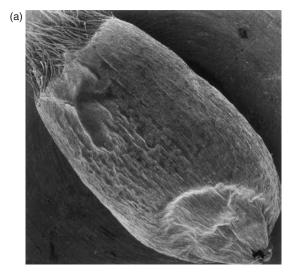
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#### Introduction

The cereal grains are defined as "flowering plants of the Grass Family (Poaceae or Gramineae), whose seeds are used as food." The name "cereal" derives from Ceres, the Roman goddess of grain. Colloquially, the word "cereal" has also come to refer to the range of breakfast foods, e.g., corn flakes, made from the cereal grains. The words "seed," "kernel," or "caryopsis" are also used to denote the cereal grain. In addition, the term "corn" is sometimes used for the grains of the cereal species in general, but more often "corn" denotes the cereal species maize, also known as "Indian corn." A wheat seed is shown in Figure 1, as it appears by scanning electron microscopy.

The cereal grain serves two contrasting functions.

1. For the plant, the mature grain is solely of significance as a seed, namely, the means by which the species is perpetuated. When the plant has died, the ongoing life of the cereal seed is in its embryo or germ (Figure 2) (see Grain, Morphology of Internal Structure and Grain and Plants, Morphology). Most of the grain's mass is taken up by the endosperm, which is the storage organ of the grain. It contains the starch and protein for the "rainy day" when moisture will trigger the dormant seed to start swelling and producing roots and a shoot. In that situation, the endosperm provides the



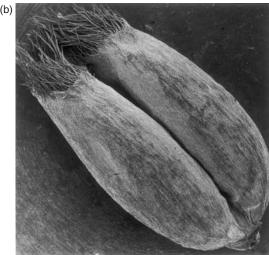
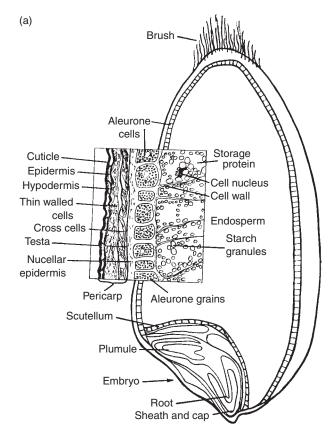


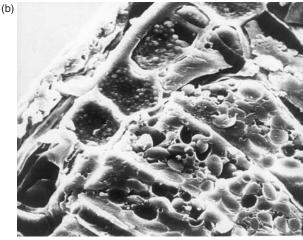
Figure 1 The wheat grain, showing the "brush" at the end opposite from the point of attachment ("hilum") to the wheat head. The "crease" extends along the ventral side of the grain, opposite to the dorsal (top) where the germ is located at the hilum end. These images were provided by scanning electron microscopy, with the grain resting on the stage of the microscope. (Reproduced with permission from Wrigley CW (2004) Cereal Sprouting. In: Goodman RM (ed.) Encyclopedia of Plant and Crop Science. New York: Marcel Dekker.)

essential nutrients for germination before the emergence of the green leaf, allowing photosynthesis to take over as the source of energy and nutrients.

2. For mankind, the mature grain is mainly of significance as an important source of nutrients, namely, an item of food for humans, and a source of feed for man's animals. Examples of these many processed foods are shown in Figure 3.

The major cereal grains of economic importance are the cool-season crops — wheat, barley, oats, and rye — and the warm-season cereals — rice





**Figure 2** Diagram of the many parts of a wheat grain, cut in half to show its subcellular structure, plus electron microscope image of the cut surface of the wheat grain.

(paddy), maize (corn), sorghum, and millet. **Table 1** lists their botanical names and major uses. The cereals are monocotyledonous plants, as distinct from the dicotyledonous members of the wider family of grains (*see* **Taxonomic Classification of Grain Species**). The dicot grain species include the oil-seeds and the pulses (grain legumes) (*see* **Oilseeds**, **Overview**. **Pulses**, **Overview**). The major oil-seeds are canola/rapeseed, sunflower, safflower, and



Figure 3 Some of the many foods made from cereals.

Table 1 The cereal-grain species (in taxonomic groups) and their uses

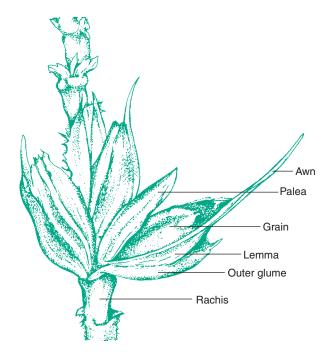
Common names	Genus and species	Uses
Pooids		
Common wheat	Triticum aestivum	Breads (leavened and unleavened), noodles, breakfast cereals (processed porridge), cakes, cookies, chapatis, snack foods, bulgur, ingredients for a wide range of foods, animal feed, industrial uses
Durum wheat	Triticum turgidum subsp. durum	Pasta (e.g., spaghetti, macaroni)
Rye	Secale cereale	Bread (often together with wheat), crispbread, spirits
Triticale	Triticosecale	Bread (often together with wheat), animal feed
Barley	Hordeum vulgare	Malt, beer, spirits, porridge, cooked grains (e.g., soup ingredient)
Oats	Avena sativa	Porridge (as rolled oats, oatmeal), muesli, oatcakes, animal feed
Oryzoids		
Rice	Oryza sativa	Cooked and fried grain, sake, extruded snack foods
Wild rice	Zizania aquatica	Cooked grain
Chloridoids		
Tef, Indian millet	Eragrostis tef	Cooked grain, porridge
Finger millet	Eleusine coracana	Cooked grain, porridge
Panicoids		3 /1 3
Corn (maize)	Zea mays	Polenta, breakfast cereals, tortilla, cornbread, porridge, snack foods, a wide range of industrial uses (e.g., corn syrup, food ingredients)
Sorghum	Sorghum bicolor	Animal feed, cooked grain, porridge, beer, flat cakes
Pearl millet	Pennisetum glaucum	Cooked grain, porridge
Common millet	Panicum miliaceum	Cooked grain, porridge
Japanese millet	Echinochloa species	Cooked grain, porridge
Foxtail millet	Setaria italica	Cooked grain, porridge

linseed (see Canola: Genetics and Breeding; Agronomy; Harvest, Transport, and Storage; Processing. Sunflower). The pulses include lupins, peas, beans, soybeans (also an oilseed), and cottonseed (family Malvaceae) (see Beans. Cottonseed. Lupin: Overview; Breeding; Agronomy. Nutrition: Soy-Based Foods. Pea: Overview. Soybean: Germplasm, Breeding, and Genetics; Agronomy; Grading and Marketing; Processing; Soy Concentrates and Isolates; Soy-Based Fermented Foods). Amaranth, buckwheat,

and quinoa are other dicot grains (see Amaranth, Buckwheat, Quinoa).

# Morphology of the Cereals – What They Look Like

Cereal grasses have narrow leaves, hollow jointed stems, and spikes or clusters of membranous flowers (see Grain, Morphology of Internal Structure and



**Figure 4** A spikelet of wheat, with the mature grain, held within glumes, and attached to the rachis. Other spikelets have been removed from the rachis for clarity. (Reproduced with permission from Fitzsimmons RW, Martin RH, and Wrigley CW (1983a) *Australian Wheat Varieties: Identification According to Growth, Head and Grain Characteristics*, 2nd edn., p. 13. Melbourne: CSIRO.)

Grain and Plants, Morphology). Figure 4 shows how the mature grain of wheat is held within glumes on the single stem of the head (ear). The threshing process at harvest separates the wheat grain (as in Figure 1) from the glumes, rachis, and other parts of the plant. The structure of the barley head is similar to that of wheat (Figure 5), but on threshing, the glumes of barley remain with the grain, whereas the wheat grain threshes free from the glumes. The rye grain also threshes free of the glumes. The flowers of oats and rice, on the other hand, are arranged in an inflorescence called a panicle (Figure 6). The grains of oats and rice do not thresh as the free seed. The term "groat" applies to the oat seed with the glumes removed. The term "paddy rice," or just "paddy," refers to the rice grain with its husks in place, the form in which it is harvested. The familiar cob (ear) of corn (maize) is distinct from the seed-bearing structures of the other cereals described above (Figure 7).

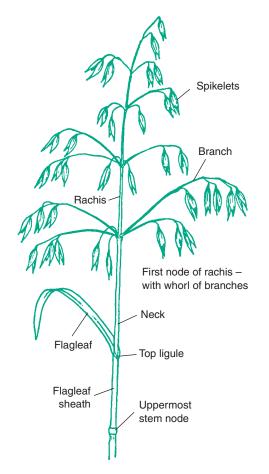
#### **World Production of the Cereals**

Cereal grains are grown extensively in all continents, with the obvious exception of Antarctica. They are the major sources of food for mankind, especially in developing countries. In addition to their use as



Figure 5 Heads of wheat (left), of two-row barley (center), and of six-row barley.

a major source of feed for animals, the cereal grains are extensively processed industrially for food and nonfood products. World production and trade in the major cereals are provided in Tables 2–4 for the year 1999. The provision of such data for one



**Figure 6** The head (panicle) of oats. (Reproduced with permission from Fitzsimmons RW, Roberts GL, and Wrigley CW (1983b) *Australian Oat Varieties: Identification of Plants, Panicles and Grains*, p. 11. Melbourne: CSIRO.)

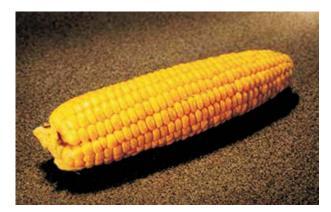


Figure 7 The cob of corn.

harvest year permits direct comparison between cereals and countries, but it should be regarded as a "snapshot" of production and trade, because of the fluctuations that occur from one year to another. Nevertheless, the tables illustrate the regional distribution of centers of production for the prominent cereals, namely, tropical countries for rice and maize, cooler regions for oats, barley, and rye.

World production of all cereal grains approaches 2 billion tons per year on average (including rice as paddy, unmilled). This represents ~700 g of grain per person per day for the world's population, assuming that all the cereal grain could be used for food, and assuming that it could be transported to all the regions where it is needed. Of course, this is neither practicable nor possible, since many of the regions of production are distant from the places of need. In addition, the world's agricultural and industrial operations are reliant on significant amounts of cereal grain.

On average, world production of wheat totals ~600 million tons (Mt) annually, grown on some 230 Mha, with a world average yield of ~2.6 t ha<sup>-1</sup>. Most of this production is used in the countries in which it is grown. Two-thirds of this production is used for food; the remainder goes to a combination of seed use, animal feed, and industrial processing. Many countries produce much more than their domestic requirements, resulting in world trade of ~100 Mt annually (Table 4). The main exporting regions are USA, Canada, Europe, Australia, and Argentina. Wheat's close relative, rye, is minor by comparison; world rye production is ~20 Mt annually, the main production regions being in eastern parts of Europe, especially Poland, Germany, and Russia.

World production of rice is nearly as great as that of wheat, if the production of unmilled paddy rice is considered. On the basis of milled rice, annual world production averages ~400 Mt (Tables 2 and 3). International trade for rice is much less than that for wheat and maize (Table 4). Rice, together with maize, provide the major energy and protein source for many cultures. Annual production of maize is similar to that of wheat. Statistically, maize is included in "coarse grains," production of which totals 900 Mt annually, harvested from over 300 Mha. World trade in coarse grains is ~100 Mt. In addition to maize, coarse grains include barley (averaging ~150 Mt annually), sorghum (50 Mt), and oats (30 Mt).

#### **Historical Perspective**

The cereal grains have the great distinction of being the catalyst that transformed man – the hunter gatherer – into man – the agriculturalist. The origins

Table 2 World production of the major cereal grains, in millions of tons (metric tons) for general regions in the year 1999

Region	Wheat	Rice <sup>a</sup>	Maize	Barley	Oats	Rye
North America	96.7	7.4	276.9	21.0	6.5	0.7
South America	16.5	13.5	50.9	1.4	0.9	
Western Europe	103.9	1.7	35.2	53.0	6.7	6.7
Eastern Europe	33.7		25.4	10.6	2.5	6.3
Former Soviet Union	57.5	0.8	5.3	21.4	6.5	6.6
Africa	18.8	9.5	40.0	5.1	0.2	
Asia	239.1	357.7	170.2	18.9	1.0	0.2
Oceania	22.4	1.0	0.5	6.1	2.0	
World	588.7	391.7	605.0	137.2	26.0	20.3

<sup>&</sup>lt;sup>a</sup>Calculated as milled rice.

Data from United States Department of Agriculture (www.usda.gov.nass.pubs.agroo).

Table 3 Production of the major cereal grains, in millions of tonnes (metric tons) for the year 1999, for prominent countries

Country	Wheat	Rice <sup>a</sup>	Maize	Barley	Oats	Rye
Argentina	12.0	1.1	13.5	0.5	0.4	
Australia	22.1	1.0	0.3	5.7	1.9	
Bangladesh	1.8	19.1				
Brazil	2.2	7.8	32.1	0.3	0.3	
Canada	24.1		9.0	12.7	4.0	0.4
China	109.7	139.1	133.0	3.5	0.7	
France	39.8		15.2	10.6	0.7	0.2
Germany	20.2		2.8	12.5	1.3	4.8
Hungary	4.9		6.0	1.3	0.1	0.1
India	65.9	84.7	10.8	1.7		
Indonesia		32.1	6.5			
Italy	7.9	0.8	8.6	1.4	0.4	
Japan	0.6	8.2		0.1		
Pakistan	18.7	4.7	1.3	0.2		
Philippines		6.7	4.9			
Poland	9.5		0.5	3.6	1.5	5.7
Russia	26.9	0.2	0.8	9.8	4.6	3.3
Spain	5.3	0.6	4.2	10.9		0.2
Thailand		15.0	4.3			
Turkey	18.5	0.2	2.3	7.6	0.3	0.2
UK	15.5			6.6	0.5	
Ukraine	14.9		2.3	5.9	0.7	1.1
USA	69.3	6.1	247.9	7.7	2.4	0.3
Vietnam		20.0	1.5			

<sup>&</sup>lt;sup>a</sup>Calculated as milled rice.

Data from United States Department of Agriculture (www.usda.gov.nass.pubs.agroo).

of civilization involved the enormous step of primitive mankind discovering that it was possible to remain in one place where cereal seed, sown intentionally, would yield a reasonably reliable source of food. As a result, people found that a fixed dwelling place could be established near the cereal crops. Further consequences were the development of permanent forms of housing, the domestication of animals (fed with the cereal grain), and the spare time to develop the many other characteristics of civilization.

Early consumption of the cereals involved merely chewing the raw grain. Then followed the primitive forms of processing, such as grinding the grain between stones and soaking the grain in water to make it easier to chew. Next, heat was discovered as a further means of easing mastication and digestion, producing a porridge or gruel. Placing a stiff porridge in the hot ashes of a fire would have made it more palatable, resulting in baked foods. A porridge left overnight would gain wild yeasts, leading to the discovery of leavening as a means of making the baked product lighter. Further refinements would have been the sieving step, to remove husks and bran particles after grinding, and the identification of wheat as the ideal grain for the baking of leavened bread.

**Table 4** Prominent exporting countries involved in international trade in wheat, rice, and maize, in millions of tons (metric tons) for the year 1999

Country	Wheat <sup>a</sup>	Rice <sup>b</sup>	Maize
Argentina	8.7	0.5	7.8
Australia	16.0	0.7	
Canada	14.4		
China		2.8	3.3
Eastern Europe	3.9		
European Union	16.0		0.3
India		2.4	
Kazakastan	2.0		
Pakistan		1.9	
Thailand		6.1	
Turkey	3.0		
USA	29.0	2.8	51.9
Vietnam		4.5	
Total	101.1	24.5	68.8

<sup>&</sup>lt;sup>a</sup>Wheat and wheaten flour.

Data from United States Department of Agriculture (www.usda.gov.nass.pubs.agroo).



Figure 8 Chinese steamed bread.

There is archeological evidence that primitive baked products were being made in the late stages of the Stone Age. Purpose-built ovens were used in ancient Egypt as far back as 2700 BC, with reasonably sophisticated milling and baking a millennium later. As a result of this long history of cereal-grain use, diverse uses have been developed worldwide for their processing, taking advantage of the specific characteristics of the individual cereal species (see Table 1 and Figures 3 and 8).

**Table 5** Major importers of wheat, in millions of tons for the year 1999

Country	Wheat	Rice	Maize
Algeria	4.4		1.1
Bangladesh	2.2	1.4	
Brazil	7.3	0.8	0.9
Egypt	7.3		3.7
European Union	3.8	0.7	3.0
Indonesia	3.0	3.9	0.5
Columbia	1.1	0.2	1.6
Iran	3.0	0.9	0.8
Iraq	2.5	0.7	
Japan	5.9	0.1	16.3
Mexico	2.5	0.4	5.6
Morocco	2.8		0.8
Pakistan	3.2		
Philippines	2.3	1.2	0.2
Russia	2.5	0.3	0.7
South Korea	4.7		7.5
USA	2.9	0.3	0.4

Data from United States Department of Agriculture (www.usda.gov.nass.pubs.agroo).

# The Diversity of Cereal Grains

#### Wheat

Wheat is prominent among the cereals, because of its unique dough-forming properties (*see* Wheat: Genetics; Breeding; Agronomy; Harvesting, Transport, and Storage; Grading and Segregation; Dry Milling; Marketing; Wet Milling). These viscoelastic characteristics are due to the gluten-protein complex (*see* Cereals: Protein Chemistry), formed from the major storage protein of its endosperm. This is the basis of man's attraction to wheat – the reason for the annual cultivation of over 10<sup>14</sup> wheat plants – because wheat gluten alone can sustain man's desire for leavened bread products. Major countries involved in production and trade are listed in Tables 2–5.

Ideal wheat-growing climates are temperate, with mean summer temperatures of  $\sim 15^{\circ}$ C, an annual rainfall of 300-700 mm, falling more in spring (during grain filling) than in summer (providing opportunity for a dry harvest). Nevertheless, the climates in which wheat can be grown vary greatly, as is exemplified by wheat growing in North and Central America. In northern parts of Europe and USA, winter wheats are sown in autumn, to lie under a snow cover throughout winter, ready for the warmth of spring to bring them back into growth and grain production.

On the Canadian prairies and the northern US plains (North Dakota and Montana), the winters are too harsh to permit the growing of the higher yielding winter-habit wheats. In these very cold regions, spring wheats are sown after the snow has melted, as soon as it is possible to access the fields in spring. Thereafter,

<sup>&</sup>lt;sup>b</sup>Calculated as milled rice.

there is very rapid growth, permitting the grain to mature for harvest just before the snows of autumn. By contrast, wheat growing in Kansas, USA, and down into Mexico may involve the likelihood of heat stress in the late stages of grain filling. Wheat growing in parts of Mexico and the Indian subcontinent involves regions of high altitude.

Yields of wheat vary considerably, depending on the rate of input of fertilizers, and especially on access to water. Some regions where high inputs are possible have "Ten-Ton clubs," involving groups of growers, who regularly achieve yields of over  $10 \text{ t ha}^{-1}$ . By contrast, regions of dry-land farming may reckon on little more than  $1 \text{ t ha}^{-1}$ . Drought and disease may reduce yields in either of these extreme cases (*see* Cereals: Grain Diseases. Plants: Diseases and Pests. Wheat: Agronomy).

Many factors determine the times of harvest in the various wheat-growing regions of the world, listed in **Table 6**. Harvest generally coincides with summer, thus reflecting whether the particular country is in the northern or southern hemisphere. Other factors include sowing date and the maturity of the particular variety sown, plus constraints such as avoidance of extremes of climate — both very cold and very hot.

There is a great diversity of uses of wheat (Table 1), such as pan breads, pocket (Arabic) breads, noodles, and steamed breads (Figures 3 and 8). In addition, there is a diversity of processing methods, especially for leavened bread. There is thus the need to breed a range of wheat varieties to suit the various processing specifications. In addition, there is the need for the appropriate variety to be grown in a suitable climate and region, with the right management, aimed at achieving target attributes of bulk density, lack of defects and contaminants, grain hardness, protein content, milling quality, and dough-forming properties. It is usual to segregate wheat after harvest into specific grades, each being suited to appropriate uses and prices. These grades carry through into world trade, forming a basis for buyers to select based on the combination of quality and price.

Table 6 Harvest times around the world for wheat

Australia October-January Canada July-September China May-September	Country	Harvest dates
	Australia Canada China England France India Italy Russia	July-September May-September August-September June-July February-May June-July July-September

Durum, a distinct species of wheat (Table 1), has a harder, larger grain, with a yellowish hue ("amber" according to the title of the international durum grade). Because its endosperm is so hard, it fragments on milling into particles larger than does common wheat. This coarse flour ("semolina") is suited for pasta manufacture (extruded from a relatively dry dough), and also for couscous, frike, and bulgar (also known as "bulghur" or "burghul"). Some of these foods are also made from common wheat in some regions.

#### Rice

Like wheat, rice is another cereal with a special place in man's diet, providing a dietary staple for over half of the world's population, thus constituting their major source of energy and protein. Unlike other cereals, rice is consumed as the whole grain, after removal of the bran layers (see Rice: Genetics; Breeding; Chinese Food Uses; Wildrice, Zizania). Rice can be grown under a wide range of climates, either in flooded irrigation or under dry-land conditions. Some 90% of the world's rice production is in Asia, especially in developing countries. International trade in rice accounts for only ~4% of world production. Prices vary greatly depending on the type of rice. For example, Basmati scented rice from Pakistan and north-west India might command 4 times the domestic price of "ordinary" rice. Quality grades of rice are partly based on dimensions – long-, medium-, and short-grain rice. Quality attributes relate to the milled grain: shape, color, translucency, and uniformity, and also the absence of broken grains.

For human consumption, paddy rice is de-hulled and milled to remove the bran layers, leaving the familiar lustrous, white grain. Brown rice, much less popular for eating, retains the bran layers, together with added nutrients. Short-grain varieties (*indica* types) usually become sticky on cooking, a characteristic that is preferred in many parts of north Asia. There is a preference in many western countries for the drier, flaky quality usually provided by the long-grain varieties (*japonica* types). Whereas milled rice is mainly consumed as the cooked grain, there are also many food products made from rice, namely, parboiled rice, rice crackers and noodles, rice cakes and snack foods, rice flour, and fermented drinks (sake and rice wine).

#### Maize (Corn)

Maize, indigenous to the Americas, is produced worldwide – mainly in tropical and warm regions (**Table 1**). It is an annual plant with an erect, leafy stalk. The ear consists of a central pithy section

(the cob, Figure 7), via which nutrients are transported from the leaves and stalk to the grains (some hundreds or up to one thousand grains per cob). The tassels of the immature cob constitute the inflorescence bearing the male flowers.

There is a wide range of types of grain (see Maize: Genetics; Breeding; Quality Protein Maize; Dry Milling; Wet Milling; Foods from Maize). Variations in grain types include size, color, and hardness. Colors range from white, through yellow to red. Endosperm types include soft and floury, through to hard and flinty. Popcorn is a special type of endosperm that expands suddenly on heating, due to vapor expansion inside the grain. Sweet corn, with an unusually high sugar content, is generally harvested immature, retaining a high water content to provide the familiar juicy mouth-feel.

There is extensive industrial processing of maize, especially in the USA and Europe. An important product is a range of breakfast cereals and snack foods. Industrial processing also involves fractionation of the maize grain by wet milling into its major chemical components. The main product of processing is cornstarch, which is the largest component of the corn endosperm. By-products are maize oil, from the germ and the protein fraction, which is traded under the inappropriate term "corn gluten." These direct products may be further processed, especially the starch, much of which is hydrolyzed to simple sugars as a sweetening agent, as well as being used to make syrups and spirits.

# **Barley**

The main uses of barley are for animal feed and for the production of malt and beer (Table 1) (see Barley: Genetics and Breeding; Agronomy; Harvesting, Storage, and Transport; Grading and Marketing; Milling and Processing; Malting). These two uses form the basis of the two main grades for trade in barley (feed and malting barley). Another major distinction is based on head morphology, namely, "two row" or "six row" depending on whether there are grains in the full set of florets in the head (six row) or there is only one set of grains on either side of the head, with the other florets sterile (Figure 5). In some barley-growing countries (Europe and Australia), six-row barley varieties are often of feed quality, but six-row varieties are used for malting in North America.

Barley is the grain used for Scotch and Irish whisky. In addition, a small proportion of the barley crop is used directly for human consumption; after pearling to remove the adhering lemma and palea, the barley grain is boiled in soup as the whole pearled grain, or it is coarsely ground and cooked as gruel. Barley flour is

also used for a range of baked products, but barley is not suitable for the production of leavened bread. Nevertheless, barley appears to have been used for bread making by ancient civilizations. Barley, for example, appeared on Greek coins several centuries BC, and it was the staple diet of Roman gladiators, who were thus known as *hordearii* (reflecting the origins of the genus name, *Hordeum*).

#### **Oats**

Oat plants thrive in temperate climates, with cooler moister conditions, compared to other cereals (Tables 1–3). Oats are often used as fodder for stock, grazing off the growing plants. In such cases, it may also be possible for the farmer to harvest a crop of grain, and often this also is used on-farm as stock feed. In addition, oats are grown as a grain crop, generally using varieties that have been bred specifically for use as grain. A large proportion of harvested oats is used for animal feed, especially for horses.

Compared to the other cereal grains, the oat grain tends to have higher contents of protein, lipid, and soluble fiber ( $\beta$ -glucan). Oats are reported to have beneficial hypocholesterolemic properties, as well as being useful in the management of the insulin response in diabetics. For human consumption, the adhering glumes are abraded from the oat grain. The resulting groat is cut, rolled, or ground to yield products such as oatmeal, rolled (flaked) oats, and oat flour, for processing into "instant oats" for porridge, oat cakes, breakfast cereal, and infant foods (*see* Oats).

The groat has a higher content of lipid (fats) than wheat, plus significant lipase activity (fat-splitting enzyme), and these factors must be taken into account in oat processing. There is the consequent risk that after milling, fat rancidity, bitterness, and a soapy taste will be produced by the actions of various endogenous enzymes, namely, lipase, lipoxygenase, and peroxidase. To prevent this, it is usual for milled oats to undergo some form of heat treatment to inactivate the lipase activity. This is often done by steam treatment, together with kiln drying.

# **Rye and Triticale**

Rye is the only cereal grain that approaches wheat with respect to bread-making properties, although the achievement of bread quality in rye breads usually requires the incorporation of a considerable proportion of wheat flour (Table 1). Rye is most popular human diet in Europe. The production of rye in Germany exceeded that of wheat from about 1940 to 1960, but more recently the production of wheat has increased considerably, and a higher

proportion of the rye crop has been used for animal feed.

Rye is particularly susceptible to contamination with ergot, a toxic fungus that replaces the grain in the rye head during development, so that a long black ergot body protrudes from the mature head (*see* Rye). Ideally, the production of ergot in the crop is avoided, but there are techniques for the removal of intact ergot bodies prior to milling.

In addition to the manufacture of rye breads, ryebased crispbread is a major use of rye, generally with rye wholemeal or from flaked rye. Other baked products include Ryvita and pumpernickel. In human nutrition, the rye grain offers an unusually high level of pentosans. This source of soluble fiber offers advantages of giving a feeling of satisfaction, thus helping in slimming diets, as well as reducing the rate of rise in the blood sugar level after ingestion.

Triticale is a man-made crop that combines the genomes of wheat and rye (Table 1). It thus tends to be intermediate between rye and wheat in its grain composition and characteristics. The expectations for the grain have been that it would contribute the disease resistance and growth hardiness of rye to wheat, whilst also combining the baking quality of wheat with the unique flavor of rye bread. Triticale has thus been used for baking; nevertheless, its main use is for animal feed (see Triticale).

#### Sorghum and the Millets

The grains of sorghum and of various millet species (Table 1) constitute a major source of protein and energy for many people in Africa and Asia, where these grains are used as porridge, boiled in water after grinding. In such situations, these cereals offer the advantage for subsistence farming of tolerance to drought and suitability for dry tropical growth environments. Pearl millet is the most widely grown of the millet family, with an estimated growth area worldwide of  $\sim$ 30 Mha. Second in importance are common millet and finger millet (Table 1).

In addition to the use of sorghum in cooking, it is also dry-milled and fermented for various forms of beer. On the other hand, the main use of sorghum in developed countries is for animal feed, and to a lesser extent for industrial processing. There are various mutant forms of sorghum with distinct characteristics that include high lysine content, waxy starch, high sugar content, and even scented endosperm (see Sorghum: Breeding and Agronomy; Harvest, Storage, and Transport; Utilization). The grain of many sorghum varieties is pigmented, largely due to tannins located in the outer layers (testa) of the grain. The high-tannin varieties are also somewhat less nutritious, because the tannins interfere with protein digestibility. Some sorghum varieties have bitter flavors, which confer resistance to attack by birds.

# Composition

Starch is the major component of the endosperm of the cereal grains (Table 7). It is the stored form of energy that is released on germination when amylase enzymes are produced to break the starch down to glucose units for the developing embryo, roots, and shoots. For feed and food, starch also provides the major source of energy, providing it in a "slowrelease" form that is well suited to our digestive systems. Although cereal-grain proteins do not have the ideal combination of essential amino acids, being slightly deficient in lysine especially, they are generally ingested together with other sources of protein whose amino-acid composition complements that of the grains. The relatively low fat content of the cereal grains is a dietary advantage, especially when coupled with the levels of nonstarch polysaccharides, which act as important sources of fiber in the human diet.

# The Supply Chain of Cereal Production and Processing

All cereal grains follow the sequence shown in Figure 9 in their production through to utilization. The initial stage involves the production of the seed for sowing. It is important for the farmer to have the most appropriate variety (genotype) that suits the expected

Table 7 Approximate composition of the major cereal grains, as percentages of dry weight of whole grain, except for rice

Component	Wheat	Rice (milled)	Maize	Barley	Oats (groats)	Rye
Starch	75–80	85–90	75–80	75–80	70-80	75–80
Protein	9-16	8-11	10-12	9-12	12-15	12-15
Fat	2-3	0.5-1.0	4-10	1-2	6-8	1-2
Crude fiber	2-3	0.2-0.6	2-3	5-6	1–2	2-3
Minerals	1–2	0.4-0.6	1-2	2–3	2–3	2–3

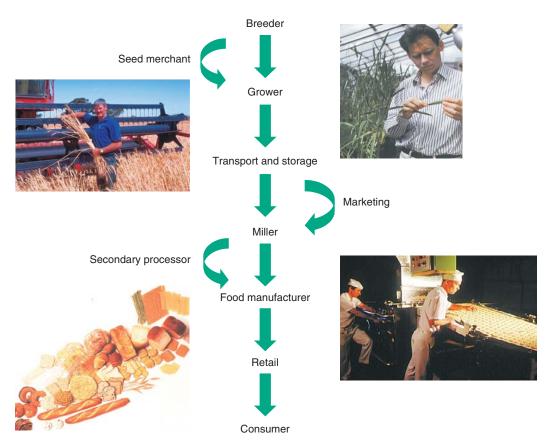


Figure 9 The grain supply chain extends from the breeding of a variety through to the food product reaching the consumer.

growth conditions, and that will produce grain with desired qualities for the planned use. Plant breeding has developed during the past century as the means of producing suitable genotypes, selecting them on the basis of the required attributes, after the use of cross-breeding to expand the genetic diversity available from which to make the selections.

Following the stages of breeding, selection, and registration (see Variety Registration and Breeders' Rights), there is the task of propagation and distribution of pure seed for sowing. Choice of the most appropriate variety is the first of many choices of farm management that must be made by the grower. Other management issues include soil preparation, fertilizer use at sowing and during growth, sowing rate, the possible use of irrigation and of herbicides and pesticides, and finally the harvest of the mature crop.

Even before harvest, the grower may have negotiated marketing of the crop, based on expectations of yield and quality attributes. Delivery arrangements might involve delivery of the harvested grain to a nearby storage facility ("elevator" or "silo"), or directly to a flour mill or feed mill (Figures 10 and 11). Further transport may involve delivery to the export terminal for loading into the hold of a ship (Figure 12). In many grain-producing regions, farmers



**Figure 10** Grain being tipped from the farmer's truck for delivery at the country storage facility.



Figure 11 Grain storage facilities are generally situated beside rail connections to facilitate transport to mill or export terminals.



Figure 12 Loading grain into the hold of a ship for international trade.

store the harvested grain on their own properties, using the postharvest period to negotiate the prices and conditions of delivery. In any case, some grain is likely to be stored on-farm for seed use in the next season and for animal feed. In situations of subsistence farming, the family will be directly dependent on the stored grain for the coming year.

Depending on what type of grain has been produced, there is likely to be the need for further storage and transport prior to milling or processing plant, followed by delivery of the product to retail outlets. The final test of any product for sale is the consumer, who provides the "proof of the pudding," whatever form the "pudding" might take.

#### **Cereal Grains and Our Health**

For some individuals, cereal-based foods may cause dietary problems. One of the best characterized of these intolerances is celiac disease, a condition caused

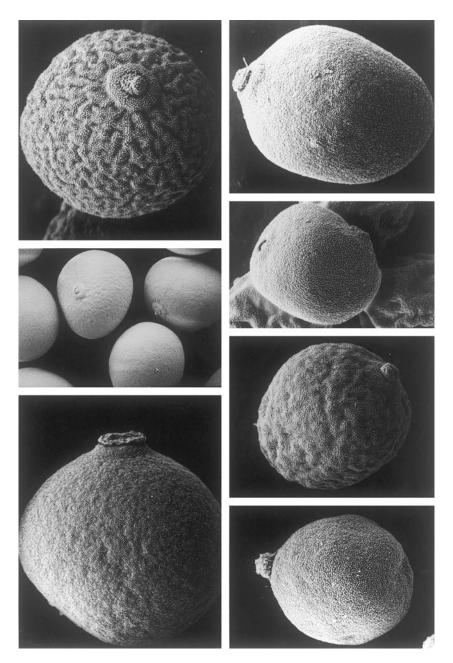


Figure 13 Pollen grains, revealed by scanning electron microscopy.

by the ingestion of wheat gluten protein, and of related grain proteins, namely, rye, triticale, barley, and sometimes oats. Despite its misleading name, buckwheat is not a grain that is toxic to celiacs (*see Celiac Disease*).

On the other hand, the cereal grains (milled or not) provide good sources of protein, fiber, and complex carbohydrates, earning for the cereal grains a major place in dietary guidelines. Additional health benefits may be provided by the inclusion of the outer layers of

the cereal grains, in whole-grain foods. This is because these parts of the grain (Figure 2) provide an additional source of vitamins and minerals, and because they contribute increased amounts of fiber to the diet (see Whole-Grain versus Refined Products).

During their growth, the cereals also produce pollen that may cause allergy problems on inhalation. The pollen grains of the various cereal flowers have distinctive shapes (Figure 13). The germination of the pollen grain commences on exposure to moisture,

normally when it alights on the stylus of a flower of the same species, but the same reaction may occur if the pollen comes in contact with the mucosa of the respiratory tract. The proteins so released may cause swelling of the mucosa, resulting in rhinitis and asthma. The cultivated cereals may be the cause of this scenario, but it is more common for inhalant allergy to be caused by a wider range of plants species, not necessarily propagated for their grain.

# **Future Prospects**

Despite the success of the cereal grains as the major food source for mankind, there are many stresses that reduce the yield and quality of the grain produced, necessitating continuing research effort to improve performance. These stresses include a range of pathogens and predators, as well as many abiotic factors (heat, cold, frost, drought, waterlogging, and heavymetal toxicity). Traditional breeding has provided great improvements and it will continue to do so. These advances are being assisted by the identification and characterization of specific remedial genes. Some of these genetic opportunities lie in plants of species distantly related to the species of interest.



Figure 14 The inscription on this old plate portrays the ongoing problem of finding enough grain to provide food for everyone in the world. The words "Altes Brot ist nicht hart – kein Brot, das ist hart!" translate as "Old bread is not hard - no bread, that is hard." (Reproduced with permission from Ng PKW and Wrigley CW (eds.) (2002) Wheat Quality Elucidation. St. Paul, MN: American Association of Cereal Chemists, p. 24.)

Novel genetic technologies may now facilitate these cross-species transfers, thus augmenting conventional breeding, leading to new varieties that can better survive stresses, plus providing novel grain qualities. The composition and functional properties of the edible grains offer a wide diversity of possibilities for new and varied end uses. Given this genetic diversity, and the consequent access to the genes responsible, combined with the capabilities of novel methods of genetic manipulation, we have many possibilities for extending the compositional diversity of any grain to provide it with new functional properties.

There remains the overwhelming problem of how mankind can share the cereal-grain harvest more equally. This problem is epitomized in the inscription on the old plate in Figure 14; the translation reads: "Old bread is not hard – no bread, that is hard."

See also: Barley: Agronomy. Cereals: Grain Diseases; Protein Chemistry. Maize: Genetics; Breeding; Dry Milling; Wet Milling. Rice: Genetics; Breeding. Wheat: Genetics; Breeding; Agronomy; Dry Milling; Wet Milling.

# **Further Reading**

Dendy DAV (ed.) (1995) Sorghum and Millets: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Fabriana G and Lintas C (eds.) (1988) Durum Wheat: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Fitzsimmons RW, Martin RH, and Wrigley CW (1983a) Australian Wheat Varieties: Identification According to Plant, Head and Grain Characteristics, 2nd edn., p. 13. Melbourne: CSIRO.

Fitzsimmons RW, Roberts GL, and Wrigley CW (1983b) Australian Out Varieties: Identification of Plants, Panicles and Grains. Melbourne: CSIRO.

Henry RJ and Kettlewell PS (eds.) (1996) Cereal Grain Quality. London: Chapman and Hall.

Juliano BO (ed.) (1985) Rice: Chemistry and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Kent NL (ed.) Technology of Cereals, with Special Reference to Wheat. Oxford: Pergamon.

McGregor AW and Bhatty RS (eds.) (1993) Barley: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Ng PKW and Wrigley CW (eds.) (2002) Wheat Quality Elucidation. St. Paul, MN: American Association of Cereal Chemists.

Pomeranz Y (ed.) (1988) Wheat: Chemistry and Technology, 3rd edn. St. Paul, MN: American Association of Cereal Chemists.

Webster FH (ed.) (1985) Oats: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists. White PJ and Johnson LA (eds.) (2003) Corn: Chemistry and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

#### **Relevant Websites**

http://www.aaccnet.org - American Association of Cereal Chemists.

http://www.awb.com.au - AWB Ltd, Melbourne, Australia.

http://www.campden.co.uk - Campden and Chorleywood Food Research Association.

http://www.cgc.ca - Canadian Grain Commission, Winnipeg.

http://www.wheat.pw.usda.gov - Grain genes.

http://www.icc.or.at - International Association for Cereal Science and Technology.

http://www.crop.cri.nz - New Zealand Institute of Crop and Food Research.

http://www.sgrl.csiro.au - Stored Grain Research Laboratory, Canberra, Australia.

http://www.usda.gov - United States Department of Agriculture.

# **Breakfast Cereals**

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#### Introduction

The breakfast cereal industry, in its history of over 110 years, has emerged as an important segment of the food industry. Annual supermarket sales of all grain-based breakfast products in the USA were about \$11 billion  $(1.1 \times 10^{10})$  in 2002. Well over half of this (\$6.6 billion) came from sales of a billion kg (2.2 billion lb) of packaged ready-toeat (RTE) breakfast cereals. This remarkable progress has been achieved through a combination of knowledge gained in human nutrition, creative formulation (including presweetening), innovative progress in cereal processing technology, and imaginative marketing. The chief driving force has been the continued market for new, convenient, nutritious,

and fun foods, many of them with special appeal to children in a discriminating, affluent, and mobile society.

Any large grocery store in the USA, Canada, the UK, or Australia is likely to stock 150 or more different grain-based breakfast cereal products. In the UK, annual sales are about £1 billion  $(1 \times 10^9)$ . Penetration of these specific products into other European, Asian, and African countries is much lower, although consumers in Asian countries may well eat rice at breakfast as they do at other meals. Consumption of breakfast cereals as such increased significantly in continental Europe and in Japan during the 1990s, in both cases associated with growing indigenous manufacture and marketing. This has continued into the current century and is forecasted to continue.

Breakfast cereals have been defined as "processed grains for human consumption." The major grains used in their manufacture are corn, rice, wheat, oats, and barley. Breakfast cereal products can be divided into: (1) those that are RTE before or after adding milk and (optionally) sugar; (2) those that are ready-to-cook, also known as hot cereals (HCs); and (3) alternative breakfast products based primarily on cereal grains, such as cereal bars (some of which border on confectionery), toaster pastries, waffles and other frozen products, muffins, and bagels. This article deals primarily with RTE and HC products.

RTE cereals are frequently made from mixtures of one or several grain components with other ingredients; they require extensive processing, are usually fortified with vitamins and minerals, and are specially packaged to protect their flavor, texture, and nutrition during storage as well as display their contents in such a way as to visually appeal to and entice the consumer. They typically represent almost 90% of the combined volume of RTE and HC products. On the other hand, HC products are typically made from a single grain component by using relatively simple processing and packaging technologies. The difference between the RTE and HC products is reflected in the price at the consumer level. The usual single serving (28–35 g, or 1 oz) of RTE cereal costs consumers about \$0.20 (0.08-0.40) at retail in the USA, whereas a typical one-ounce serving of HC costs consumers about half of that (\$0.06-0.24, for bulk-packaged product to instant single-serving pouches, respectively).

While annual sales in the USA of the various grainbased alternative breakfast products had grown to substantially over \$3 billion  $(3.25 \times 10^9)$  by 2002, the per capita annual consumption of RTE cereals had leveled off to under 5 kg (11 lb), and that of HC products (at one time over 1 kg) had declined to less than 0.6 kg (1.3 lb). HC consumption would have been even lower were it not for the emergence and proliferation of so many instant portion-pack products. The latest per capita annual consumption figures in the UK were 5.9 kg (13 lb) and 0.6 kg (1.4 lb) for RTE and HC, respectively, with similar numbers in Canada, Australia, and New Zealand as contrasted with as low as 0.1 kg in other parts of the world.

#### **Methods of Manufacture**

#### **RTE Cereals**

RTE breakfast cereals are primarily grain formulations, suitable for human consumption without further cooking. Manufacturing technology varies according to type of cereal, whether flaked, puffed, shredded, granola, extruded, expanded, or baked. Representative samples are shown in Figure 1.

Flaked cereals Flaked RTE breakfast cereals can be divided into two groups: flakes rolled from whole grains or parts of the whole grains, and flakes made from more finely ground materials that are first extruded into pellets and then rolled into appropriate size flakes.

The whole grains (typically wheat or rice) or major components (such as grits from de-germed yellow maize or corn) are cooked with flavorings such as sugar, salt, and malt; they are then dried and tempered

to a firm but slightly plastic state, flaked by passing between rolls, and toasted or dried to a final specified moisture content. Cooking of grits or whole grains for traditional flakes is usually done in batches. The moisture content of the cooked mass at the end of cooking is usually ~28%. The cooked material should not be mushy, soft, or sticky. After cooking, the mass of material is cooled, dried at  $\sim$ 120°C, and tempered (held) for several hours at a final equilibrated moisture content of 10–18%, depending upon the grains. The tempering process is important, not only to allow equilibration of moisture within the cooked grains or pellets, but also to allow sufficient starch recrystallization or retrogradation to provide a suitable texture for flaking (or for shredding, as discussed next).

Flaked RTE cereals are also made from extruded pellets rather than cooked grits or whole grains. A variety of floury or finely ground grain products such as whole wheat or oat flour can be mixed with sugar, salt, and malt syrup or other flavoring and coloring ingredients to form a dough. This is then extruded to form pellets about the same size as cooked grits or whole grains, the cooking having taken place in the extruder. Pellet moisture is in the range of 18–24%. Conditioning or tempering prior to flaking may or may not be required.

Whether consisting of cooked grits, cooked whole grains, or extruded pellets, the tempered material is then flaked. Tremendous pressures are necessary to flatten the prepared material into thin flakes, which traditionally are toasted by keeping them suspended



**Figure 1** Some common RTE cereals. Clockwise from the top left, they are, respectively, flaked, puffed, shredded (bite-size), granola, extruded expanded, and baked products.

in hot air between 270°C and 330°C for  $\sim$ 90 s. Certain properties of the finished flakes can be modified by a heating or steaming step immediately before flaking (e.g., infrared heating immediately before flaking will increase the tendency of flakes to crinkle rather than remain flat), while other properties – such as blistering, color, crispness, tenderness, and flavor of the final product – are influenced by toasting conditions. The moisture content of the finished product is  $\sim$ 1–3%.

Puffed cereals The predominant grains used for making puffed cereals are wheat and rice. The milled rice that is used for puffing is either short- or mediumgrain white rice. Such rice usually requires no pretreatment other than the hull and bran removal that occurs during milling or pearling. Wheat, on the other hand, is usually decorticated (bran removed), either by pretreatment with concentrated brine solution or by pearling. In the pearling operation, the grain is passed through a revolving cylinder inside which are mounted silicon carbide or carborundum stones.

Usually hard wheat, preferably durum, is used for puffing. A grain flour mixture can also be formed into desired shapes by an appropriate extrusion process and dried to a moisture content of 9-12% for subsequent gun puffing.

Puffing guns are vessels capable of holding very high temperature and high-pressure steam. The critical feature of any puffing gun vessel or barrel is a quick opening lid that seals the vessel. Operation of a simple single-shot gun consists of placing the grain in the vessel, closing the lid, and raising the pressure and temperature in the vessel to  $\sim$ 1380 kPa (200 psi). The quick-opening lid is then activated, causing rapid pressure drop, which in turn instantaneously causes moisture in the grain to "flash off" as steam, and the kernels of grain or preformed pellets to expand to as much as 16 times their original volume. Modern puffing guns can be multiple shot automatic, or can be continuous in operation. In the latter case, the grain kernels or preformed pellets are conveyed through a pressure vessel to which they are admitted and from which they are ejected via an orifice or mechanical device without loss of pressure inside the vessel.

Another group of RTE products is made by oven puffing. Specially prepared rice or corn grains or their mixtures are oven-heated to 290–340°C in order to puff them. Puffed rice cereal marketed in the USA is either oven-puffed (e.g., Rice Krispies, prepared from short-grain rice) or gun-puffed (e.g., Puffed Rice). Whether gun- or oven-puffed, the grains are then screened to remove any unpuffed kernels, loosened

bran, fine dust, and broken kernels, and the product is heated to lower the final moisture to 1-3%. Processes such as sugar coating or the addition of vitamin enrichment can be carried out during this drying operation.

Shredded cereals Shredded wheat, one of the oldest mass-produced breakfast cereals, is still an important product. Soft white wheat is the primary grain used. Other classes of wheat, as well as other cereal grains such as rice, corn, or oats, or mixtures thereof, can also be used to make shredded products. The grains are cleaned, cooked in boiling water, partially dried, and tempered. The shredding operation consists of passing the tempered grain between two rolls. One roll is grooved and the other is smooth. Strong forces in the range of 7000 kPa (1000 psi) along the roll length are often required to maintain the rolls in tight contact. The tempered kernels are squeezed into the grooves of the roll and emerge as strands roughly 1 mm in diameter. These strands are accumulated in multiple layers below multiple pairs of rolls, and formed into biscuits or bite-sized pieces. A refinement of the shredding process is the use of a roll with added cross-grooves to provide lateral strands that can tie the shreds together in a coherent sheet. Alternatively, mixtures of ground cereal products with sugar, salt, malt, and flavoring can be cooked by extrusion, formed into pellets, tempered, and then shredded in the same way as cooked whole grain kernels.

The formed biscuits or pieces are oven dried and baked to desired color, texture, and moisture. The baking procedure is done in a specialized manner to impart fluffy texture, particularly for products containing rice or corn. Other minor ingredients — such as sweetening or other flavorings, nutrients, or antioxidants — can also be added prior to drying and baking.

Granola cereals The main grain used to make granola cereals is either regular or quick-cooking (thin-flaked) rolled oats. The cereal products are mixed with other desired ingredients such as nut pieces, coconut, corn syrup, brown sugar, honey, malt extract, dried milk, dried fruits, and/or vegetable oil. Liquid and dry ingredients are prepared separately, then mixed together and spread in a uniform layer on the band of a continuous oven, with baking temperatures in the range of 150–220°C. When the product is uniformly toasted to a light brown color and the moisture is reduced to ~3% after baking, the product is broken into small pieces and packaged.

Extruded expanded cereals Successful application of extrusion technology for producing RTE cereal products from grain flours or meal mixtures by formulating, continuous preconditioning, extrusion cooking, and forming has been an important achievement of the breakfast cereal processing industry. The extruders can be single screw, twin screw, low shear, high shear, and combinations of these or other features. Extrusion technology was developed to produce flaked, puffed, shredded, and expanded breakfast cereals. For extruded expanded cereals, cereal flour mixtures having ~25-30% moisture are preconditioned, cooked, and extruded. Perforations in the die and design of the cutoff knives at the end of the extruder, as well as temperature, pressure, dwell time, and shear in the extruder barrel, all influence the shape, size, and texture of the final product. The sudden change from high pressure and temperature to ambient conditions as the product exits the extruder imparts the necessary expansion, texture, and other characteristics. The extruded products may be coated with solutions of vitamins and minerals, and with sweetening and flavoring materials to enhance their sensory appeal (especially to children), and are then ovendried to desired moisture levels.

Other RTE cereal types A breakfast cereal can be manufactured using essentially baking or cookie technology to form and cook the pieces. Another type is compressed-flake biscuits, formed from the compression of previously cooked and flaked grains, usually wheat flakes with added sugar, salt and malt, or other flavorings as described above. So-called muesli RTE cereals are mechanical mixtures of several ingredients. In Europe, the major ingredient is quick-cooking rolled oats mixed with sweetening and flavoring components but not otherwise processed. In the USA, a major component can also be processed and toasted flakes from various grains. Either kind is eaten with added milk and sugar.

#### **Hot Cereals**

HCs are products of a single grain or a simple mixture that require cooking or heating in water before consumption. The main such products are rolled oats and related oat products, farina and other wheat fractions, and corn grits.

After cleaning, oat kernels are de-hulled by an impact process. The resulting groats are steamed, primarily to deactivate lipolytic enzymes, then rolled, dried, and packaged. Quick-cooking rolled oats are

made by steel cutting groats into four or five pieces before the steaming and flaking process. Instant oatmeal is made by subjecting groats to a special process that results in rapid cooking extra-thin flakes. Incorporation of 0.1–1.0% of an edible gum during processing helps to achieve this objective. Distribution of the gum and necessary salt without damaging the flakes is accomplished during portion-control packaging. Such instant products require only the addition of boiling water, stirring, and a short standing time to make the product ready for consumption. The industry has successfully incorporated dried fruits, nuts, and other flavorings to make instant oatmeal products more attractive to consumers.

Wheat farina is another important HC. In the USA, farina and enriched farina must meet respective legal standards of identity. Farina is basically wheat endosperm, preferably obtained from hard red spring or winter wheat, the granules of which stay intact during cooking at home. Farina is normally obtained by drawing off chunks of endosperm during milling of wheat into bread flour. In the UK it can also be called semolina, but in the USA that term is reserved for a similar product from durum wheat intended for macaroni and other pasta products. Farina is heated at  $\sim 60^{\circ}$ C for 15 min before packaging, to enhance flavor and head off infestation. Quick and instant farina products are prepared by using disodium phosphate, enzymes, and other treatments including precooking and drum drying. Wheat germ, bran, malted barley, cocoa, and other flavorings can be mixed into faring to enhance the consumer appeal.

Other important HC products include wheat products such as cracked wheat and bulgur, and corn grits. Grits and bulgur are generally used as cereal accompaniment to other meal menu components such as eggs, sausage, meat, and related preparations, whereas cracked wheat products are cooked for breakfast. Grits usage is mostly in southern parts of the USA, garnished with butter and salt rather than with sugar and milk as for traditional breakfast cereals. Grits are produced by dry milling of white de-germed corn and can be fortified with vitamins and minerals. Bulgur or parboiled wheat is one of the oldest cereal-based foods and has been consumed for centuries in Turkey, Syria, Jordan, Lebanon, and Egypt. To prepare it, common hard or soft wheat varieties are soaked in water and heated to ~90°C for 2-3 h. The cooked wheat is then cooled, dried, moistened, peeled (optional), re-dried, cleaned, and sized. Bulgur has good shelf life, because cooking destroys insects present in harvested crops and distributes nutrients uniformly throughout the kernel. It does not render the final product quick-cooking or "instant."

# Significance in the Diet and Fortification

Breakfast cereals are one of the most highly fortified foods in both vitamins and minerals. One serving often can supply up to 25% of the daily values (DVs) of most vitamins and trace minerals as these are defined in the USA for labeling purposes. Some products supply 100% of all established DVs, making them as much nutritional supplements as breakfast foods. The cereal industry justifies these practices on the basis that they fulfill a genuine need to improve the overall nutritional needs of consumers. Past food consumption surveys showed that some of the US population had inadequate intakes of several vitamins and minerals, particularly vitamin A, folic acid, calcium, and iron, and possibly vitamin B<sub>6</sub>, zinc, and magnesium.

In the US, the practice of fortification of cereal foods started in 1941, when the Food and Drug Administration (FDA) established a standard of identity for enriched flour to include the addition of thiamin, niacin, and iron. Riboflavin was subsequently included, and addition of calcium and vitamin D was optional. Such recommendations developed after extensive discussions by various committees of the American Medical Association, hearings sponsored by the FDA, and the Food and Nutrition Board. As a result, criteria for fortification of foods were developed:

- 1. the intake of nutrient is below the desirable level in the diet of a significant number of people;
- 2. the food used to supply the nutrient is likely to be consumed in quantities that will make a significant contribution to the diet of the population in need;
- 3. the addition of nutrient is not likely to create imbalance of essential nutrients;
- 4. the nutrient added is stable under proper conditions of storage;
- 5. the nutrient is physiologically available from the food; and
- 6. there is reasonable assurance against excessive intake to a level of toxicity.

Various studies have confirmed that fortification of BC foods is appropriate and has contributed significantly to improving overall nutrition. Fortification of flour and other grain-based foods with folic acid became a requirement in the USA in 1998 because of evidence that it may help prevent neural tube defects.

Many nutritionists consider eating breakfast regularly as one of the important health habits associated with subsequent favorable health status and reduced mortality. People who had breakfast were found to do significantly more work in the morning than those who did not. Children and the elderly (above 65 years

old) are the largest consumers of breakfast cereals. Half or more of the school-age children in the USA prepare their own breakfast, because most of their parents work outside the homes. These groups are more likely to skip breakfast. Studies have confirmed that consumers of RTE breakfast cereals are likely to skip fewer breakfasts and that they obtain higher levels of vitamins and minerals than others. Some products contain up to 5 g of dietary fiber per serving. Some high-fiber products can even provide 10 g or more of fiber and thus help consumers to improve their dietary fiber intake to meet recommendations of 25-30 g daily dietary fiber intake. Oatbased breakfast cereals containing sufficient  $\beta$ -glucan soluble fiber to provide 3 g or more of that substance daily, or other products containing sufficient psyllium to provide 7 g of soluble fiber from that source daily, have been shown to decrease blood cholesterol by a small but significant amount, and in the USA may carry label health claims to that effect. Recent studies have shown that diets rich in whole-grain foods (such as breakfast cereals with whole grains as a major constituent) and other plant foods that are low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and cancer.

Most breakfast cereals are not high in protein, and the protein in some of them, such as gun-puffed or flaked products, may have little or no biological value due to excessive Maillard browning. RTE cereals as marketed may also be high in sugar and/or salt (sodium) and may contain added saturated fat, but with milk and fruit still make an inexpensive, nutritious, and easy-to-prepare breakfast.

#### Packaging, Storage, and Spoilage

The industry over the years has developed efficient processing, packaging, storage, and shipping technologies to supply consumers with cereal foods that routinely have 6 months or longer shelf life. It is indeed quite a feat to have such an array of vitamins and minerals, which often interact with each other, in a highly processed food and yet end up with a wholesome product with a desirable flavor and texture. Novel techniques have been developed for vitamin preservation and for the incorporation of antioxidants such as BHT directly or via the packaging material. The package not only protects the product against moisture, oxygen, and insects during shipping and storage, but also provides attractive consumer appeal at the point of purchase. The industry has accomplished this by following "good manufacturing practices" and other quality control measures, and by instituting hazard analysis and critical control point (HACCP) systems for food safety purposes.

Computer control of processing and attention to environmental issues also characterize most breakfast cereal manufacturing operations, and certainly the newer ones.

# **Future Developments**

The Nutritional Labeling and Education Act of 1990 and subsequent amendments in the USA undoubtedly contributed to re-evaluation of product development, marketing, and advertising strategies by the breakfast cereal industry as a result of stricter control of nutrient claims, ingredient listing, and other label data. Among other moves, the industry has developed new products made from whole grains that are also low in fat, sugar, and salt (sodium), with encouragement provided by FDA approval of a health claim for products containing one or more whole grains as a majority constituent. Other product developments or modifications are likely to be influenced by such regulatory developments as well as by advances in technology or nutritional knowledge.

See also: Cereals: Overview. Cultural Differences in Processing and Consumption. Extrusion Technologies. Nutrition: Guidelines for Grain-Based Foods; Mineral Composition; Vitamin Composition.

# **Further Reading**

Web pages of breakfast cereal manufacturers may contain useful information. Addresses of these are frequently included in package copy, and the reader is referred to them for specific information, such as the ingredient list or nutrition facts pertaining to any product of that manufacturer.

Cho S, DeVries J, and Prosky L (1997) *Dietary Fiber Analysis and Applications*. Gaithersburg, MD: AOAC International.

Corlett DA (ed.) (1998) *HACCP User's Manual*. Gaithersburg, MD: Aspen Publishers.

Faridi H and Faubion JM (eds.) (1990) Dough Rheology and Baked Products Texture. NY: Van Nostrand.

Fast RB (1999) Origins of the US breakfast cereal industry. *Cereal Foods World* 44: 394–397.

Fast RB and Caldwell EF (eds.) (2000) Breakfast Cereals and How They Are Made, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Hoseney RC (1994) *Principles of Cereal Science and Technology*, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Marquardt L, Slavin JL, and Fulcher RG (2002) Whole-Grain Foods in Health and Disease. St. Paul, MN: American Association of Cereal Chemists. McCleary B and Prosky L (2000) Advanced Dietary Fibre Technology. St. Paul, MN: American Association of Cereal Chemists.

Nelson AL (2001) High-Fiber Ingredients. St. Paul, MN: Eagan Press.

Slade L and Levine H (1991) Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. *Critical Reviews in Food Science and Nutrition* 30: 115–360.

Whistler RL and BeMiller JN (1997) Carbohydrate Chemistry for Food Scientists. St. Paul, MN: American Association of Cereal Chemists.

Wood PJ (1993) Oat Bran. St. Paul, MN: American Association of Cereal Chemists.

#### **Relevant Websites**

http://www/aaccnet.org — American Association of Cereal Chemists.

http://www/asbe.org — American Society of Bakery Engineers.

http://www/fdli.org — Food and Drug Law Institute. http://www/gmabrands.com — Grocery Manufacturers of America.

http://www/ift.org — Institute of Food Technologists. http://www/ilovepasta.org — National Pasta Association.

# **Chemistry of Nonstarch Polysaccharides**

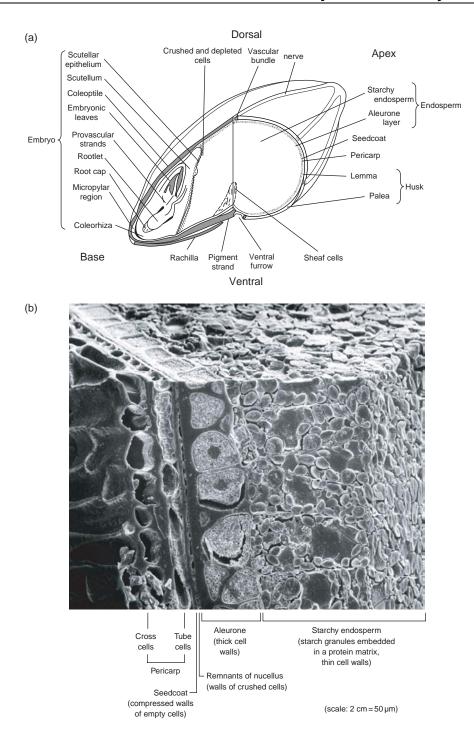
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#### Introduction

The nonstarch polysaccharides of cereal grains include cellulose,  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans, heteroxylans (arabino-xylans), glucomannans, xyloglucans, pectic polysaccharides, callose, fructans, and arabinogalactan-proteins. With the exception of the fructans and the arabinogalactan-proteins, all are key components of the walls of cells in the various tissues that comprise the grain (Figure 1a) (see Grain, Morphology of Internal Structure. Grain and Plants, Morphology). The cells of the various tissues have diverse functions during grain development, dormancy, and after germination. The pericarp and



**Figure 1** (a) Barley grain, showing component tissues. (Reproduced with permission from Briggs DE (1978) *Barley*. London: Chapman and Hall, figure 1.1.) (b) Scanning electron micrograph of the outer portion of a wheat grain, showing starchy endosperm, aleurone and overlying nucellar remnants, seedcoat and inner pericarp. The outer pericarp has been lost from the preparation. Preparation and electron microscopy by Susan Joyner. (Reproduced with permission from Fincher GB and Stone BA (1986) Cell walls and their components in cereal grain technology. *Advances in Cereal Science and Technology* 8: 207–295.)

seedcoat tissues are concerned with the protection of the seed during development and dormancy. However, at grain maturity, these tissues are dead and consist almost entirely of cell walls. The nucellar tissue between the seedcoat and the aleurone surface is involved in transfer of nutrients to the developing grain but has collapsed at maturity to leave cell-wall remnants. Cells of the starchy endosperm are dead, but

Table 1 Composition of cell walls of cereal grains (% by weight)

Cereal	Origin of cell wall	Component						
		Cellulose	Glucomannan	(1→3, 1→4)- β-D-Glucan	Heteroxylan	Pectin	Lignin	Protein <sup>a</sup>
Wheat (Triticum aestivum L.)	Aleurone	2	2	29	65			1
	Starchy endosperm	2	2	20	70			
	Bran (pericarp, seedcoat, aleurone)	29		6	64		8.3	9.2
	Beeswing bran (outer pericarp)	30			60		12	6
Barley ( <i>Hordeum</i> vulgare L.)	Aleurone	2	2	26	71			6
,	Starchy endosperm	2	2	75	20			5
Rice ( <i>Oryza</i> sativa L.)	Starchy endosperm	28	15 <sup>b</sup>	20 (xyloglucan/ma	27 annan)	3		18
,	Bran .	9	3	6	60 <sup>′</sup>	8	12	8
Maize ( <i>Zea mays</i> L.)	Bran (pericarp, seedcoat, aleurone)	23			67			

<sup>&</sup>lt;sup>a</sup> Values for protein may include cellular proteins.

are packed with starch and storage protein and are usually thin-walled. In contrast, the thick-walled, nucleated, aleurone cells are alive at grain maturity, and are packed with protein bodies and lipid droplets. The living embryonic tissues of the germ are differentiated into the functional cell types found in shoot and root. At the interface of the starchy endosperm lies the scutellum, which delivers nutrients to the developing endosperm and during germination transfers digestion products of the endosperm reserves to the developing embryo.

The cell-wall composition and organization of the cell types found in the tissues of the grain reflect these different functions and in turn determine their nutritional impact and the behavior of various grain parts in technical operations during grain utilization.

Together the nonstarch polysaccharides usually constitute less than 10% by weight of the grain (Table 1), but can nevertheless be key determinants of grain quality. Although the precise physical relationships between individual nonstarch polysaccharides and other wall components have not been described, it is generally considered that in the wall, microfibrils of cellulose are embedded in a matrix phase of noncellulosic polysaccharides and protein (Figure 2). Wall integrity is maintained predominantly through extensive noncovalent interactions, especially hydrogen bonding, between the matrix phase and microfibrillar constituents. In the walls of some grain tissues,

covalent associations between heteroxylans, lignin, and proteins are present. The extent of covalent associations between components also varies with the wall type and genotype. The relative ease of extraction of noncellulosic polysaccharides from the different wall types in cereal grains is strongly influenced by these interactions.

Noncellulosic polysaccharides, especially heteroxylans and  $(1\rightarrow 3, 1\rightarrow 4)-\beta$ -D-glucans, constitute a relatively high proportion of the walls of the aleurone and starchy endosperm, and probably also of the scutellum. Cellulose contents are correspondingly lower (Table 2). The generally low cellulose content of these walls, together with the fact that they contain no lignin, might be related to a limited requirement for structural rigidity of walls in central regions of the grain, and to a requirement to rapidly depolymerize wall components following germination of the grain. In walls of cells in the multiple tissue layers of the maternally derived, pericarp-seedcoat, which provide a protective coat for the embryo and endosperm and which are not mobilized during germination, cellulose, and lignin contents are much higher and the concentrations of noncellulosic polysaccharides are correspondingly lower.

In this article, the chemistry and physico-chemical properties of nonstarch polysaccharides from cereal grains, which are important in human and animal nutrition and which have an impact in grain utilization and in cereal technology, will be described.

<sup>&</sup>lt;sup>b</sup>Some genotypes had very low glucomannan content.

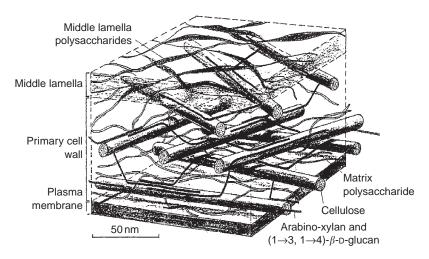


Figure 2 Organization of polymeric components in cell walls. A simplified schematic representation of the spatial arrangement of polymers in a primary wall, for example, from a starchy endosperm cell. Note the cellulosic microfibrils are embedded in a network of noncellulosic polysaccharides (arabinoxylans and  $(1 \rightarrow 3, 1 \rightarrow 4) - \beta - p$ -glucan) and protein (not shown). The noncellulosic polysaccharides can form associations with surfaces of several microfibrils and provide cohesive interactions. In some cells, e.g., those of the pericarpseedcoat, a secondary wall is deposited between the primary wall and the plasma membrane and the water in the matrix of both the primary and secondary walls is replaced by lignin, a phenylpropanoid polymer that overlies and encrusts the cellulose microfibrils and noncellulosic polysaccharides and may be covalently bonded to the latter. (Reproduced with permission from McCann MC and Roberts K (1991) Architecture of the primary cell wall. In: Lloyd CW (ed.) The Cytoskeletal Basis of Plant Growth and Form, pp. 109–129. London: Academic Press.)

**Table 2** Content of  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -p-glucan and heteroxylan in whole cereal grains (g per 100g DW)

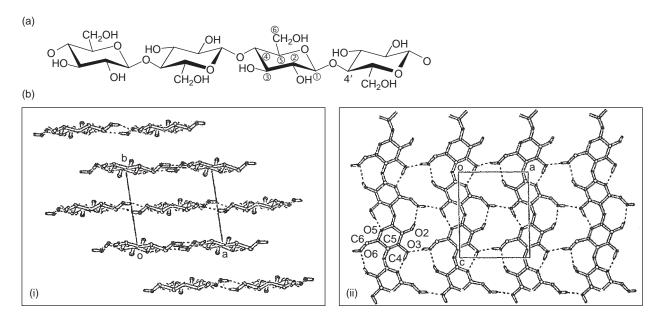
Cereal	<i>(1→3,</i>	1→4)-β-⊳-Glucan	Heteroxylan		
	Total	Water extractable	Total	Water extractable	
Wheat (Triticum aestivum L.)	0.5-2.3	0.02	4.0-9.0	0.3-0.9	
Durum ( <i>Triticum durum</i> )	0.5 - 0.6	0	?		
Spelt ( <i>Triticum aestivum</i> ssp. <i>spelta</i> )	0.57		?		
Barley (Hordeum vulgare L.)	2-10	up to 50%	4-8	0.4	
Hull-less	3.9 - 5.4	1.0-2.7			
Waxy hull-less	6-15				
Rice (Oryza sativa L.)	0.13	0.03	?		
Sorghum (Sorghum bicolor L.)	1.1-6.2		?		
Rye (Secale cereale L.)	1.0-2.0		7.1-12.2	0.6 - 2.4	
Rye-derived wheats (1B/1R chromosome translocations)			5.2-5.5		
Maize (Zea mays L.)	0.8-1.7		5.1-6.8	4.6	
Oats (Avena sativa L.)	3.8-6.1	3.7-5.0	2.2-4.1	0.2	
Triticale	0.34 - 1.8	0	5.8 - 7.4		

<sup>? =</sup> data unavailable.

#### Cellulose

Cellulose is a component of all cell walls in cereal grains. However, its concentration varies widely. Cellulose is usually a minor constituent of the primary walls of starchy endosperm and aleurone cells of cereal grains, but its content is much higher in secondary walls of the pericarp and seedcoat cells (Table 1). It is a linear homopolymer composed of  $\beta$ -D-glucopyranosyl residues, all of which are

 $(1\rightarrow 4)$ -linked (Figure 3a). Individual molecules have a degree of polymerization of up to 6000 in primary walls and 14000 in secondary walls. The molecular chains have an extended, ribbon-like conformation that allows parallel packing of chains into three-dimensional, fibrillar aggregates stabilized by extensive intermolecular hydrogen bonding and van der Waals interactions (Figure 3b). These aggregates, termed microfibrils, are easily visible under the electron microscope, and may reach diameters



**Figure 3** Cellulose structure. (a) Portion of a cellulose molecule. (b) Crystal structure of cellulose I: (i) projection down the fiber axis c showing the layers (sheets) of cellulose chains hydrogen-bonded in the ac plane but lacking intersheet bonding. The intersheet bonding involves van der Waals interactions between the hydrophobic faces of the glucose units. (ii) View of a layer approximately perpendicular to the ac plane showing the two intra-molecular hydrogen bonds in the direction of the fiber axis c and the inter-chain hydrogen bonds. (Reproduced with permission from Kroon-Batenburg LMJ and Kroon J Carbohydrates in Europe No. 12, 15–19.)

of 3 nm in primary walls and 5–10 nm in secondary walls. Within microfibrils, individual cellulose molecules are mostly packed into parallel, highly ordered, crystalline arrays, although there are usually regions where molecular alignment is less ordered. In particular, the surface molecules may have a different conformational and hydrogen-bonding arrangement compared with molecules within the more crystalline interior.

Cellulose microfibrils may also associate with water and matrix polysaccharides such as the  $(1\rightarrow3, 1\rightarrow4)$ - $\beta$ -D-glucans, heteroxylans (arabino-xylans), and glucomannans.

Cellulose is insoluble in water but swells in concentrated sodium hydroxide solutions and may be brought into solution by powerful hydrogen-bond-breaking reagents such as N-methylmorpholino-N-oxide. Thermal and shear treatments, followed by alkaline peroxidation and shearing to remove lignin, have been used to convert cellulose-rich maize bran (Table 1) to a cellulosic gel for use as a dietary fiber supplement.

## (1 $\rightarrow$ 3, 1 $\rightarrow$ 4)- $\beta$ -D-Glucans

 $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans, also referred to as mixed-linkage or cereal  $\beta$ -glucans, occur almost exclusively in members of the monocotyledon family Poaceae, to which the cereals and grasses belong, and in related

families of the order Poales (sensu stricto). They are important constituents of the walls of the starchy endosperm and aleurone cells of most cereal grains, where they can account for up to 70% by weight of the walls (Table 1). The concentration of  $(1\rightarrow 3,$ 1→4)- $\beta$ -D-glucans in cereal grains depends on the genotype, the position of the grain on the spike and environmental factors such as planting location, climatic conditions during development, and soil nitrogen. Barley, oat, and rye grains are rich sources of  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans, whereas wheat, rice, and maize have lower concentrations of the polysaccharide (Table 2). The  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans are relatively minor components of walls in vegetative tissues of cereals and grasses. A structurally related  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan, lichenin, is found in the walls of the fungal component of the lichen, Iceland moss (Cetraria islandica).

#### **Structure**

 $(1\rightarrow 3,\ 1\rightarrow 4)$ - $\beta$ -D-glucans are linear, unbranched polysaccharides in which  $\beta$ -D-glucopyranosyl monomers are polymerized through both  $(1\rightarrow 4)$ - and  $(1\rightarrow 3)$ -linkages (**Figure 4a**). The ratio of  $(1\rightarrow 4)$ - to  $(1\rightarrow 3)$ -linkages is generally fairly constant and in the range 2.2-2.6:1, although in the  $(1\rightarrow 3,\ 1\rightarrow 4)$ - $\beta$ -D-glucan from sorghum endosperm, the ratio is 1.15:1. The two types of linkages are not arranged in regular, repeating sequences. Single  $(1\rightarrow 3)$ -linkages

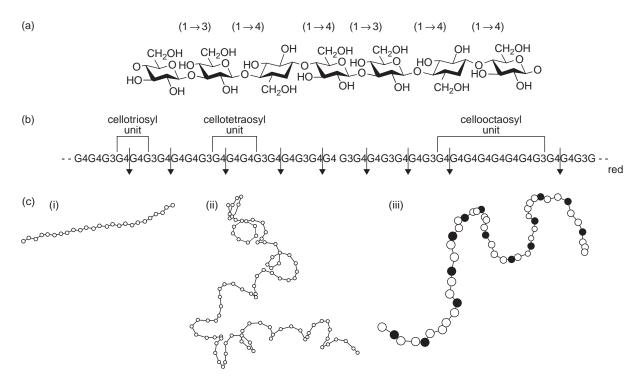


Figure 4 (a) Portion of a  $(1 \rightarrow 3, 1 \rightarrow 4)$ - $\beta$ -D-glucan molecule. (b) Distribution of linkages in a  $(1 \rightarrow 3, 1 \rightarrow 4)$ - $\beta$ -D-glucan. (G,  $\beta$ -glucosyl unit; 3,  $(1\rightarrow 3)$ -linkage,  $4(1\rightarrow 4)$ -linkage; red, reducing end, arrows, site of hydrolysis by  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan endohydrolase [EC 3.2.1.73]. (c) Perspective drawings of computer-generated, instantaneous conformations of  $\beta$ -glucans: (i)  $(1 \rightarrow 4)$ - $\beta$ -D-glucan (cellulose), (ii)  $(1 \rightarrow 3)$ - $\beta$ -p-glucan (callose), (iii) (1 $\rightarrow$ 3, 1 $\rightarrow$ 4)- $\beta$ -p-glucan ( $\bigcirc$ ), (1 $\rightarrow$ 4)-linked residues ( $\bullet$ ), (1 $\rightarrow$ 3)-linked residue. (Reproduced with permission from Fincher GB and Stone BA (1986) Cell walls and their components in cereal grain technology. Advances in Cereal Science and Technology 8: 207-295.)

are separated by two or more  $(1\rightarrow 4)$ -linkages. Regions of two or three adjacent  $(1\rightarrow 4)$ -linkages predominate, but again there is no regularity in the arrangement of these units. The linkage sequence does not depend on preceding linkages further away than two glucose units and follows a second order Markov chain distribution. Moreover, up to 10% of the chain may consist of longer stretches of 5-20 adjacent  $(1\rightarrow 4)$ -linkages. Thus, cereal  $(1\rightarrow 3,$ 1→4)- $\beta$ -D-glucans may be considered as (1→3)- $\beta$ -linked copolymers of cellotriosyl (G4G4G<sub>Red</sub>), cellotetraosyl (G4G4G4G<sub>Red</sub>) units, and longer  $(1\rightarrow 4)$ - $\beta$ -D-oligoglucosyl units (**Figure 4b**). The ratio of tri- to tetrasaccharide units varies between cereal species, as follows: wheat (3.0-4.5:1), barley (2.9-3.4:1), rye (2.7:1), and oats (1.8-2.3:1). The ratios vary according to the temperature and conditions of extraction.

#### **Molecular Size**

The average molecular masses reported for cereal  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans range from 48 000 (degree of polymerization, DP, ~300) to 3000000 (DP  $\sim$ 1850), depending on the cereal species, cell-wall type, extraction procedure, and the method used for molecular mass determination. They are invariably polydisperse with respect to molecular mass and this is illustrated by a weight average to number average molecular mass ratio (M<sub>w</sub>/M<sub>n</sub>) of 1.18 for barley  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan. Certain barley  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans are covalently associated with small amounts of protein and have estimated molecular masses of up to 40 000 000.

#### Solubility

The extractability of  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans from walls of cereal grains is a function of their degree of self-association and their association with other wall polysaccharides and proteins. In particular, extractability depends on the molecular mass and linkage distribution in the  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan chains. Extensive association with other polymers and very high molecular masses will render the  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans more difficult to extract from the grain.

A portion of the  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan from barley, oat, and rye flours is extracted by water at pH 7.0 and 40°C. These flours consist predominantly of aggregates of starchy endosperm cells. Further fractions can be solubilized at higher temperatures. The proportion of total  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan that is

water-soluble at 40°C varies within and between species. For example, waxy (high amylose) barleys have a higher proportion of water-soluble  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan than normal barleys.  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-Glucans extracted from barley at 40°C have a slightly lower tri- or tetrasaccharide ratio (1.7:1) than those extracted at  $65^{\circ}$ C (2.0:1). Complete extraction of cereal  $(1 \rightarrow 3, 1 \rightarrow 4)$ - $\beta$ -D-glucans from grain requires the use of alkaline extractants such as 4 M NaOH or aqueous Ba(OH)<sub>2</sub>, containing NaBH<sub>4</sub> to prevent alkali-induced degradation from the reducing terminus. Alkali-extracted barley  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan fractions have higher molecular masses, higher ratios of  $(1\rightarrow 4):(1\rightarrow 3)$  linkages, more contiguously linked  $(1\rightarrow 4)$ -linked segments and higher tri-: tetrasaccharide ratios than their water-extractable counterparts. Other extractants, such as dimethylsulphoxide, hot perchloric acid, trichloroacetic acid, N-methylmorpholino-N-oxide, and dimethylacetamide-LiCl, have also been used to solubilize  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans, but all are liable to cause some depolymerization or degradation of the polymer. Once extracted with hot water or alkali, the  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans are soluble at neutral pH and room temperature. However, upon cooling, the  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans slowly aggregate and precipitate.

#### **Conformation in Solution**

In aqueous media  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans adopt a partially extended, somewhat rigid, reptate (snakelike) chain conformation (Figure 4c) with a cross-sectional diameter of 0.45 nm and a length: width (axial) ratio of  $\sim 100$ .

#### **Solid-State Conformation**

X-ray fiber diagrams of the water-soluble barley  $(1\rightarrow 3,\ 1\rightarrow 4)$ - $\beta$ -D-glucan can be interpreted as a  $(1\rightarrow 3)$ -linked cellotriosyl polymer with a threefold screw axis and a fiber repeat of 4.2 nm. The shape of the chain is reminiscent of cellulose in the cellotriosyl and longer cellosaccharide units, but the  $(1\rightarrow 3)$ -linkages provide some helical character.

#### Viscosity of Solutions of (1 $\rightarrow$ 3, 1 $\rightarrow$ 4)- $\beta$ -D-Glucans

The intrinsic viscosities of  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan solutions are dependent on the cereal species from which the polysaccharide is extracted and on the solvent used. Intrinsic viscosity values for barley  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans in water range from 4.6-6.9 dl g<sup>-1</sup> and from 2.0-9.6 dl g<sup>-1</sup> for oat  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans. The extractability, solubility, and viscosity characteristics of cereal  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans can be understood by reference to their fine structures. Long runs of adjacent  $(1\rightarrow 4)$ - $\beta$ -D-glucosyl units in

the  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan chain will form extended ribbon-like cellulosic stretches, but the insertion of a  $(1\rightarrow 3)$ -linked glucosyl unit between them causes a "kink" in the molecule (Figure 3c). Chain flexibility arises principally from the isolated  $(1\rightarrow 3)$ - $\beta$ -linkages. Because the  $(1\rightarrow 3)$ -linked  $\beta$ -glucosyl units do not occur regularly along the chain, the polysaccharides are not able to align over extended regions and hence remain "dispersed" and soluble in aqueous media. The overall extended polysaccharide conformation is due to the predominance of  $(1\rightarrow 4)$ -linkages and results in the occupancy of a high volume of solvent (high hydrodynamic volume). This results in aqueous solutions of high viscosity.

The viscosity of solutions of cereal  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans depends strongly on concentration. At concentrations of oat  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan where the total volume occupied by the chains is, in aggregate, less than the total volume of the solution, there is contact between individual chains, but the viscosity of the solution is independent of shear rate. At higher concentrations, there is interpenetration of the chains in response to increasing space occupancy. The viscosities of such aqueous solutions are reduced with increasing shear rate, a phenomenon known as "shear thinning." These different concentration-dependent rheological properties of  $(1\rightarrow 3,$ 1→4)- $\beta$ -D-glucan solutions may be of significance in their physiological responses as food ingredients and in their use in food formulations.

#### Gelation

 $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan solutions at concentrations of 5% (w/v) or more form elastic gel networks. The gels are thermoreversible, exhibit broad melting transitions and show syneresis. Their melting temperatures depend on the source of  $(1\rightarrow 3, 1\rightarrow 4)-\beta$ -Dglucan, as follows: lichenin, 73°C; barley, 65°C, and oats, 62°C. These values are again related to tri-: tetrasaccharide ratios, which in these examples are 22:1, 3:1, and 2:1, respectively. In the gel state, the  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan chains interact to form three-dimensional networks. Association between chains at junction zones, through pairs of consecutive cellotriosyl units, is proposed. This accounts for the higher melting temperature of lichenin gels, where cellotriosyl units make up most of the molecule. In the cereal  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans, consecutive cellotriosyl units are less frequent, although the longer runs of adjacent  $(1\rightarrow 4)$ - $\beta$ -D-glucosyl units, not present in lichenin, may also participate in junction zone formation.

The induction time for gelation depends on mobility and diffusivity. It increases with the molecular

mass of  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan and decreases as the concentration increases. Gelation is repressed at high and low temperatures, where molecular motion is high and low, respectively. Gelation may be induced by homogenization, centrifugation, and by freezing and thawing.

## Structure-Function Relationships in Cereal **Processing and Nutrition**

The viscosity-generating properties of soluble cereal  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans are critical determinants in many aspects of cereal processing. For example, incompletely degraded  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans from malted barley and cereal adjuncts can contribute to wort and beer viscosity and are associated with problems in wort separation and beer filtration. In other applications,  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans can be cast as biodegradable films, have foam- and emulsionstabilizing ability and, when used as a flour supplement, increase water absorption in doughs. Soluble cereal  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans have "antinutritive" effects in monogastric animals such as pigs and poultry. The "antinutritive" effects have been attributed to the increased viscosity of gut contents, which slows both the diffusion of digestive enzymes and the absorption of degradative products of enzyme action. This, in turn, leads to slower growth rates. Moreover, in dietary formulations for poultry, high  $(1\rightarrow 3,$ 1→4)- $\beta$ -D-glucan concentrations are associated with "sticky" feces, which are indicative of the poor digestibility of the  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans and which may present major handling and hygiene problems for producers.

Cereal  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans are important components of dietary fiber in human and animal diets. Humans and monogastric animals produce no enzymes that degrade  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans, although there are indications that some depolymerization occurs in the stomach and small intestine, presumably due to the activity of commensal microorganisms. By comparison, the soluble  $(1 \rightarrow 3, 1 \rightarrow 4)$ - $\beta$ -D-glucans and other nonstarch polysaccharides are readily fermented by colonic microorganisms and make a small contribution to digestible energy.

In contrast to their "antinutritive" effects in monogastric animals, oat and barley  $(1\rightarrow 3, 1\rightarrow 4)-\beta$ -Dglucans at high concentrations in human foods have beneficial effects, especially for noninsulin-dependent diabetics, by flattening glucose and insulin responses that follow a meal. High concentrations of  $(1\rightarrow 3,$  $1\rightarrow 4$ )- $\beta$ -D-glucans (20% w/v) in food have also been implicated in the reduction of serum cholesterol concentrations, by lowering the uptake of dietary cholesterol or resorption of bile acids from the intestine.

#### **Immuno-Modulatory Effects**

 $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-Glucans, in common with a number of other polysaccharides, in particular  $(1\rightarrow 3)$ - $\beta$ -Dglucans, modify immunological responses in humans by a process that is mediated through binding to receptors on cells of the reticulo-endothelial system (leucocytes and macrophages). In addition, they may have the capacity to activate the proteins of the human complement pathway, a system that is invoked as a first line of defense before circulating antibodies are produced.

## **Analysis and Detection**

Specific reagents for the analysis and detection of cereal  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans include enzymes, fluorochromes, and monoclonal antibodies. The  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan endohydrolase (EC 3.2.1.73) from Bacillus subtilis has been adopted for the specific quantitation of  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan in grain or grain extracts. This enzyme cleaves only  $(1\rightarrow 4)$ -linkages that adjoin  $(1\rightarrow 3)$ -substituted glucosyl units in the glucan chain and therefore liberates a series of oligoglucosides with  $(1\rightarrow 3)$ -linked reducing units (**Figure 3b**). These may be quantified by  $\beta$ -glucosidase cleavage and measurement of the released glucose with the glucose oxidase-peroxidase reagent, by separation and quantitation by high-performance liquid chromatography (HPLC), or by matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF-MS). The latter procedures allow an assessment of the profile of oligosaccharides making up the primary structure of the  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan and, in particular, the tri-: tetrasaccharide ratio.

 $\beta$ -D-Glucans, including the  $(1\rightarrow 4)$ - $\beta$ -D-glucan, cellulose, the  $(1\rightarrow 3)$ - $\beta$ -D-glucan, callose, and cereal  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans, form complexes with the diphenyldiazo dye, Congo red, and members of the Calcofluor family of diaminostilbene sulphonates. These reagents complex poorly with heteroxylans and arabinogalactan-proteins. Irradiation of the complexes induces a brilliant red fluorescence in the case of Congo red (emission  $\lambda_{max}$  590 nm, green excitation 470 nm) and a bright blue fluorescence with Calcofluor (emission  $\lambda_{\text{max}}$  420, 442 nm; blue excitation 350 nm). The fluorochromes complex with and precipitate soluble  $\beta$ -glucans. Although complexing with the fluorochromes is not specific for  $(1\rightarrow 3,$  $1\rightarrow 4$ )- $\beta$ -D-glucans, it is nevertheless useful in quantifying soluble  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans with molecular weights of greater than 10 kDa in grain extracts and in products such as wort, where cellulose will not be present.

The fluorochromes and the  $(1\rightarrow 3, 1\rightarrow 4)-\beta$ -Dglucan-specific monoclonal antibody can be used to locate  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans in tissue sections in histological studies. Although near-infrared reflectance (NIR) spectra of polysaccharides are complex, making their direct interpretation difficult, principal component analysis of the spectra of different polysaccharides enables characteristic NIR wavelengths to be determined. NIR spectroscopy has been applied to the determination of  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans in ground barley grain and this method forms the basis of rapid, routine  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan estimation.

#### **Genetics**

 $(1\rightarrow 3,\ 1\rightarrow 4)$ - $\beta$ -D-glucan concentrations in grain are influenced by both genotype and environment. Quantitative trait locus (QTL) mapping has enabled regions of the barley genome that control  $(1\rightarrow 3,\ 1\rightarrow 4)$ - $\beta$ -D-glucan concentrations to be identified and placed on high-density genetic maps. Furthermore, the conservation of gene order (synteny) across cereal genomes means that regions controlling  $(1\rightarrow 3,\ 1\rightarrow 4)$ - $\beta$ -D-glucan concentrations in cereals other than barley can often be deduced from the genetic positions of barley QTLs. This is of particular relevance when wheat, rice, or oats are to be used for human or animal nutrition.

The multigenic control of  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan content in ungerminated barley grain is reflected in the mapping of QTLs to both chromosomes 1H and 2H. Although genes that contribute to  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan content in ungerminated barley grain have not yet been identified, it is likely that at least some will encode  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan synthases. Malt  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan concentrations are determined not only by genetic factors that control their synthesis in the developing grain, but also by the speed and level of synthesis of degradative enzymes in the germinated grain. Thus, QTLs for  $(1\rightarrow 3, 1\rightarrow 4)-\beta$ -Dglucan content in malt extracts are found on barley chromosomes 1H, 3H, 4H, 5H, and 7H. Some of these QTLs may be related to concentrations of the various  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan exo- and endohydrolases that degrade the polysaccharides in germinated grain and to factors that control grain germination more generally.

#### **Heteroxylans**

The cereal heteroxylans, or pentosans, are of two main types: the arabino-xylans and glucuronoarabinoxylans. They are characteristically abundant in walls of vegetative tissues of cereals and grasses. The arabino-xylans are the major noncellulosic polysaccharides in walls of the starchy endosperm cells and of the aleurone layer of cereal grains, whereas

the glucuronoarabinoxylans are characteristically found in walls of the pericarp—seedcoat tissues (Table 1). Within a cereal species, heteroxylan concentration is influenced by both genotypic and environmental factors.

#### Structure

Cereal heteroxylans have a  $(1\rightarrow 4)-\beta$ -D-xylan backbone that has the same shape as the  $(1\rightarrow 4)$ - $\beta$ -D-glucan, cellulose (Figure 3a). The  $\beta$ -xylopyranosyl (Xylp) units of the xylan backbone are modified by sidechain branching (substitution) (Figure 5). The major side-chains comprise single α-L-arabinofuranosyl (Araf) units, situated predominantly at C(O)3, but in certain species also at C(O)2 of the Xylp units. In some cases, Araf substitutions occur at both C(O)3 and C(O)2. The frequency of Araf substitution depends on the cereal species and wall type and is reflected in a very wide range of Xylp: Araf ratios (0.89-13:1). The Xylp units of cereal arabino-xylans may be acetylated. In sorghum heteroxylans, acetyl groups are present on both the C(O)2 and C(O)3 of xylopyranosyl residues. Heteroxylans from cell walls in vegetative tissues, especially lignified walls such as those from parenchyma, sclerenchyma fibers and the pericarp-seedcoat tissues that are found in cereal brans, have low degrees of Araf substitution and therefore high Xylp: Araf ratios. On the other hand, heteroxylans from the aleurone layer and starchy endosperm have relatively low Xylp: Araf ratios.

The distribution of Araf units along the xylan chain is not regular. In one wheat arabino-xylan fraction, three major types of substitution patterns have been identified in different regions of the xylan backbone

**Figure 5** Structure of portion of a  $(1\rightarrow 4)$ - $\beta$ -D-xylan chain substituted at O3-L-arabino of one residue with a 5-O-trans-feruloyl-L-arabinofuranosyl substituent. (Reproduced with permission from Fincher GB and Stone BA (1986) Cell walls and their components in cereal grain technology. *Advances in Cereal Science and Technology* 8: 207–295.)

(Figure 6). In region I, isolated unsubstituted Xylp units are separated by one or two mono- or di-substituted units. The region II contains high amounts of Araf units linked at C(O)3 and region III contains high amounts of contiguous (up to 6 and possibly more) unsubstituted Xylp units.

Other monosaccharides found on the xylan backbone include D-glucuronic acid (GlcAp) and its 4-O-methyl ether, linked to the C(O)2 of Xylp units. GlcAp units are found on endosperm and bran heteroxylans of rice and sorghum, and on bran heteroxylans of wheat, rye, and maize. The barley husk arabino-xylan has 4% by weight GlcAp. Various oligomeric chains are also found as substituents on arabino-xylans (Table 3).

Characteristically, a proportion of the Araf units in the arabino-xylans of cereals, grasses, and several other families of monocotyledons, are esterified with the hydroxycinnamic acids, ferulic acid (FA), and to a lesser extent, its nonmethoxylated analogue p-coumaric acid (pCA) (Figure 5). These hydroxycinnamates are found at C(O)5 of Araf units that are linked to C(O)3 of the Xylp units. Hydroxycinnamates constitute 1.8% (w/w) in walls of wheat aleurone cells and the FA: pCA ratio is 9:1. In contrast, walls of the starchy endosperm cells contain only 0.04% (w/w) FA, with a trace of pCA.

During wall formation enzyme-catalyzed, radical (oxidative) coupling of ferulovl units on neighboring arabino-xylan chains occurs, and leads to their crosslinking. At least five different cross-linking ferulic acid dehydrodimers are encountered in fiber from wheat, barley, rye, spelt, oats, rice, and millet. Their structures are shown in Figure 7.

In walls of pericarp and seedcoat tissues, dehydrodiferulate bridges also cross-link arabino-xylans and in lignified walls the arabino-xylans are cross-linked to lignin through ferulic acid and dehydrodiferulate ester-ether bridges (Figure 8). Thus, the lignified walls in the pericarp-seedcoat layers and husk of cereal grains contain significant concentrations of lignin-heteroxylan complexes. Wheat bran, which consists largely of pericarp-seedcoat and aleurone, contains 0.09-0.18% (w/w) diferulate residues. This arises almost exclusively from the pericarp seedcoat, because the contribution of FA dimers from the aleurone is very low (0.03% w/w).

The heteroxylans in maize bran contain 1.3% (w/w) dehydrodiferulates, which represents  $\sim 15$ dehydroferulate cross-bridges per heteroxylan molecule. Lignin-heteroxylan associations through esterether FA and di-FA cross-links also occur in lignified walls in stem tissues of cereals and grasses.

In walls of wheat aleurone, highly-branched arabino-xylan appears to be covalently associated with protein, possibly through ester-linked FA that is linked to tyrosine in wall proteins by radical

**Table 3** Substituents on xylan backbone of heteroxylans.

```
α-L-Araf-(I-
4-Me-D-GlcpA-(I-
D-GlcpA-(I-
\beta-D-Galp-(I\rightarrow5)-L-Araf-(I-
\beta-D-Xylp-(I\rightarrow2)-L-Araf-(I-
B-D-Xylp-(I\rightarrow3)-L-Araf-(I-
\beta-D-Galp(I\rightarrow 4)-\beta-D-Xyl-(I\rightarrow 2)-L-Araf-(I-
  (or L)
4\text{Me-D-GlcA-}(1\rightarrow 4)-D-Xylp-(1\rightarrow 4)-D-Galp-(1-4)
```

Figure 6 Model of distribution of substituents on an arabinoxylan fraction from wheat endosperm showing three regions with different substitution patterns. (Reproduced with permission from Izydorczyk MS and Biliaderis CG (1995) Cereal arabinoxylans – advances in structure and physico-chemical properties. Carbohydrate Polymers 28: 33-48.)

**Figure 7** Ferulated arabino-xylan chains in cell walls of grasses become cross-linked by radical coupling of ferulate monomers into ferulate dehydrodimers (structures 1–6). Dotted arrows indicate potential sites for further radical coupling with hydroxycinnamyl alcohols or lignin oligomers, resulting in cross-linking of arabino-xylans to lignin. "Ara" is an arabinofuranosyl residue on an arabino-xylan. (Reproduced with permission from Grabber JH, Hatfield RD, Ralph J, Zon J, Amrhein N (1995) Ferulate cross-linking in cell walls isolated from maize cell suspension cultures. *Phytochemistry* 40: 1077–1082.)

coupling. Heteroxylans in maize and rye brans are also covalently associated with protein. The covalent bridges between heteroxylans, wall proteins, and lignins are important determinants of the cohesiveness of the wall fabric and of the resistance of lignified walls to digestion by polysaccharide and ester hydrolases.

#### **Molecular Parameters**

Average molecular masses of cereal heteroxylans range from 65 000 to 5 000 000 (DP 500–38 000) and, as is the case with the  $(1\rightarrow 3,\ 1\rightarrow 4)$ - $\beta$ -D-glucans, the value depends on the cereal species, cell-wall type, extraction procedure, and the method used for molecular mass determination. The cereal heteroxylans are highly polydisperse with respect to molecular mass as indicated by  $M_w/M_n$  values that range from 1.3–4.2 for alkalisoluble wheat arabino-xylan, 4.1 for water-extractable wheat arabino-xylan, and 8.5 for rye arabino-xylan.

#### **Solution Properties**

As with the  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans, a portion of the arabino-xylans from walls of cereal grains is

soluble in water at 40°C and higher temperatures, but other extractants are required to bring all the arabino-xylan into solution. Whereas 0.05 M Na<sub>2</sub>CO<sub>3</sub>, 8 M urea, and dimethylsulphoxide extract only a small part of the water-insoluble arabino-xylan, most can be extracted with 1 M NaOH, 1 M hydroxylamine hydrochloride, saturated Ba(OH)<sub>2</sub> or 4-methylmorpholino-N-oxide. Of these, saturated Ba(OH)<sub>2</sub> containing NaBH<sub>4</sub> is the most successful extractant. Oxidative degradation with chlorite or alkaline H<sub>2</sub>O<sub>2</sub> is effective in extracting arabino-xylan cross-linked to lignin from cereal brans. Arabino-xylans extracted under alkaline conditions may lose all or part of their acetyl and hydroxycinnamate substituents.

#### **Solid-State and Solution Conformations**

Unsubstituted  $(1\rightarrow 4)$ - $\beta$ -D-xylans can adopt a twofold, helical conformation with only one hydrogen bond (O5'-H-O3') between adjacent Xylp units. This conformation is more flexible than the twofold helix of the  $(1\rightarrow 4)$ - $\beta$ -D-glucan, cellulose, which has two hydrogen bonds (O2'-H-O6') and O5'-H-O3'

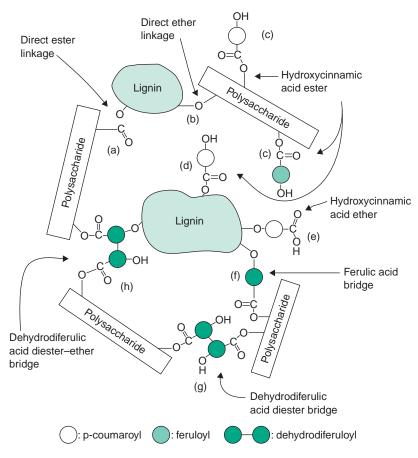


Figure 8 Schematic diagram showing possible covalent cross-links between polysaccharides and lignin in walls. (()) p-coumaric acid; ( ) ferulic acid; ( ) dehydrodiferulic acid. (a) Direct ester linkage; (b) direct ether linkage; (c) hydroxycinnamic acid esterified to polysaccharide; (d) dehydrocinnamic acid esterified to lignin; (e) hydroxycinnamic acid etherified to lignin; (f) ferulic acid ester-ether bridge; (g) dehydrodiferulic acid diester bridge; and (h) dehydrodiferulic acid diester-ether bridge. (Reproduced with permission from liyama K, Lam TB-T and Stone BA (1994) Covalent cross-links in cell wall. Plant Physiology 104: 315-320.)

between adjacent glucosyl units (Figure 3b). The absence of cooperative inter-residue associations in xylans allows the molecules to be conformationally more versatile. Indeed, in the solid state the unsubstituted  $(1\rightarrow 4)-\beta$ -D-xylan chain preferentially exists as a fully-extended, threefold (i.e., three Xylp units per helical turn), left-handed helix or a "slowly twisted ribbon" (Figure 9a). Araf substitution of the backbone Xylp units does not substantially change the basic threefold helical conformation (Figures 9b and 9c). However, substituted xylans cannot assume the twofold helical conformation because of significant steric interactions between the Araf units and the xylan backbone. Nevertheless,  $(1\rightarrow 4)-\beta$ -D-xylans with low degrees of substitution interact reversibly with the surfaces of cellulose microfibrils and may do so in the twofold helical conformation. With increased Araf substitution the affinity for cellulose decreases, presumably due to the increased incidence of the threefold helical conformation, which would be unfavorable for association with cellulose and because of the steric hindrance to interaction imposed by the substituents themselves.

The length of the backbone chains of arabinoxylans is  $\sim 3-5$  nm. In solution, the arabino-xylans behave as partially stiff, worm-like, cylindrical molecules whose flexibility is largely unaffected by Araf: Xylp ratios, which are generally in the range 0.39 - 0.82 : 1.

#### **Solution Properties**

The intrinsic viscosities of wheat arabino-xylans range from 0.8 to 5.5 dl g<sup>-1</sup>, depending on the degree of substitution. These values may be compared with  $0.21 \,\mathrm{dl}\,\mathrm{g}^{-1}$  for dextran, 0.19 for beet arabinan, 0.120.25 for gum arabic, and 4.6-6.9 and 2.0-9.6 for barley and oat  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans, respectively. The behavior of the arabino-xylans in solution is influenced not only by the overall asymmetry of the molecules and their DP, but also by the specific arrangement of Araf units along the xylan backbone.

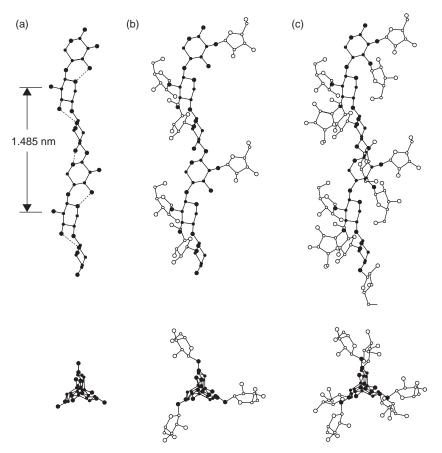


Figure 9 Arabino-xylan conformations. Projections perpendicular (top) and parallel (bottom) of (a) (1→4)-β-D-xylan backbone, (b) backbone with single arabinofuranosyl side groups, and (c) backbone with two arabinofuranosyl side groups. Hydrogen bonds are shown by dotted lines. In each case the backbone is a left handed, threefold helix. (Reproduced with permission from Atkins EDT (1992) Three dimensional structure, interactions and properties of xylans. In Visser J, Beldman G, Kusters-van Someren MA, and Voragen AGJ (eds.) (1992) *Xylan and Xylanases*, 576pp. Amsterdam: Elsevier.)

Thus, stretches of unsubstituted Xylp units may permit intermolecular alignment over these sequences and lead to formation of chain associations that are stabilized by hydrogen bonds.

As with  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans, the properties of arabino-xylan solutions are strongly concentration-dependent. At low concentrations, their viscosities are not shear-dependent but become so at higher concentrations. This behavior is typical of polysaccharides with extended, asymmetric conformations. Removal of Araf units, either by dilute acid or by  $\alpha$ -L-arabinofuranosidase action, leads to aggregation and precipitation of the residual  $(1\rightarrow 4)$ - $\beta$ -D-xylan or of partially substituted arabino-xylan molecules.

#### Gelation

At high concentrations, arabino-xylan solutions form thermo-reversible gels that are stabilized by noncovalent interactions. Gelation of arabino-xylan

solutions can also be induced by dimerization (cross-linking) of feruloyl units on neighboring arabino-xylan chains by radical coupling (Figure 7). The dimerization can be catalyzed by peroxidase/hydrogen peroxide, manganese-dependent peroxidase, laccase, ammonium persulfate, ferric chloride and other metal chlorides, halogens, linoleic acid/lipoxygenase, but not by iodate or bromate.

Well-developed, three-dimensional gel networks are obtained with arabino-xylans containing high FA contents, high molecular masses, and relatively unsubstituted xylan backbones. The latter facilitate the initial contact between feruloyl groups on neighboring arabino-xylan chains. The rate of gelation depends on temperature, pH, and concentration of oxidizing agent. The gel-forming abilities of water-extractable arabino-xylans from rye and barley are higher than for those from wheat and triticale. Covalently cross-linked arabino-xylans may hold up to 100 g water per 1 g polysaccharide. Gels formed by treatment of maize bran with peroxide/peroxidase

are brittle, probably due to the high content of cellulose.

## Structure-Function Relationships in Cereal **Processing and in Nutrition**

Arabino-xylans, together with  $(1\rightarrow 3, 1\rightarrow 4)-\beta$ -D-glucans, contribute to wort and beer viscosity, which may impede wort separation and beer filtration. Arabinoxylans are also components of some beer hazes.

The water-soluble arabino-xylans in barley, rye, and wheat contribute an "antinutritive" effect in dietary formulations for poultry. In human diets, cereal arabino-xylans are components of the dietary fiber fraction. Water-extractable fractions from rye, wheat, and spelt (Triticum spelta) are mainly arabinoxylan, whereas those from barley and oats are mainly  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucans. Ingestion of arabino-xylanenriched, wheat flour by-products reduces the glucose response following a meal in normal subjects. Waterextractable cereal fractions have a very low content of dehydrodiferulate and are partially fermented by anaerobic bacteria in the colon to produce short-chain fatty acids. Water-unextractable cereal fractions contain variable proportions of dehydrodiferulate; maize and millet have the highest content and oat wheat and spelt the lowest. Water unextractable cereal fractions are poorly fermented in the colon which may be related to the relatively high diferulate cross-linking of arabino-xylan chains that prevents swelling and attack by bacterial polysaccharide hydrolases. High concentrations of water-unextractable fractions in the diet are effective in increasing stool weight, through its bulking capacity.

Arabino-xylans have well-demonstrated impacts in bread making. Added water-extractable arabinoxylans have positive effects on the water absorption of doughs, especially after oxidative gelation, and also enhance loaf volume. In both cases, the amounts and molecular masses of the added arabino-xylan are important. Added water-extractable arabino-xylans are reported to slow starch retrogradation and to produce less firm breadcrumbs. The effect on breadcrumb texture is attributed to the increased moisture content of the samples. Water-extractable arabino-xylans from wheat bran are good emulsion stabilizers. They increase the strength and elasticity of gluten-starch films surrounding gas bubbles in doughs. This leads to a higher retention rate of CO<sub>2</sub> in the bubbles and produces a positive effect on the fineness and homogeneity of crumb texture. On the other hand, water-unextractable arabino-xylans have detrimental effects on dough rheology and loaf volume. Waterextractable rye arabino-xylans are inhibitors of ice crystal growth and are thus candidate cryostabilizers. Arabino-xylan—lipid mixtures may be cast as films.

## **Analysis and Detection**

The determination of arabino-xylans in cereal and cereal products relies on the phloroglucinol-HCl or orcinol-HCl colorimetric reactions that show a high, but not absolute, specificity for pentoses. In addition, NIR can be used to estimate arabino-xylan content in grain and NIR peak intensity ratios at 1164 and 990 cm<sup>-1</sup> allow the degree of Araf substitution in arabino-xylans to be defined. Arabino-xylans in grain tissues can be sensitively detected by UV-induced, bright blue fluorescence ( $\lambda_{\text{max}}$  415–445 nm, excitation 310 nm) due to esterified FA residues. Lignins also fluoresce under UV irradiation but may be distinguished from ferulate esters by examining walls at a high pH; where the lignin continues to fluoresce blue, but ferulate esters fluoresce green. Arabinoxylans may also be located in tissue sections through immuno-gold labeling with polyclonal antibodies raised against 4-O-methyl glucuronoxylan or arabino-xylan, or with gold-labeled xylanases. Polyclonal antibodies raised specifically against highly or sparsely substituted glucuronoarabinoxylans can also differentiate these types of arabino-xylan in cell walls.

#### **Genetics**

Despite the availability of high-density genetic maps for barley, no QTLs for arabino-xylan (or pentosan) content in barley are recorded in the major databases. However, a QTL accounting for  $\sim$ 35% of variation in both Araf: Xylp ratio and extract viscosity has been found on the long arm of chromosome 1B of bread wheat. The observation that there is no apparent change in arabino-xylan concentrations in the wheat 1BL/1RS translocation lines suggests that the genes involved are located outside the segment of that chromosome that was introgressed from rye. A total of five QTLs controlling acid detergent fiber (ADF) have been identified in barley and although ADF consists predominantly of cellulose and lignin from the pericarp-seedcoat, some of the effects might be related to arabino-xylan and/or  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -Dglucan content. Three QTLs, with relatively large effects, map close to one another on chromosome 2H, whereas the other two QTLs are on chromosomes 4H and 1H.

#### Glucomannans

Glucomannans are linear copolymers of  $\beta$ -D-glucopyranose ( $\sim$ 30%) and its 2-epimer,  $\beta$ -D-mannopyranose ( $\sim$ 70%), joined by (1 $\rightarrow$ 4)-linkages (Figure 10a) to form linear chains with DPs ranging from less

Figure 10 Structural features of three noncellulosic polysaccharides found as minor constituents in cell walls of cereal grains: (a) glucomannans, (b) xyloglucans, and (c) pectic polysaccharides.

than 100 to several thousand. In some examples, the backbone has  $(1\rightarrow 6)$ -linked  $\beta$ -D-galactopyranosyl substituents and is generally esterified with acetyl groups. The shape of the glucomannan polymer is similar to cellulose (Figure 3a) and it has therefore been suggested that the chains may associate strongly with surfaces of cellulose microfibrils. The glucomannans are extractable from walls with alkaline borate solutions that act by complexing with the mannopyranosyl units.

Glucomannans are minor constituents of walls of most aleurone and starchy endosperm cells of cereals (Table 1), but in certain *indica* rice cultivars the endosperm walls contain up to 17% glucomannan.

#### **Xyloglucans**

Xylo- $(1\rightarrow 4)$ - $\beta$ -D-glucans (heteroglucans) are a family of side-chain-branched heteroglycans (**Figure 10b**) consisting of a cellulose-like  $(1\rightarrow 4)$ - $\beta$ -D-glucan backbone side-branched at regular intervals by α-D-xylopyranosyl units at C(O)6 of the glucosyl residues. Some side-branch xylosyl units carry  $\beta$ -D-galactopyranosyl and α-L-fucopyranosyl substituents. Compared with xyloglucans from dicotyledonous plants, those

from grasses are less substituted with xylose, contain little galactose, and have stretches of unsubstituted backbone. Xyloglucans have DPs of 600–700 and readily associate with surfaces of cellulose microfibrils. After extraction with dilute alkali, they are water-soluble. Xyloglucans are generally considered to be of minor significance in walls of cereal grain tissues, although procedures used in earlier analyses may have underestimated their relative abundance in wall preparations.

#### **Pectic Polysaccharides**

The pectic polysaccharides are a diverse group of side-chain-branched heteroglycans. The backbone is a copolymer of alternating  $(1\rightarrow 4)$ -linked  $\alpha$ -galacturonosyl and  $(1\rightarrow 2)$ -linked  $\alpha$ -L-rhamnopyranosyl units (Figure 10c). The rhamnogalacturonans are variously side-branched on the rhamnosyl units by arabinan, arabinogalactan, and more complex branched oligosaccharides. In addition, variable amounts of  $(1\rightarrow 4)$ - $\alpha$ -linked homogalacturonan may be present in the same backbone chain. There is also variable methyl esterification and hydroxyl acetylation of the galacturonosyl units.

The homogalacturonan domain complexes with Ca<sup>2+</sup>, which promotes gelation by forming Ca<sup>2+</sup> bridges between unesterified galacturonosyl units on adjacent chains. Pectic polysaccharides are minor constituents of the walls of vegetative tissues and grains of cereals, although measurable amounts have been reported in walls of rice endosperm cells (Table 1).

## **Callose**

Callose is a  $(1\rightarrow 3)$ - $\beta$ -D-glucan. Like cellulose, it is a linear homopolymer and is insoluble in water. However, callose is soluble in dilute NaOH. Callose can be recognized in tissue sections by an intense yellow, UV-induced fluorescence ( $\lambda_{\text{max}}$  495 nm, excitation 380 nm) when associated with the aniline blue fluorochrome, a sulphonated 4,4'-dianilinobenzophenone, or by its binding to a specific monoclonal antibody. In mature barley endosperm, callose occurs as small bead-like deposits on the inner surfaces of walls throughout the starchy endosperm but especially at the aleurone/subaleurone interface. The deposition of callose is a well-known consequence of stress and wounding. Thus, the endosperm deposits may arise as a consequence of plasmolysis during the drying of the grain. The callose content of barley endosperm wall preparations is  $\sim 1\%$ . Callose is found in the developing walls of rice endosperm during the early stages of cellularization, but is not a component of mature walls. It is also found in the nucellar projection and in the vascular tissue of the crease of developing barley, but at maturity remains only in the vascular tissue. In the nucellar projection and the vascular tissue, callose deposition might be related to the control of assimilate transport into the developing endosperm.

#### **Fructans**

Fructans from cereals consist of short chains of up to  $4-5 \beta$ -D-fructofuranosyl units in  $(2\rightarrow 6)$ - $\beta$ -linkage to the fructosyl residue of the disaccharide sucrose. Some branching through  $(2\rightarrow 1)$ - $\beta$ -linkages may also occur. Cereal fructans are soluble in water and in boiling 80% (v/v) ethanol. During the early development of wheat grain, fructans can account for as much as 20% dry weight. In mature grains, the contents are low and variable: wheat, 1.3-2.5%; rye, 4.6-6.6%; barley, 0.8%; and oats, 0.1%. In the grain, fructans are found both in the endosperm and the pericarp/embryo, with higher concentrations in the latter fraction. Fructans are important carbohydrate reserves in the vegetative tissues of temperate cereals, particularly in young seedlings where they can account for 70% of the dry weight.

Cereal fructans are not digested in the monogastric stomach or small intestine but are readily fermented by microorganisms in the lower gut. Thus, they may be considered to be part of cereal dietary fiber. Fructans may be quantified by NIR or by a specific enzymatic procedure, and their size distribution determined by HPLC or MALDI-TOF-MS.

## **Arabinogalactan-Proteins**

Arabinogalactan-proteins (AGPs) are widely distributed in plants. They consist of a protein backbone that is rich in hydroxyproline and to which arabinogalactan polysaccharides are covalently attached. In wheat grains, the major AGP is an arabinogalactan-peptide, with a peptide backbone that is much smaller than most AGPs. The arabinogalactan-peptide from wheat flour constitutes 0.27-0.38% on a dry-weight basis and has an average molecular mass of 22 000, although higher molecular mass AGPs have been detected in other cereal grains, including barley. The polysaccharide portion of the wheat arabinogalactan-peptide constitutes 92% of the molecule, and consists of branch-on-branch,  $\beta$ -D-galactosyl units (Galp) linked by (1 $\rightarrow$ 3)and  $(1\rightarrow 6)$ -linkages (Figure 11). The branched

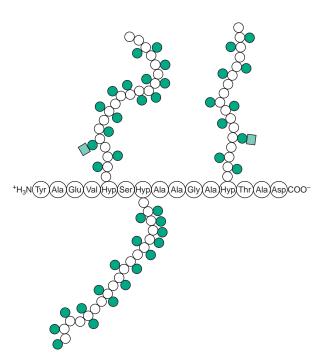


Figure 11 Structure of wheat arabinogalactan-peptide. (()) (1 $\rightarrow$ 3)-linked- $\beta$ -D galactopyranosyl unit, ( $\bigcirc$ ) (1 $\rightarrow$ 6)-linked  $\beta$ -Dgalactopyranosyl unit, and ( $\blacksquare$ ) terminal  $\alpha$ -L-arabinofuranosyl unit. (Structure based on data of Fincher GB, Sawyer WH, and Stone BA (1974) Chemical and physical properties of an arabinogalactan-peptide from wheat endosperm. Biochemical Journal 139: 535-545 and Van den Bulck K, et al. (2002) 79: 329-331.)

 $\beta$ -D-galactan backbone is substituted by single  $\alpha$ -Larabinofuranosyl units. The arabinogalactan has an Araf: Galp ratio of 0.63-0.72:1. In wheat endosperm, the arabinogalactan component of the molecule is covalently linked to a peptide of molecular mass 7800 at each of the three hydroxyproline residues in the peptide sequence. The same peptide sequence is found at the NH<sub>2</sub> terminus of a protein associated with the surface of wheat starch granules. Among eight Canadian wheat cultivars, the peptide constituted between 6.5% and 14.3% of the AG-peptide molecule.

The wheat AG-peptide is very soluble in water and is not precipitated at saturating ammonium sulfate concentrations. Its solutions are of low intrinsic viscosity  $(0.045-0.062 \,\mathrm{dl}\,\mathrm{g}^{-1})$ . Neither its subcellular location in the endosperm nor its physiological function are known. The wheat AG-peptide, unlike its larger AGP counterparts, does not bind to the phenylazo dye,  $\beta$ -glycosyl Yariv reagents. The effects of wheat AG-peptides on bread-making properties are of equivocal significance.

## **Concluding Remarks**

There is a great deal of information available on the chemistry of nonstarch polysaccharides in cereal grains. This is directly attributable to the central importance of these polysaccharides in large-scale food processing activities that include brewing, baking, and stock feed manufacture. Moreover, the nonstarch polysaccharides of cereals have attracted renewed interest in recent years because of their potentially beneficial effects in human nutrition. Despite this interest, there remain major gaps in our knowledge of the genes and enzymes that control nonstarch polysaccharide biosynthesis in the cereal grain. It is likely that large scale, high throughput functional genomics programs, many of which are focused on cereal quality determination, will soon lead to the identification of the genes and enzymes responsible for  $(1\rightarrow 3,$ 1→4)- $\beta$ -D-glucan and arabino-xylan biosynthesis in developing cereal endosperm, and eventually to an understanding of the factors that control fine chemical structure of the polysaccharides. This information can be confidently expected to provide valuable molecular markers through which breeders will track key genes in their breeding programs and improve quality characteristics related to nonstarch polysaccharides in commercially important cereals.

#### **Acknowledgments**

The authors thank D. Mather, J. Dufour, and K. Valenta for assistance in the preparation of material for this manuscript. We are grateful to Dr. D. Mares and Dr. P. Harris for their comments on the manuscript. We are also indebted to the Australian Research Council and the Grains Research and Development Corporation for providing research support over many years.

See also: Animal Feed. Barley: Genetics and Breeding; Malting. Cereals: Grain – Quality Attributes. Genome Mapping. Grain, Morphology of Internal Structure. Grain and Plants, Morphology. Millet: Pearl. Rye. Sorghum: Utilization. Triticale.

## Further Reading

Atkins EDT (1992) Three-dimensional structure, interactions, and properties of xylans. In: Visser J, Beldman G, Kusters-van Someren MA, and Voragen AGI (eds.) Xylan and Xylanases, 576pp. Amsterdam: Elsevier.

Bamforth CW (1994)  $\beta$ -glucans and  $\beta$ -glucanses in malting and brewing: Practical aspects. Brewers Digest 69: 12-16.

Basic A, Harris PJ, and Stone BA (1988) Structure and function of plant cell walls. In: Preiss J (ed.) The Biochemistry of Plants, pp. 297-371. New York: Academic Press.

Bhatty RS (1993) Non-malting uses of barley. In: MacGregor AW and Bhatty RS (eds.) Barley: Chemistry and Technology, pp. 355-417. St. Paul, MN: American Association of Cereal Chemists.

Briggs DE (1978) Barley. London: Chapman and Hall.

Carpita N and Gibeaut D (1993) Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. The Plant Journal 3: 1-30.

Cui SW (2001a) Cereal non-starch polysaccharides I:  $(1\rightarrow 3)$   $(1\rightarrow 4)$ -β-D-glucans. In: Cui SW (ed.) *Polysac*-Gums from Agricultural Products. pp. 104–165. Lancaster, UK: Technomic Publishing Co.

Cui SW (2001b) Cereal non-starch polysaccharides II: Pentosans/Arabino-xylans. In: Cui SW (ed.) Polysaccharide Gums from Agricultural Products, pp. 167–227. Lancaster, UK: Technomic Publishing Co.

Evers T and Millar S (2002) Cereal grain composition and development: some implications for quality. Journal of *Cereal Science* 36: 261–284.

Fincher GB and Stone BA (1986) Cell walls and their components in cereal grain technology. Advances in Cereal Science and Technology 8: 207–295.

Fincher GB, Sawyer WH, and Stone BA (1974) Chemical and physical properties of an arabinogalactan-peptide from wheat endosperm. Biochemical Journal 139: 535-545.

Grabber JH, Hatfield RD, Ralph J, Zon J, and Amrhein N (1995) Ferulate cross-linking in cell walls isolated from maize cell suspension cultures. *Phytochemistry* 40: 1077-1082.

Iiyama K, Lam TB-T, and Stone BA (1994) Covalent crosslinks in the cell wall. Plant Physiology 104: 315-320.

Izydorczyk MS and Biliaderis CG (1995) Cereal arabinoxylans - advances in structure and physico-chemical properties. Carbohydrate Polymers 28: 33-48.

Kroon-Batenburg LMJ and Kroon JJ (1995) The crystal and molecular structures of cellulose. Carbohydrates in Europe No. 12, 15-19.

MacGregor AW and Fincher GB (1993) Carbohydrates of barley grain. In: MacGregor AW and Bhatty RS (eds.) Barley: Chemistry and Technology, pp. 73-130. St. Paul, MN: American Association of Cereal Chemists Inc.

McCann MC and Roberts K (1991) Architecture of the primary cell wall. In: Lloyd CW (ed.) The Cytoskeletal Basis of Plant Growth and Form, pp. 109-129. London: Academic Press.

Skendi A, Biliaderis CG, Lazaridou A, and Izydorczyk MS (2003) Structure and rheological properties of water soluble  $\beta$ -glucans from oat cultivars Avena sativa and Avena bysantina. Journal of Cereal Science 38: 1–17.

Stone BA and Clarke AE (1992) The Chemistry and *Biology* of  $(1\rightarrow 3)$ -β-Glucans. Victoria, Australia: Latrobe University Press.

Van den Bulck K, Looseveld A-MA, Courtin CM, Proost P, Van Damme J, Robben J, Mort A, and Delcour JA (2002) Amino acid sequence of wheat flour arabino galactan-peptide, identical to part of grain softness protein GSP-1 leads to improved structural model. Cereal Chemistry 79: 329-331.

Vinkx CJA and Delcour JA (1996) Rye (Secale cereale L.) arabino-xylans - a critical review. Journal of Cereal *Science* 24: 1−14.

Visser J, Beldman G, Kusters-van Someren MA, and Voragen AGJ (1992) Xylan and Xylanases, 576pp. Amsterdam: Elsevier.

## **Grain Defects**

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#### Introduction

This article begins with a brief introduction to the stages through which grain travels from field crop to commodity. Similar symptoms may result from more than one cause. Therefore, it is important to be familiar with normal, healthy cereal grains before trying to recognize defects in them. The recognition of defects that are important to merchants or processors who buy the grain is explained with examples of how a poor raw material would affect an end product. An explanation of why certain

defects result in sample rejection is provided where possible. Causes of physical and chemical defects are not always obvious; many result from pest infestation or disease infection earlier in the growing season of the crop. Grain defects that have not resulted from damage during development of the crop may be due to problems encountered at harvest, subsequent storage, or processing. Measures to prevent some of the main causes for rejected grain samples are suggested.

As there are other articles in this encyclopedia devoted to a cereal overview, descriptions of grain morphology, nutritional composition of grains, and diseases of various individual crops are intentionally kept brief.

#### **Production of Sound Grain**

#### **Growth and Harvest**

In order to recognize defective grain, it is first necessary to know the qualities of sound (healthy or intact) grain. Cereal crops can be defined as any plants cultivated for seed as human food, although they tend to include only crops closely related to grass. In most cases, they require physical processing (cutting and threshing, or combining) to remove individual grains from the spike or ear in which they have grown. Barley, millet, oats, rice, rye, triticale, and wheat have grass-like grains when harvested. The seed of maize and grain sorghum is produced on larger, sturdier plants that are less grass like and better adapted to climates with brighter sunlight. Barley and the majority of oat varieties retain their husks when threshed, although several varieties of naked oats are also available whose husks are removed when they are harvested. The other cereals require threshing to remove their husks at harvest, prior to storage. Barley, oats, rye, triticale, and wheat all have a crease on one side of their grain as a result of the way in which they develop. It is this complication in grain morphology that has led to the evolution of a complicated milling process to extract white flour from wheat. The more uniform shape of other grain-producing plants allows them to be processed more simply.

## **Drying**

When cereals are grown in the temperate European climates where summer weather cannot be guaranteed to be dry, it is often necessary to reduce the moisture of grain once it has been harvested from the field. This can be done by a range of methods involving the flow of air through the grain, causing it to dry out. The air temperature may be raised in

order to speed up the drying process, but if an internal grain temperature of  $60^{\circ}\text{C}$  is exceeded, there is a risk of protein denaturation. Drying at high temperatures can damage germination potential. The grower must ensure that the grain is dried enough to reduce or prevent fungal growth during storage (usually below 15% moisture content). The crop may be stored on the farm by the grower or sold on to a merchant or cooperative and bulked up with other similar types of grain. In drier climates, premature ripening may occur due to stress from drought. In such cases, grains are unlikely to reach their full potential size.

#### **Storage**

The reason why cereal grains are so attractive to humans for food is because they have pleasant flavor, high nutritional value, and versatility. It is not surprising then that a number of pests have developed to exploit grain at whatever stage of production they are able to gain access to it. Assuming a grain crop has been protected during growth and has been successfully harvested into a clean storage area, it must then be protected from marauding birds, rodents, and insects. Secure grain silos or barns, which protect against pest invasion, are necessary. In addition to this, the temperature and humidity of the produce must be kept to suitably low levels to prevent conditions suitable for storage fungi, mites, and insect pests. In this way, even if the store is breached, the environment within the grain will not allow it to be spoiled. Regular monitoring of the store conditions, particularly the temperature, is vital. If a problem is to be dealt with in a timely and effective manner, its early detection is vital. Specific and detailed information may be found in the article on harvesting, storage, and transport of barley.

#### **Detection of Grain Defects**

#### Sampling

Defects are assessed at intake points where grain consignments are delivered to the buyer or merchant, prior to processing or bulking with other samples that have been produced independently. Sampling correctly is vital as a precursor to any type of assessment in order to ensure that the original load is being correctly represented (Figure 1).

It is worth taking time to appreciate the importance of this aspect of grain management. If a scoop full of grains were to be removed from the top of the sample after it has been transported to the intake point, the lightest and least dense material would be removed, rather than a mixture of the whole consignment. This is likely to contain unthreshed grains, broken grains, weed seeds of similar size, but lower density to the crop, ergot and insects as well as some healthy, but lighter crop grains. By analyzing such a sample, the bulk load may be rejected without having had a fair trial. If a subsample were taken from the bottom of the bulk load, it would be likely to contain the most dense and heaviest fractions, including stones, heavy dust, and small, dense weed seeds as some of the heavier (larger) crop grains. Analysis of this sample could produce results indicating the whole load to be of better quality than it really is. To avoid such misrepresentations, a strategy for accurate sampling is necessary.

A sample for analysis should consist of several individual subsamples taken with a sampling spear that penetrates the entire depth of the transport container, at intervals across it. These subsamples should then be thoroughly mixed and subdivided in order to give a suitably sized sample for analysis. The subdivision should be carried out in such a way as to keep the

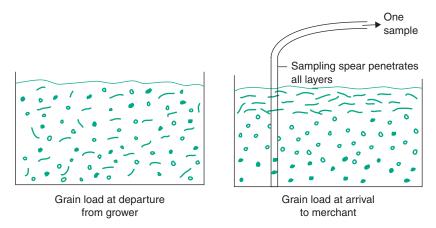


Figure 1 Stratification of grain during transit and sampling spear.

sample representative of the entire bulk. The use of a grain splitter enables this process to be carried out quickly and efficiently (Figure 2). The sample is tipped into the top of the splitter and half is diverted into one collection tray, the other half into the second tray. A number of tests are routinely carried out in order to check for grain suitability; these are outlined here, but explained in detail in the article on analysis of cereal quality.

#### **Visual Assessment**

Once a representative sample has been obtained, an evaluation by a trained individual is the quickest and most effective way to identify many potential defects. With experience, it is even possible to identify differences between varieties or quality types of the same cereal at this stage.

Sample appearance The kernels themselves should appear healthy and fit for purpose; generally they should be intact (with no sign of having started to germinate), bold (not shriveled), and bright (not dull or dead looking). If there is evidence that germination (or sprouting) has occurred, even if it has stopped, this is an indication that problems could arise during processing. In bread-making wheat, this would be as a result of excess enzyme activity from the germination process, or in malting barley,

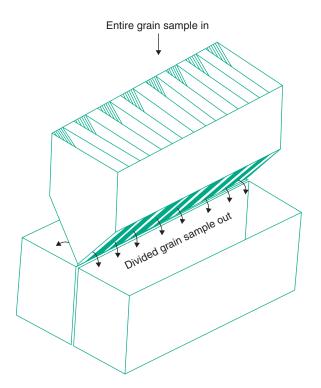


Figure 2 Sample division using a grain splitter.

prevention of further growth during malting. Skinned barley grains cannot be fully malted and are not acceptable for malting samples; the condition occurs during harvesting, when all or part of the protective covering layer (husk) is torn away. Similarly, broken grains occurring during harvest will reduce the quality of any cereal sample, as they will be removed during cleaning.

Sample purity Sample purity is particularly important where the purchaser has set specific requirements. Although contaminants may not relate directly to the integrity of the grains themselves, they provide the main contribution to impurities within the sample. Easily visible signs of contamination include broken grains, ergot, insects, unthreshed grains, other cereal grains, weed seeds, soil, and stones. Dividing a predetermined weight of grain into grain and nongrain material is used as an objective measurement of impurities within a sample. Each category of nongrain material is then weighed and expressed as a percentage of the total sample. This type of assessment is particularly important for high-quality crops such as the high-protein bread-making wheats produced in Australia, Canada, and the USA. Contaminants mixed in with grain consignments can be the cause of reduction in price paid by the purchaser, or in outright rejection of the sample. Although the majority of contaminants can be removed by cleaning the grain, the cost of this (to the processor) together with the cost of waste disposal must be recoverable in the final product market price or discounted when purchasing the grain.

Grain should be free from pests (live or dead); if adults are present, they will be obvious during the visual appraisal, but certain larvae develop within stored grain, with the only indication to their presence being a small hole in the side of the affected kernel. The sense of smell can be used to detect odors, which may be symptomatic of mold (musty), mite infestation (minty or sour), or animal droppings; none of these should be present.

Discoloration If a pale, uniform product is to be made from the raw material, it is important that the entire sample is uniformly clean and does not contain discolored grain. Grains may become brown in places either as a result of physical "bin burning" or fungal staining (black point). The latter is particularly noticeable in whiter types of wheat such as the Australian Prime Hard, and its severity is associated with cereal variety. The germ end of the kernel becomes darkened, varying from a pale watermark to almost black, the affected area ranging in size from a small speck to covering over half of the grain.

A proportion of unripe, green grains may be present in the sample. Field fungi that have remained on the grain after harvesting may produce either pink (*Fusarium* species) or black (sooty molds) areas on the grain. The presence of black mold in malting barley samples is potentially problematic, as the malting process is carried out under conditions which may encourage the proliferation of these fungi. Storage fungi that have developed after harvest may cause blackening and a musty sour smell. Again, it is important to be familiar with the crop in question, as the color of healthy grain is not always golden; for example, sorghum grains naturally ripen to a great variety of colors.

#### **Grain Analysis**

**Specific weight** This measurement of grain density is the weight of the grains that exactly fill a defined volume (generally the weight in kg of a sample calculated to fill 1 hl or 20 l) and it takes into account the type of cereal grains being measured. If samples contain a high proportion of small, unthreshed, or damaged grains, or if the moisture content is high, the grains will not pack efficiently and will give a low specific weight. If grains are larger, dry, and undamaged, they will give a higher specific weight. There will be some variation between seasons due to the weather and other environmental factors, also between different varieties, or types of each crop. Merchants and cooperatives will generally set acceptance values, below which a discounted price may be paid to the grower or the load may be rejected as unfit for use.

**Moisture** If a sample is too wet, it will not be safe to store for any length of time without risking rapid deterioration of quality. The most commonly used method for its determination at grain intake is by near-infrared reflectance (NIR); this indirect method is calibrated using the oven moisture method. The moisture content is simply the amount (weight) of moisture present in a sample expressed a percentage of the initial weight. The oven moisture method involves weighing a sample accurately, drying it under specified conditions, and weighing the dried sample. The calculation of the moisture can then be made as the difference in weight divided by the initial weight and multiplied by 100 to give it as a percentage. High moisture content is the precursor to many problems within grain, including growth of storage fungi, pest infestation, and germination. Although the acceptable moisture of a sample will be agreed between a producer and merchant, generally, if a consignment of grain is too wet, it will require drying before it is fit to be processed further. If a sample is dry, it will store well, but may require the addition of large quantities of water over a long period of time to enable processing (particularly in the case of milling wheat). Also, dry grain will weigh less than the same quantity of wetter grain; this means that the grower will effectively be selling the produce at a lower price as price is based on weight, not number of grains.

The support of fungal growth by moist conditions is of particular concern in connection with mycotoxin production. The presence of the wrong sort of fungi in warm damp conditions can lead to them producing toxic substances such as ochratoxin. By ensuring that the moisture content is kept suitably low, the conditions needed for the production of mycotoxins are prevented from occurring.

**Protein** The percentage protein content of a sample of cereal grains is likely to be an important factor in determining its sale value. At grain intake, a rapid protein measurement is taken with a suitably calibrated instrument. The amount of protein present is expressed at a specified moisture content, an example might be 13% protein on a dry matter basis (i.e., at 0% moisture). For the majority of grains, the higher the amount of protein (or nitrogen) present, the better, although, for malting barley, a lower nitrogen content is preferred as this indicates a higher carbohydrate content. The protein quality is also important when considering bread-making wheat types; if the specification for this is not met, the consignment will not be suitable for processing into bread. In order to ensure that a consignment of wheat is only accepted if it is suitable for processing, there are various tests that could be carried out. The simplest of these is the gluten-washing or wet gluten test. The starch is washed out of a ground wheat sample (using water or weak salt solution) and the remaining gluten is checked for color, elasticity, and extensibility.

**Starch** This is the main component of cereal grains; in some crops it has the highest value of all components. In wheat it is important during bread making as it is broken down by enzymes (α-amylase in particular) to feed the yeast during the baking process and enables the production of gas to give a risen loaf. At the subcellular level, there are different types of starch granules that give different characteristics to the starch depending on the ratio in which they occur. Cornstarch (from maize) is an effective thickening agent; it is relatively easy to extract and gives a consistent product. Modified maize starch can be found on the ingredient list of many modern food products. Starch is also used in the preparation of paper, wallpaper paste, and many pharmaceutical products.

Enzyme activity Green wheat grains can have high levels of  $\alpha$ -amylase activity. As wheat grains develop, the enzyme activity reduces to a minimum level around the time of harvesting when the grains reach maturity. During germination, enzyme level rises again as it is involved in the breakdown of starch granules into polysaccharides. The Hagberg falling number (HFN) method is used at grain intake as an indirect measure of wheat enzyme content and it is able to detect the very early stages of enzyme activity that have no visible external signs. High levels of the enzyme α-amylase will cause starch to break down into its constituent polysaccharides. When starch is mixed with water at high temperatures it forms a gelatinous paste, and the time taken by a plunger to fall through the mixture indicates the integrity of the starch present within the flour. If the plunger falls more quickly, it indicates that the starch has been broken down.

#### **Causes of Grain Defects**

#### **Diseases**

**Black point** Black point appears to be a response by the living tissue over the germ of the grain, to invasion or infection, whereby pigmented lignin precursors are laid down, blocking the progress of the invading mechanism. It has been linked to species of Alternaria by some work, but shown to be nonpathogen specific by other studies.

Bunt or stinking smut Spores of bunt (Tilletia caries) may be present in wheat grain samples as they are often released during harvesting if they were present within the crop. The fungus enters the germinated seedling and develops within the plant, resulting in misshapen grains that contain black, foul-smelling spores instead of white, starchy endosperm.

**Ergot** Ergots are the fruiting bodies of a fungal pathogen (Claviceps purpurea) that invades the developing florets of all grass species. It is spread by insects, rain-splash, or strong gusts of wind, then grows into the developing ovaries and gradually forms a solid purple-black mass that is often over twice the length of a normal grain. Ergotoxin is present within these fruiting bodies and, if ingested, can result in many unpleasant (if not deadly) symptoms including hallucinations, convulsions, abortion, and gangrene. Prevention is by the use of ergot-free grains or by burying any ergots from previous crops by deep plowing and crop rotation to prevent germination of ergots in the following year's crop.

Fusarium sp. Pink grains often result from the infection of cereal ears by Fusarium sp., which will sporulate freely under the right conditions resulting in a salmon pink tinge to the grain. The mycotoxin "deoxynivalenol" (DON) is produced by Fusarium sp. when conditions are suitable. DON levels indicate the gushing (foaming) potential of beer.

Loose smut Florets of developing grain plants are infected with spores of *Ustilago nuda*; symptoms of infected plants include yellowing of the flag leaf and a covering of thick black spores over the entire ear. Seed dressing can be used to prevent reoccurrence in the following crop. Common smut of maize (*Ustilago* maydis) is more resilient to control, the best defense being choice of a resistant maize variety.

#### **Environment**

Drought During grain development, a certain amount of moisture is required prior to harvest to enable grain filling to take place. Under very dry conditions, grains will become severely shriveled and will be very small and light when they are harvested.

Frost damage Kernels that have been affected by frost may become thin and distorted with a bluegray discoloration in severe cases.

Genetic and environment interaction The malformation of grains may be induced by a combination of genetic makeup and environmental factors. It was this that led to the sterility seen in the wheat variety Moulin in the UK in the 1980s.

Green grains The presence of green grains within a grain sample indicates either an early harvest, or a late wet period that stimulated the crop to produce back tillers that did not have sufficient time to ripen prior to harvest.

Sprouting (pregermination) Wet weather prior to harvest can have the effect of retarding ripening by keeping the crop green for longer and this may lead to the crop lodging (or falling down) due to the weight of the plants and the damp soil allowing roots to become loosened. Once areas of a crop have become lodged, the ripe, damp grains are prone to germination (or sprouting) whilst remaining in the ears. This process may be halted at the onset of dry conditions either in the field or due to drying of harvested seeds. The evidence that germination had begun remains visible to the trained eye, however, and should be picked up at sample intake.

Weed competition High populations of competitive weeds in crops will prevent them from realizing their full potential in terms of yield and grain size. Also, the presence of weed seeds within the yielded crop will add to impurities and reduce the quality of the grain vield.

#### **Pests**

Field insect larvae Orange blossom midge larvae enter the wheat floret before anthesis and live on the developing green grain. If the affected kernels reach maturity, they are shrunken and have a deformed, flattened (and often darkened) dorsal side.

Storage insect larvae Some storage pests ensure their survival by burrowing a hole into a cereal grain and laying a single egg in it; when the egg hatches, the larva is surrounded by a plentiful food supply. As it develops, the larva hollows out the grain, eventually leaving just a husk of bran without the starchy endosperm.

#### **Crop Processing**

Drying Heat damage of proteins due to incorrect drying is mainly a problem in bread-making quality wheat. The functional gluten proteins are denatured, which causes them to lose their extensible and elastic properties and may cause a darkening of the flour produced upon grinding the grain. Heat damage of barley can reduce the malting quality of the grain.

**Harvesting** Harvesting when too dry or with incorrect settings of the combine can lead to broken grains.

Storage Discoloration of grains known as "bin burning" occurs when stored grain remain in constant contact with silo walls heated in localized areas, for example, by sunlight on the outside of these walls.

#### **Prevention of Grain Defects**

By addressing the causes of grain defects outlined above, many of the symptoms can be reduced, but due to the nature of crop growth, they are unlikely to be eliminated. The careful selection of variety to suit the local environmental conditions, good husbandry in the field, management of diseases and pests both during field growth and storage, and care during harvesting and processing will ensure a healthy, good quality crop.

See also: Barley: Harvesting, Storage, and Transport; Grading and Marketing. Cakes, Pastries, Muffins, and Bagels. Cereals: Overview; Grain - Quality Attributes. Cookies, Biscuits, and Crackers: The Diversity of Products. Contaminants of Grain. Grain, Morphology of Internal Structure. Grain and Plants, Morphology. Starch: Analysis of Quality. Wheat: Grading and Segregation.

## **Further Reading**

Kent NL and Evers AD (1994) Technology of Cereals: An Introduction for Students of Food Science and Agriculture, 4th edn. Butterworth-Heinemann.

Matz S (1991) The Chemistry and Technology of Cereals as Food and Feed, 2nd edn. Texas, USA: Pan-Tech International.

Morris PC and Bryce JH (eds.) (2000) Cereal Biotechnology. Cambridge, UK: Woodhead Publishing.

Owen G (ed.) (2001) Cereals Processing Technology. Cambridge, UK: Woodhead Publishing.

## **Grain Diseases**

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Published by Elsevier Ltd.

#### Introduction

Plant diseases are major constraints to crop production, affecting yield and quality. In extreme circumstances, contamination with pathogens or their metabolites can cause unpleasant odors and tastes, and in some instances, toxicoses of food and fodder products. Diseases can be subdivided into those with abiotic or biotic causes.

Abiotic diseases include mineral deficiencies and toxicities, and the effects of soil salinity, acidity, and alkalinity. In some instances, mineral deficiencies and toxicities can carry over to harvested products, and to the food and fodder products derived from them. Biotic diseases are caused by fungi, bacteria, nematodes, viruses, and mycoplasmas. Many farm management practices are aimed at alleviating the effects of disease through agronomic, chemical, and genetic means. It has been estimated that one-half of the cost of plant improvement involves "maintenance" breeding, i.e., breeding to maintain current yield and quality levels to meet

the challenges of degrading growing environments and evolving pathotypes of major pathogens.

This article will outline some of the internationally important diseases that affect cereal production and will emphasize those with greatest effects on commerce and endpoint quality.

#### **Abiotic Diseases of Cereals**

All plant species have an optimum range of soil conditions in which they thrive, and performance declines with digression from these optima. Marginal environmental conditions can be alleviated by cultural practices and fertilizers. Some species may be better adapted to prevailing conditions. For example, cereal rye and some triticale genotypes are tolerant to low pH (high aluminum) conditions. Within each species there is also a range of genetic variation that can be exploited in breeding programs, resulting in cultivars that perform better under specific nutrient regimes, e.g., cultivars more tolerant to low pH, or more tolerant to high or to low levels of soil boron or manganese. In these latter cases, mechanisms such as mineral exclusion that lead to a genotype's ability to tolerate high levels of a mineral, may be the basis of the poor ability of that genotype to tolerate a deficiency of the same mineral.

Mineral deficiencies are more easily rectified than toxicities. Generally, mineral fertilizers applied to the soil or leaves will overcome deficiencies even though breeding solutions may also be available. Toxicities can be alleviated by amelioration of the environment to reduce availability of the mineral or by selection and use of more tolerant genotypes. In many circumstances, more tolerant cereal species may be preferred.

In a broader context, disease-like symptoms may be caused by extreme or abnormal weather conditions such as drought, heat, and frost, as well as pesticides. Agronomists and farmers need to be able to distinguish symptoms of environmental stress from diseases caused by both abiotic and biotic agents. Finally, certain genetically determined "disease mimics" are expressed as physiologic spotting or blotching. These are more likely to be encountered in breeding populations than in released cultivars, as breeders obviously select against such phenotypes. However, they may appear in cultivars grown in environments different from those in which they were originally selected. Genetic analyses have shown that some forms of physiologic spotting involve single genes or the complementary interaction of unlinked genes.

## Black Point/Kernel Smudge: Abiotic or Biotic Diseases?

Winter cereals, particularly those grown outside a classic Mediterranean climate, often encounter a complex of harmful weathering conditions during grain development. Winter crops in the subtropics, and summer crops in temperate climates, often ripen with high humidity or rain. High humidity alone can stall or prolong ripening, initiate germination, induce excessive enzyme activity, and establish colonization by saprophytic fungi and bacteria. In wheat, high humidity induces a clean edged symmetrical melanized pattern in the pericarp and testa cells covering the germ and adjacent areas. In barley, the glume tissue covering the germ may become melanized. Prolonged wet periods may smudge the melanization and induce vigorous saprophytic growth on the grain surface affecting its color and brightness. Pink pigments exuded from Eppicoccum spp. and sporulation of fungi such as *Cladosporium* spp. are often included in the black point/kernel smudge complex.

Grain can also be directly infected by foliar and root pathogens. For example, *Bipolaris sorokiniana* causes shriveling and browning of infected tissue in wheat and barley grain and *Phaeosphaeria avenaria* f. sp. *avenaria* causes similar symptoms in oats. *Pyrenophora tritici-repentis* and *Fusarium* spp. produce a pink pigment in wheat, often referred to as kernel smudge, and can invade the living aleurone tissue and attack the embryo.

Although various fungi are associated with black point/kernel smudge, there is no conclusive evidence that black point melanization is a direct result of fungal action. The often reported association between symptoms and the most common grain colonizer, *Alternaria alternata*, is not strong and several studies have been unable to confirm any link. Pigmentation first appears when the pericarp and testa cells are crushed to form the seedcoat and the grain begins to lose moisture. Prolonging or reversing the drying process allows interaction between enzymes and substrates that normally lie dormant until subsequent germination.

Black point melanization alone does not affect flour or end product quality of bread wheat. Recent research has shown that flour color and speckiness does not deteriorate despite black point levels of up to 10% and that black point only affects the bran, and that flour protein has a greater effect on flour color than does black point. Even with very high levels of black point, careful milling can extract flour with an acceptable color for most bread-making purposes. However, black point in durum poses a greater problem with many reports suggesting that low levels of black point can cause visible dark specks in pasta

products. Kernel smudge caused by saprophytic and/ or pathogenic fungi is also an indicator of weather damage, and as such, can have a severe effect on grain quality and viability. Pink smudge caused by *Fusar*ium graminearum may contain mycotoxins.

Under wet conditions, all cereal grains can develop symptoms of black point/kernel smudge, but only wheat, durum, barley, rye, and triticale produce black point melanization. However, similar discolorations are sometimes found in rice, sorghum, and other cereals.

While fungicide application has been used in some countries to control black point, there is little evidence of success. Genetic variation required to reduce or eliminate black point melanism has been identified in wheat, durum, and barley. However, despite variation, genetic improvement has proven very difficult due to a lack of understanding of the underlying mechanisms, the complexity of interactions with environmental factors, and a lack of reproducible and reliable screening techniques. Consequently, most breeding programs rely on opportunistic selection.

## Significant Biotic Diseases of Cereal Grain Crops

An exhaustive list of all diseases of the major cereal grain crops is beyond the scope of this article. Some of the more important and widely dispersed diseases of wheat, barley, rye, oats, maize, and rice are listed in **Table 1**. However, numerous diseases of each crop and of triticale, sorghum, and millets that may be very important on a local, or even regional, basis have been omitted. Infection processes and groups of diseases based on means of infection will be discussed.

Triticale, a hybrid crop combining the genomes of tetraploid wheat and rye is more prone to the diseases of wheat than those of rye. Infection and spread of many diseases is enhanced by wet conditions and the use of cereal rye and sorghum and millets in areas considered too dry for wheat and barley, or corn, respectively, results in reduced risks of epidemics on those crops, in addition to the distinctive resistances they may possess.

#### Soil-Borne Diseases

True soil-borne diseases are probably relatively few in number as pathogens are more likely to be associated with decomposing plant material. Fungi such as the take-all pathogen, *Gaeumannomyces graminis* var. *tritici*, attacking wheat, and to a lesser extent, triticale and barley, can be considered to be soil-borne — or at least located below the soil surface — although they may be associated with the decaying residues

of previous crops and certain grass species. The spores of a number of smut species can be soil-borne as a result of previously smutted crops. They are then available to infect the roots, crowns, and coleoptiles of germinating seeds. These include all of the bunts (except karnal bunt) and smut pathogens except the flower-infecting loose smuts of wheat, barley, and oats. Most nematodes survive in the soil before attaching to cereal roots. The most important nematodes of cereals are cereal cyst nematode (cereal root eelworm) of the winter cereals and the root lesion nematodes of wheat.

#### Seed-Borne Diseases

Some diseases such as the loose smuts of winter cereals and barley stripe are exclusively seed-borne. In the case of loose smut, infections occur at flowering and the mycelia are actually present within the seed tissue and the pathogens develop with growth of the host. The primary means of dispersal for many pathogens is with seed, either as, or on, contaminating material, present on seed surfaces or in infected seed that may or may not be viable. These pathogens are then available to infect germinating seedlings to produce seedling blights, or to induce stress symptoms at later growth stages. Alternatively, fungal pathogens present on the soil surface may produce conidia that can be splash-dispersed to above ground tissues. Some bacterial and virus pathogens are also seed-borne.

#### **Residue-Borne Diseases**

This group is probably the largest and most important group of pathogens of cereals and their significance has greatly increased in large-scale agriculture with its increased monoculture and minimum tillage, or notillage, practices where residue is retained on the soil surface to prevent soil erosion. Fungal pathogens of this type survive from season to season as mycelium and resting structures on host debris. While deepplowing and burning can give marked reductions in levels of many of these diseases, such practices are not encouraged, especially where soil surfaces remain vulnerable to erosion.

Diseases in this group often infect young seedlings, giving rise to early damping off or wilting symptoms (e.g., *Fusarium* blights) or may remain in the root, subcrown, and crown regions to develop disease symptoms at later growth stages, especially at times of short-term or long-term moisture stress. In the winter cereals, these symptoms result in premature death, wilting, "white heads," and lodging. Examples of such diseases include common root rot, eyespot and crown rot of wheat. Wheat crown rot is caused by

 Table 1
 Major diseases of cereal grain crops, causative agents, means of infection, spread, survival, pathogenic variability, and host range

Crop	Disease name and pathogen type <sup>a</sup>		Pathogen: anamorph (teleomorph)	Disease type <sup>b</sup>	Means of infection	Path var <sup>c</sup>	Methods of control	Overseason survival	Infection of other cereal crops
Wheat and durum	Take-all, firing	F	Gaeumannomyces graminis var. tritici	Soil-borne	Mycelial penetration of roots and crown	1	Crop rotation, take-all decline	Volunteer cereals and grasses, residues	Barley, triticale, rye
	Cereal cyst nematode	N	Heterodera avenae	Soil-borne	Penetration of roots	3	Nematicides, crop rotation, resistance	In soil	Barley, oats, triticale, rye
	Root lesion nematode	N	Pratylenchus spp.	Soil-borne	Parasite of roots	1	Crop rotation, some resistance	In soil, other host species	No
	Crown rot	F	Fusarium pseudo- graminearum, F. culmorum, Fusarium spp.	Residue-borne	Stem base and subcrown internode	1	Rotation, stubble management, partial resistance	Crop residues	Barley, triticale, oats (symptomless)
	Common root rot, Heminthosporum leaf blight	F	Bipolaris sorokiniana (Cochliobolus sativus)	Seed and residue-borne	Crown, coleoptile, and leaves	1	Rotation, stubble management, resistance	Crop residues	Barley
	Common bunt, stinking smut	F	Tilletia spp.	Soil and seed- borne	Germinating seedlings	4	Seed dressings, resistance	Soil	No
	Loose smut	F	Ustilago nuda f. sp. tritici	Seed-borne	Flowers	4	Seed dressings, resistance	Seed	No
	Karnal bunt	F	Neovossia indica syn. Tilletia indica	Seed and soil- borne	Flowers	2	Clean seed (quarantine), resistance	Soil and seed	No
	Septoria tritici blotch	F	Mycosphaerella graminicola	Residue-borne	Splash-dispersed conidia	3	Stubble management, resistance	Crop residues	No
	Septoria nodorum blotch	F	Leptosphaeria nodorum = Phaeosphaeria nodorum	Residue- and seed-borne	Splash-dispersed conidia	1	Stubble management, seed dressings, partial resistance	Crop residues	No
	Tan spot, yellow leaf spot	F	Drechslera tritici-repentis (Pyrenophora tritici-repentis)	Residue-borne	Splash-dispersed ascospores and conidia	3	Stubble management, crop rotation, resistance	Crop residues	No
	Fusarium head scab	F	Fusarium graminearum, (Gibberella zeae), F. culmorum, Fusarium spp.	Residue- and seed-borne	At anthesis by splash-borne conidia or ascospores	2	Stubble management, rotations with nonhosts, foliar sprays, partial resistance	Perithecia and hyphal structures in crop residues and seed	Barley, triticale, corn, others, saprophyte on rice residues
	Stem (black) rust	F	Puccinia graminis f. sp. tritici	Airborne	Leaf, stem, spike tissues by uredospores	4	Foliar sprays, resistance	Green-bridge on wheat and a few grasses	Triticale, barley
	Leaf (brown) rust	F	P. triticina	Airborne	Leaf, stem, spike tissues by uredospores	4	Foliar sprays, resistance	Green-bridge on wheat and a few grasses	Triticale
	Stripe (yellow) rust	F	P. striiformis f. sp. tritici	Airborne	Leaf, stem, spike tissues by uredospores	4	Foliar sprays, resistance	Green-bridge on wheat and a few grasses	Triticale

Table 1 Continued

Сгор	Disease name and pathogen type <sup>a</sup>		Pathogen: anamorph (teleomorph)	Disease type <sup>b</sup>	Means of infection	Path var <sup>c</sup>	Methods of control	Overseason survival	Infection of other cereal crops
	Powdery mildew	F	Blumeria graminis f. sp. tritici	Airborne	Conidia, ascospores	4	Foliar sprays, resistance	Green-bridge, cleistothecia on residues	No
	Barley yellow dwarf	٧		Vector-borne	Several aphid spp.	2	Control of aphids, partial resistance	Green-bridge for aphids	Other cereals, grasses
	Wheat streak mosaic	V		Vector-borne	Mites, Aceria spp.	1	Control of Grass hosts	Green-bridge for mites on cereals and some grass spp.	Barley, corn
Barley	Cereal cyst nematode	N		Soil-borne	Penetration of roots	3	Nematocides, resistance	In soil	Wheat, oats, triticale
	Common root rot, Spot blotch	F	Bipolaris sorokiniana (Cochliobolus sativus)	Seed- and residue-borne	All tissues by conidia	1	Seed dressings, rotation, stubble management, resistance	Crop residues	Wheat, triticale
	Crown rot	F	Fusarium pseudo- graminearum, F. culmorum, Fusarium spp.	Residue-borne	Stem base and subcrown internode	1	Rotation, stubble management, partial resistance	Crop residues	Wheat, triticale, oats (symptomless)
	Net blotch – net form	F	Drechslera teres f. teres (Pyrenophora teres)	Seed-, residue-, and soil-borne	Wind and splash	1	Seed dressings, stubble management, resistance	Residues	No, wild <i>Hordeum</i> spp.
	Net blotch – spot form		D. teres f. maculata (Pyrenophora teres)	Seed-, residue-, and soil-borne	Wind and splash dispersed conidia and ascospores	1	Seed dressings, stubble management, resistance	Residues	No, wild <i>Hordeum</i> spp.
	Scald	F	Rynchosporium secalis	Seed- and residue-borne	Splash dispersed conidia	2–3	Seed dressings, stubble management, resistance	Green-bridge, residues	No, wild <i>Hordeum</i> spp.
	Barley stripe	F	Helminthosporium graminum (Pyrenophora graminea)	Seed-borne	Systemic infection of seedlings	2–3	Disease-free seed, seed dressings, resistance	Seed	No
	Fusarium head scab	F	Fusarium graminearum, (Gibberella zeae), F. culmorum, Fusarium spp.	Trash- and seed-borne	At anthesis by splash-borne conidia or ascospores	2	Stubble management	Crop residues, other species?	Barley, triticale, corn, others, saprophyte on rice residues
	Stem (black) rust	F	Puccinia graminis f. sp. tritici	Airborne	Uredospores	4	Foliar sprays, resistance	Green-bridge	Wheat, triticale
	Leaf (brown) rust	F	P. hordei	Airborne	Uredospores	4	Foliar sprays, resistance	Green-bridge	No

	Stripe (yellow) rust	F	P. striiformis f. sp. hordei	Airborne	Uredospores	4	Foliar sprays, resistance	Green-bridge	No, wild <i>Hordeum</i> spp.
	Barley yellow dwarf	٧		Vector-borne	Several aphid spp.	2	Control of aphids, partial resistance	Green-bridge for aphids	Other cereals, grasses
	Powdery mildew	F	Blumeria graminis f. sp. hordei	Airborne ascospores and conidia	Leaf	4	Foliar sprays, resistance	Cleistothecia on residues, green-bridge	No
	Barley stripe mosaic	٧		Seed-borne	Infected seed, mechanical transmission	1	Virus-free seed	Infected seed	Wheat, oats, grasses
Oats	Cereal cyst nematode	N	Heterodera avenae	Soil-borne	Penetration of roots	3	Nematocides, crop rotation, resistance	In soil	Barley, oats, triticale
	Stem (black) rust	F	Puccinia graminis f. sp. avenae	Airborne uredospores	Leaf, stem, and inflorescence	4	Resistance	Green-bridge	No, wild oats important
	Leaf (crown) rust	F	Puccinia coronata f. sp. avenae	Airborne uredospores	Leaf	4	Resistance (extremely ephemeral)	Green-bridge	No, wild oats important
	Barley yellow dwarf	٧		Vector-borne	Several aphid spp.	2	Control of aphids, partial resistance	Green-bridge for aphids	Other cereals, grasses
	Powdery mildew	F	Blumeria graminis f. sp. avenae	Airborne ascospores and conidia	Leaf	4	Foliar sprays, resistance	Cleistothecia on residue, green-bridge	No, wild oats
Cereal rye	Ergot	F	Claviceps purpurea	Seed- and soil-borne	Flowers	1	Control of grass weeds	Sclerotia in soil or seed lots	Other winter cereals and grasses
	Stem (black) rust	F	Puccinia graminis f. sp. secalis	Airborne uredospores	Leaf, stem, and spike	4	Resistance	Green-bridge	No, some grasses
	Leaf (brown) rust	F	Puccinia recondita f. sp. recondita	Airborne uredospores	Leaf, stem, and spike	4	Resistance	Green-bridge	No, some grasses
Corn	Gibberella stalk and ear rot	F	Fusarium graminearum, (Gibberella zeae), Fusarium spp.	Seed- and trash- borne	All growth stages by ascospores and conidia	1	Stubble management, crop rotation, resistance	Perithecia and hyphal structures in residue and seed	Wheat, barley, grasses, possibly other crops
	Diplodia stalk and ear rot	F	Diplodia maydis	Seed- and trash- borne	Seedlings and nodal regions by pycnidiospores	1	Stubble management, crop rotation, resistance	Pycnia on seed and residues	No
	Fusarium stalk and ear rot	F	Fusarium venticillioides	Seed- and trash- borne	Hyphal infections of most tissues	1	Measures to reduce stress	Hyphae in seed, hyphae and conidia in residues	Can affect sorghum and millet
	Northern leaf blight	F	Helminthosporium turcicum	Splash-borne	Conidial infection of leaves	2	Stubble management, crop rotation, resistance	Possibly conidial structures on residues	Sorghum, some related grasses
	Southern leaf blight	F	Helminthosporium maydis	Splash-borne	Conidial infection of all tissues	2	Resistance, especially avoidance of T-cytoplasm	Conidia on residues	No
Sorghum	Fusarium stalk rot	F	Fusarium thapsicum	Seed- and residue-borne	Hyphal infections of most tissues	1	Measures to reduce stress	Hyphae in seed, hyphae and conidia in residues	Primarily grain sorghum

Tabl	Table 1 Continued								
Crop	Crop Disease name and pathogen type <sup>a</sup>		Pathogen: anamorph (teleomorph)	Disease type <sup>b</sup>	Means of infection Path Methods of control var <sup>c</sup>	Path var <sup>c</sup>	Methods of control	Overseason survival	Infection of other cereal crops
Rice	Rice Blast	Ш	F Pyricularia oryzae	Residue-borne	Conidial infection of 4	4	Resistance	Residues, green-bridge No (different	No (different
	Bacterial leaf blight	В	Xanthomonas oryzae		Entry via wounds	4	Resistance	Green-bridge	No No
	Tungro	>		Vector-borne	Leaf hopper, <i>Nephotettix</i>	ო	Resistance	Green-bridge	No (West Asia)
	Hoja blanco	>		Vector-borne	virescens Sogatodes orizicale		Resistance	Green-bridge	No (Central and South America)

Pathogens: F = fungal, N = nematode, V = virus, B = bacteria.

"Residue" refers to partially decomposed material either on the surface or within the soil. Residues may carry saprophytic fungal mycelia or resting structures.

Pathogenic variability: 1 = no significant variability to 4 = high degree of variability.

Fusarium spp. It is of interest that the Fusarium head blight pathogen, Gibberella zeae, is not a significant cause of crown rot although it can produce seedling wilt. Crown rot of wheat and barley is caused by F. pseudograminearum in warmer areas and by F. culmorum and other species in cooler areas. Other members of this group of pathogens are dispersed as conidia or ascospores by raindrops or wind to infect leaf tissues or even inflorescences. They include most of the fungal leaf spot, blotch and streak pathogens and their spread within the canopy is dependant on periods of consistent light rainfall. These diseases are often present but not damaging. However, when environmental conditions are optimal they can be very damaging with total loss of green leaf tissue and subsequent grain losses of up to, and sometimes exceeding, 50%. Many of these diseases are cyclical in occurrence involving combinations of susceptible host genotypes and sequences of years of favorable conditions during which there is a gradual increase in inoculum loads. Examples include the southern corn leaf blight epidemic in the USA in 1969–70 and the wheat yellow leaf spot epidemic in eastern Australia in 1998. The corn leaf blight epidemic involved susceptible corn hybrids based on T-cytoplasm and disease occurrence and risk was reduced by a return to normal cytoplasm and mechanical detassling of female parents for hybrids. The vellow leaf spot problem in Australia declined with less favorable environmental conditions and release and recommendations of less susceptible cultivars. Sporadic epidemics seem to be associated with sequences of unusually favorable seasons combined with a suite of susceptible cultivars that are approved during the intervening periods.

The most damaging diseases of this group are eventually those that progress to the inflorescence or involve the inflorescence. Examples include Septoria nodorum blotch of wheat and Fusarium head scab (FHB) of wheat and barley. FHB is caused by G. zeae in high temperature areas with wheat: maize rotations (e.g., mid-western and eastern USA, central and southern Europe) and wheat: rice rotations (e.g., in the Yangtze valley in China), by F. culmorum at moderate temperatures and by Microdochium nivale at lower temperatures. G. zeae also causes stalk rot and pink ear rot of corn. On the other hand, G. zeae is not a common pathogen of rice; the fungus behaves as a saprophyte on rice stubble (and probably sorghum), thus maintaining high inoculum levels for subsequent wheat crops. Barley, especially two-row genotypes, is less susceptible to FHB than wheat suggesting the rate of spread is influenced by spike structure. Blast, probably the most important fungal disease of rice, spreads by splash-borne conidia. The blast pathogen also causes grey leaf spot of turf grasses (perennial ryegrass) and has recently become a pathogen of wheat in Brazil. However, the isolates from grasses are nonpathogenic on rice and the form attacking wheat is more closely related to those attacking grasses than isolates from rice. Despite a teleomorph stage (*Magnaporthe grisea*), dispersal of blast in nature is exclusively by conidia. Under extreme conditions when infections by splash-borne organisms reach the upper canopy, their epidemiological dynamics may change to become more like air-borne diseases typified by long distance spore dispersal.

#### **Airborne Diseases**

This group includes the rusts of all major cereals except rice and the powdery mildews of wheat, barley, and oats. The rusts are among the main diseases affecting oats and wheat with powdery mildew being more important on barley. Both the rusts and powdery mildew pathogens (as well as some splash-borne pathogens such as the rice blast pathogen) are highly variable and occur as *formae speciales* or specialized taxonomic varieties on individual species as well as pathotypes or pathogenic races within those groups.

Most of the rusts are heteroecious species with the telial stages occurring on cereals and the aecial stages on other (dicotyledonous) species. The rust pathogens cannot survive for long periods away from a living host, although they can survive as uredial infections in most areas provided a "green-bridge" is available. This green-bridge includes self-sown or regrowth plants from earlier crops or along transport corridors (roads, railway lines, storage sites) and a restricted range of "accessory" grass hosts that varies for each pathogen. In addition, uredospores can be transported over long distances by wind as documented by studies in North America and Australia. In certain rusts and in some locations where the aecial stage contributes to overall epidemiology (note the stripe rust pathogens have no known aecial stage), the black telia that develop on maturing host leaf and stem tissues provide a resting stage for overwintering. In early spring, the teliospores from within the telia germinate, and undergo meiosis to form basidiospores that infect the new spring growth of the alternate hosts and form pycnia. There, the different mating types undergo fertilization to produce a dikaryotic aecium and aeciospores reinfect the grass host species. This cycle, when it occurs, has two main effects on epidemiology. First, it provides a mechanism for survival and early infections in areas where uredial survival is prevented by cold winters and second, meiosis allows reassortment of the genotype providing an efficient means of creating new pathotypes. Despite an absence of alternate hosts

in many cereal-growing areas the rust pathogens, including *P. striiformis*, are nevertheless highly variable, with mutation and somatic hybridization (asexual recombination) the likely mechanisms.

The powdery mildew pathogens are ascomycetes. Survival between seasons is by green-bridging and by cleistothecia that develop on ripening host tissues and survive on the debris. Karyogamy and meiosis occur in the cleistothecia and the released ascospores infect young cereal plants on which the organisms recycle as conidia. Again, meiosis allows for genetic assortment, but the powdery mildew fungi and the blast pathogen are monoploid (n) organisms in contrast to the rust pathogens that are dikaryons (n + n) and functionally similar to diploids (2n).

#### **Vector-Borne Diseases**

This group includes the many virus diseases that affect cereals in different locations. However, relatively few virus diseases of cereals are significant on a worldwide basis. Tungro of rice and barley yellow dwarf (BYD), cereal yellow dwarf (CYD) and wheat streak mosaic on small grain winter cereals are among the most important. Viruses are transmitted from plant to plant by vectors that include insects, particularly aphids and leaf-hoppers, mites, and fungi. Knowledge of the biology and migratory behavior of vectors, and of specific virus strain: vector relationships, is essential to understanding the epidemiology of virus diseases and to the development of control strategies. In addition, some viruses can transmit between neighboring plants through contact of injured tissues.

BYD can be caused by one or more of several luteoviruses spread by a range of cereal aphid species, hosted by a number of grasses including corn. This complex of variable viruses, aphid species, host species (cereals, pasture species, native grasses), and environments adds to the problems of managing this disease, especially in mixed farming areas. WSM, vectored by the aerially dispersed wheat curl mite Aceria tulipae, and infecting a range of cereals and grasses that host the mite, is responsible for significant losses in wheat and barley in North America, eastern Europe and the Near East. Autumn or early winter infections of BYD, CYD, and WSM in winter cereals or autumnsown spring cereals can result in very significant yield reductions; spring infections when plants are more developed lead to lower losses.

# Diseases of the Inflorescence, Spike, and Seed

These diseases are the most important for commerce. They include the black point/kernel smudge complex

already discussed, the smuts, leaf diseases that also affect the spike and glumes, head scab and ear rots, and also the non or weakly pathogenic molds and rots. There are two aspects: firstly the effects of seedborne diseases on the seed industry, and secondly, the effects on trade and endpoint use. The smuts destroy the inflorescence and thereby reduce yields. Some smuts such as common bunt and karnal bunt of wheat produce unpleasant odors and flavors. The scab and cob rots, especially those caused by Fusarium spp., may carry a variable complex of compounds (including vomitoxin = deoxynival anol (DON), nivalanol (NIV), and related trichothecenes) that are toxic to man and animals. In the various fungal species, these compounds appear to act as virulence factors, enhancing the ability of the pathogens to colonize the hosts. The particular compounds and their amounts not only vary among the species but also between isolates within species. In addition, there are various molds and rots of stored grains caused by a range of weakly pathogenic and nonpathogenic fungi that come into contact with the grain during ripening and harvesting. These tend to be associated with grain, ripened or harvested under wet conditions or harvested at high moisture content. Shriveled seed harvested from crops with a range of diseases may have qualities that differ from grain harvested under optimal conditions.

#### Plant Quarantine Issues

Countries vary in regulations that apply to imported seed for either propagation or commerce. Plant pathogens can be carried either as infections within the seeds (certain smuts, seed-borne viruses), as contaminants on the seed surface (many bacteria and fungi), or on plant debris or soil that may be present as admixtures with grain. To reduce risks, preborder measures such as local import permits and phytosanitary certificates issued by the originating country may be required. On arrival in a receiving country, seed for propagation may be inspected before being grown in quarantine greenhouses or approved isolated field areas. Grain in large quantities for commerce may require special treatments to kill the embryos or may be milled in approved locations away from local cereal production areas.

With the recent large-scale rapid movement of goods and people around the globe even nonseed-borne pathogens can move over large distances as contaminants of traded goods or persons. For example, following the introduction of the wheat stripe pathogen into eastern Australia, probably from Europe, in 1979, there have been at least two further incursions. In 1998, a *Puccinia striiformis* 

form more adapted to barley grass (*Hordeum leporinum*, *H. murinum*), than to wheat or barley was observed for the first time, and in 2002, a distinct pathotype of *P. striiformis* f. sp. *tritici* appeared in Western Australia, which previously had been free of stripe rust. The origins of these new forms have not been established. They are assumed to have arrived on contaminated clothing or footwear.

#### The Hazards of Chemical Control

Many diseases, or the vectors that transmit pathogens, especially viruses, can be controlled effectively with chemicals applied as seed dressings or as foliar sprays at appropriate growth stages. In high-yielding environments, one or more chemical applications are often included as part of a routine management package. Under these circumstances, favorable returns can be achieved even where disease levels appear to be low. As environments become less favorable and yield levels decline, or for lower value grains for fodder, the costs of chemical applications encroach on profitability to the extent that their use on crops with yield potentials below  $2-3 \, \text{t ha}^{-1}$  may be uneconomic. This is a very significant factor of production in major exporting countries such as Australia and Canada with relatively low-average long-term wheat yields, for example, of 1.7 and 2.3 t ha<sup>-1</sup>, respectively. Under these production systems, agronomic and genetic means of disease control are more attractive.

A further major consideration is the increasing emphasis on quality standards and declining chemical residue limits placed on traded grain and grain products. Presumably, the later in growth that a chemical is applied, the greater the likelihood of residues exceeding specified limits, leading to down-grading or blending of grain to achieve specified standards.

#### **Control of Diseases by Plant Breeding**

Many of the diseases of cereal crops have and continue to be controlled by resistant genotypes. Resistance has the advantage of being residue-free and of contributing to lower production costs for the farmer. No cultivar of any crop has resistance to all disease threats nor provides complete resistance under all conditions. Hence, chemicals to target single diseases, or to provide prophylactic protection to a range of threats, are often used, especially under high-yielding conditions. A recurrent problem with resistance has been the emergence of resistance-breaking pathotypes as a result of selection of previously infrequent variants, sexual and/or nonsexual recombination in nonhaploid pathogens, and mutation. Retrospective

genetic analyses of hosts and pathogens in situations where both resistant and susceptible host genotypes, and both avirulent and virulent pathogen genotypes were available showed that expression of resistance is dependent upon the presence of a corresponding gene for avirulence, a gene-for-gene relationship. Assumptions of a gene-for-gene relationship in disease systems where genetic information is lacking permits postulation of resistance and avirulence genotypes (phenotypes) and thereby assists the breeding of resistant cultivars based on gene combinations. However, major gene combinations have usually not provided long-term solutions and breeders have had to continue the search for even more resistance genes, or to seek alternative breeding and deployment strategies. These include genotype (and crop) mixtures, minor resistance gene combinations, and various partial resistances with a history of durable resistance. Nevertheless, some resistances, represented by single genes (*mlo* for powdery mildew resistance in barley, Sr2 for stem rust resistance in wheat) or by more complex resistance sources (flagsmut resistance in wheat), have remained effective over long periods. Resistance to some diseases (e.g., take-all of wheat) does not exist and to others (e.g., crown rot of wheat and barley) only relatively low levels of partial resistance (or tolerance) are available. Nevertheless, such variability can be used very effectively in integrated disease control to improve farm profitability.

#### **Future Prospects**

Cereal crops will continue to be affected by diseases as no breeding program can develop cultivars with acceptable levels of resistance to all diseases under all conditions. Emphasis on resistance is expected to increase as fungicide use and residues become less acceptable. Molecular biology is providing tools for more accurate diagnostics of pathogens at both species and subspecies levels, some pathogenicity factors have been cloned and their gene products identified. In the host species, resistance genes have been mapped and cloned. Mapping information is being used for marker-assisted selection, aimed at developing resistance gene combinations to provide more durable and effective resistances. Genetic transformation will provide opportunities for transferring resistance genes from species that cannot be hybridized with individual cereal species as well as possibilities for synthesizing new genes in the laboratory. Genetic engineering seems likely to provide resistance to diseases for which adequate and effective resistance is currently not available.

See also: Cereals: Grain Defects. Cookies, Biscuits, and Crackers: The Diversity of Products. Contaminants of Grain. Plants: Diseases and Pests. Nutrition: Effects of Food Processing.

## **Further Reading**

Anonymous (1977) A Compendium of Corn Diseases. St. Paul, MN: APS Press.

Anonymous (2001) Crop Protection Compendium. (on CD). Oxon, UK: CAB International.

Doggett H (1988) Sorghum, 2nd edn. UK: Longman Group. Ellis S (1999) Measurement and Control of Black Point. Project Report No. 210. London, UK: Home-Grown Cereals Authority (HGCA).

Harder DE and Haber S (1992) Oats diseases and pathologic techniques. In: Marshall HG and Sorrells ME (eds.) Oat Science and Technology. Madison, Wisconsin: American Society of Agronomy Inc.

Keisling RL (1978) The diseases of barley. In: Rasmussen DC (ed.) *Barley*. Madison, Wisconsin: American Society of Agronomy Inc.

Leonard KJ and Bushnell WR (eds.) (2003) Fusarium Head Blight of Wheat and Barley. St. Paul, MN: The American Phytopathological Society.

Leslie JF (2002) Sorghum and Millets Diseases. Ames, Iowa: Iowa State Press (Blackwells).

Mathre DE (1997) Compendium of Barley Diseases, 2nd edn. St. Paul, MN: APS Press.

Ou SH (1985) *Rice Diseases*, 2nd edn. Kew, Surrey: Commonwealth Mycological Institute.

Summerell BA, Leslie JF, Backhouse D, Bryden WL, and Burgess LW (2001) Fusarium: Paul E. Nelson Memorial Symposium. St. Paul, MN: APS Press.

Ullstrup AJ (1977) Diseases of Corn. In: Sprague GF (ed.) Corn and Corn Improvement. Madison, Wisconsin: American Society of Agronomy Inc.

Weise MV (1977) Compendium of Wheat Diseases. St. Paul, MN: APS Press.

Williams RJ, Frederiksen RA, and Girard JC (1978) Sorghum and Pearl Millet Disease Identification Handbook. Information Bulletin No. 2. Patancheru, AP, India: ICRISAT.

Zillinsky FJ (1983) Common Diseases of Small Grain Cereals: A guide to Identification. Mexico DF: CIMMYT.

#### **Relevant Websites**

http://wheat.pw.usda.gov — Wheat Diseases and Pests: a Guide for Field Identification. Original text by: J.M. Prescott, P.A. Burnett, E.E. Saari, J. Ranson, J. Bowman, W. de Milliano, R.P. Singh and G. Bekele. International Maize and Wheat Improvement Center (www.cimmyt.org). Centro Internacional de Mejoramiento de Maiz Y Trigo. Lisboa 27, Apdo. Postal 6-641,06600 Mexico, D.F., Mexico. HTML version developed with permission from CIMMYT by: J. Wong, USDA-ARS-WRRC, Albany, California;

D.E. Matthews, Dep. of Plant Breeding and Biometry, Cornell University, Ithaca, New York and K.D Kephart, Dep. of Agronomy, University of Missouri, Columbia, Missouri, USA.

http://pdc.unl.edu — Plant Disease Central. J. Stack, UNL Extension Specialist, University of Nebraska, Lincoln. Describes and illustrates diseases of wheat, corn and sorghum.

http://btny.purdue.edu — Crop Diseases in Corn, Soybean, and Wheat. K. Rane and G. Ruhl. Department of Botany and Plant Pathology. Purdue University, West Lafayette, IN 47907, USA.

http://www.ext.nodak.edu — Symptoms and Controls of Crop Disease. PP-533 (Revised), March 1977. M.P. McMullen, Extension Plant Pathologist, H. Arthur Lamey, Extension Plant Pathologist. NDSU Extension Service, North Dakota State University, Fargo, ND, 58078, USA.

## **Grain** — Quality Attributes

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Published by Elsevier Ltd.

## Why Is the Quality of Cereals Analyzed?

Because cereal grains (and the products derived from them) are biological entities and can vary dramatically. Further, the ways in which different samples of the same cereal grain vary can have a profound impact on processing, nutrition, and consumer appeal.

So, if it is important to analyze quality, what is "quality"?

Quality in this context is "the degree or grade of excellence." Quality may also be thought of as *suitability*. Consequently, to understand the analysis of cereal quality, one must appreciate the array of end uses of the various cereals. The major cereals and their uses are presented in **Table 1**. The organization of this chapter follows this general scheme. Feed uses of all cereals are discussed separately. First, some general considerations.

## **General Considerations**

The success of cereals as food and feed crops stems from the ability to store easily the grain produced from them for a long period of time. Compare this feature to fruits, vegetables, and meats. Add to this the productivity and broad adaption of most cereal crops, and their relatively high nutritive characteristics, and it is clear why cereals play a prominent, central role in agriculture. This article covers the analysis of quality of the major cereal crops. Their prominence is evidenced by their production: ~590 mmt (million metric tonnes) annually for wheat, 400 mmt for rice (milled), 590 mmt for maize, 140 mmt for barley, 51 mmt sorghum, 25 mmt for the millets, 26 mmt for oats, 20 mmt for rye, and 10 mmt for triticale.

Cereal quality can be considered at two levels: (1) characteristics of grain lots or samples, and (2) the intrinsic quality of the grain itself which results from an interplay of genetics and the weather, management, postharvest storage, pathogens, insects, etc. The first of these characteristics of grain lots includes not only the intrinsic quality of the cereal grains themselves, but such things as the presence of seeds of other species, seeds of different types of the same species (e.g., soft red wheat contamination in hard white wheat), presence of dirt, stones, chaff, animal filth, or insects. This article assumes that a given lot of grain has been cleaned and is free of these sorts of contaminants. However, in practice, the complete removal of some of these factors is either physically impossible or economically impractical. Consequently, standards for inspecting and grading grain often severely penalize these factors. The second level of quality consideration is at the intrinsic level. As mentioned above, all intrinsic properties result from the interplay of the genetic makeup of the plant and the way in which it interacts with (or responds to) external factors. Some external factors exert a very dramatic effect on quality and the range of effects may be an order of magnitude greater than that observed among a group of varieties. In the other extreme, the expression of some quality genes is essentially minimally affected by external influences such that variation among grain lots is essentially controlled by differences in genes. Between these two extremes is a full range of quality traits that have greater or lesser degrees of genetic versus environmental influence. Table 2 lists a few examples of quality traits that are variously affected by external factors.

Since all of the cereal grains are botanically related, and all of the grains are technically caryopses, some general description of cereal grains can be made. All cereal grains are composed primarily of an endosperm attached to a germ (embryo) via a scutellum, and enclosed in several layers of tissues collectively referred to as the bran. These three different parts have quite different biological roles, and hence quite different compositions and structures. Because of these differences, humans utilize each in markedly different ways. Additionally, rice and most barley and

Table 1 Major cereals and their uses

Common name	Genus and species	Major uses
Wheat		
Bread wheat	Triticum aestivum L.	Many styles of pan breads, hearth breads, noodles, starch and gluten, animal feed
Soft wheat	Triticum aestivum L.	Cookies (biscuits), cakes, pastries, noodles, animal feed
Durum wheat	Triticum turgidum L. subsp. durum Desf.	Pasta, couscous
Rice	Oryza sativa L.	Polished rice (staple food)
Maize (corn, US)	Zea mays L.	
Dent, floury, flint Popcorn		Animal feed, dry milling (production of grits), wet milling Snack food
Sweet corn		As a cooked vegetable
Barley	Hordeum vulgare L.	
Malting barley Feed barley		Beer and brewing Animal feed
Rye	Secale cereale L.	Animal feed, bread
Triticale	×Triticosecale sp. (Wittmack.)	Animal feed
Oats	Avena sativa L.	Animal feed, porridge, snacks
Grain sorghum	Sorghum bicolor L. (Moench.)	Animal feed, food, brewing
Millet(s)	Various species	Food, animal feed

**Table 2** Quality traits in cereals that range from highly to minimally affected by external factors

Quality traits highly influenced by external factors	Intermediate	Quality traits minimally influenced by external factors
Test weight Moisture content Protein content Presence of mycotoxins Preharvest sprouting "Bug" protease (Eurygaster spp.) Frost damage	Seed size Milling quality Dough rheology Baking quality	Seed color Starch composition Protein composition Grain hardness Tannin content

oat varieties are also enclosed in adhering lemma and palea when harvested. These are removed during their processing for human food.

The endosperm is the key feature of cereal grains. For the cereal crops of significance, most have endosperms comprised of starch (60-75%), protein (6-19%), fiber (3-8%), and oil (1-4%). As opposed to most grain legumes, tree nuts, and horticultural crops, cereal crops store most of the energy for germination and seedling emergence in the form of starch. Nitrogen reserves are deposited in the grain in the form of protein "bodies" and "matrix" protein, which surrounds the starch granules and protein bodies.

Cereal germs are relatively rich in oil. In the case of wheat, millers desire to remove the germ because

its oil contributes to rancidity of flour. In maize, the germ contributes to the feeding value (energy) of whole grain and is the source of food oil in the milling process.

The bran and husk (lemma and palea) are of little direct value since they are largely cellulose and are undigestible by humans and most livestock. However, the husk is an important part of the filtration process in brewing with barley malt, and can protect the seed — especially the germ — from mechanical damage during harvest.

#### Wheat

The analysis of the quality of wheat is by far the most complex of all the cereal grains. This complexity reflects the tremendous diversity of the genetics of wheat, the tremendous diversity of the uses of wheat, and the central role that wheat plays in the human diet. Wheat has distinguished itself from all other cereals and grain legumes because of the unique properties of its endosperm storage proteins. These proteins, classified as glutenins and gliadins, when hydrated and mixed (by hand or machine) form "gluten." Gluten is unique in that it exhibits both viscous and elastic properties. These properties allow gluten to form a continuous membrane which can retain the gases produced in fermenting doughs. The retention of these gases greatly decreases the density of doughs, and upon baking, this light, airy structure is fixed.

An almost limitless number of foods are made from wheat. Wheat flour, bran, etc., are also important food ingredients. Table 1 lists the major uses of wheat and flour. The quality of a particular flour is not necessarily low or high until it is judged in the context of a particular end use. For example, a flour that exhibits very high bread baking quality generally also has very poor cookie or cake baking quality. Therefore, wheat quality is defined by an almost infinite number of different food products which contain flour, starch, gluten, bran, whole and cracked grain, etc.

The majority of wheat is first milled into flour, and consequently the miller is in a central and critical position in the utilization of wheat as a food. The miller takes a raw material of biological origin and converts it into a food ingredient for countless numbers of diverse food products. Much of the day-to-day success of the baker is a direct result of the miller's technical ability and expertise. In this regard, the overriding aspect of flour quality is consistency of performance. This endeavor is by no means simple or straightforward. Wheat is a biological entity and as such can vary dramatically over geographical locations, over years and importantly, among different cultivars. In one sense it is the miller's task to remove this variation and thereby produce a consistent product. Flour milling is more extensively presented in Wheat: Dry Milling.

The various categories of wheat foods described in this article include pan, hearth and other fermented (leavened) breads, steamed breads, flat breads and crackers, cookies, cakes, and other soft wheat products, noodles, breakfast foods, and pasta and durum wheat products.

## Fermented (Leavened) Breads

Leavened breads include traditional white pan, variety, hearth and sourdough breads, sweet goods including yeast-leavened doughnuts, and bagels. Rolls are simply smaller-sized products and may be similar to other, larger breads and sweet goods. Leavened breads are characterized by a crumb texture that is light, airy, and porous, yet chewy. One of the primary determinants of this texture is the quantity and quality of gluten protein.

By far the most common type of leavened bread in the West is white pan bread made from straight-grade or patent flours of hard spring and winter wheats. White pan breads require high flour water absorption, medium to strong dough mixing strength, extensible gluten, and good fermentation and mixing tolerances. Large loaf volume and fine, smooth crumb grain are desirable. A smaller version of pan bread is the sandwich bun. McDonald's alone sells over 8 billion sandwich buns per year worldwide, nearly 5 billion in the US. The US portion of sales consumes nearly 200 million kg of flour  $-\sim 1\%$  of all the flour produced in the US. Variety breads are embellished versions of white pan. These breads are prepared using whole-wheat flour and/or as many as a dozen different ancillary ingredients.

Hearth breads were the forerunners of pan breads. Hearth breads are baked on the oven floor, or sole. As a consequence, they range from having an ovate or nearly spherical shape to one approximating a horizontal cylinder. In addition to affecting the shape, the absence of baking pans exposes the entire bread surface to the oven environment. This large, exposed surface area produces the greater crust-to-crumb ratio characteristic of hearth breads. Perhaps the most famous hearth bread is the French baguette. Many hearth breads are characterized by long fermentation and no antistaling additives.

Sourdough bread is similar to white hearth bread except for its characteristic sour, acidic flavor, and extra-chewy texture. Sweet goods, on the other hand, are characterized by rich formulas high in fat and sugar and contain milk solids and eggs. These products are more varied but generally require flours similar to other leavened breads. Products include yeasted doughnuts, cinnamon rolls, coffee cakes, Danish and puff pastries, and French brioche.

Doughnuts are of two general types: yeast leavened and cake. The unique characteristics of doughnuts is their traditional ring shape and the method of frying in hot oil. Yeast-leavened doughnuts are similar to bread in that they are made from yeast-fermented doughs.

Bagels are doughnut-shaped rolls. The word bagel comes from the Austrian—German word *bugel*, meaning stirrup. Bagels were invented over 300 years ago to honor Jan Sobieski, king of Poland and famed equestrian. Bagel doughs are retarded for up to 20 h and then boiled just prior to baking. A traditional style of bagels requires very chewy texture.

#### **Steamed Breads**

Asian steamed breads are simply breads that are steamed rather than baked. As such, they lack the brown crust that is characteristic of oven-baked breads. They may be plain or with a sweet bean paste or savory meat filling. Steamed breads are traditional wheat foods in China, Japan, The Philippines, and other East Asian countries. Two main types are consumed in China: northern style and southern style. Northern style is larger and chewier, and is made from the higher-protein, stronger-gluten wheats of that

region. Southern style is smaller, of lower density, and often sweeter.

#### **Flat Breads and Crackers**

Flat breads and crackers are usually no more than 6 cm and often less than 3 cm thick. Most flat breads are higher in water content than crackers, and are therefore chewy as opposed to crisp. Most crackers have no discernable crumb and crust. The majority of flat breads are consumed in North Africa, the Middle East, and the Indian subcontinent. Major types include "chapati," "roti," "naan," "paratha," "poori," "balady," "pita," and "barabri." These breads typically have high crust-to-crumb ratios and limited crumb. They are generally baked at very high temperatures for very short time periods (e.g., 550°C for 30 s). Flat breads are typically produced from high extraction (75–90%) or whole wheat flours. Due to the higher bran content of these flours, white wheat is preferred for lighter color. Much less gluten strength is required compared to pan and hearth breads for two reasons: (1) the limited crumb does not require much gas-holding capacity, and (2) many of these breads are prepared by hand and weaker gluten is easier to mix and handle. Due to their geometry and structure, tortillas, pizza crust, English muffins, crumpets, and pretzels can be considered flat breads. Tortillas, which are traditionally made from maize, are flat breads indigenous to Mexico, Central America, and the southwestern US.

Pretzels are unique because they receive an intermediate processing step. Two types of pretzels are popular. The first tend to be small ( $\sim 10$  cm), and may be in the characteristic twisted shape or other configurations such as short, straight sticks or rings. These products are baked and then dried to  $\sim 2-3\%$ moisture content and have a crunchy texture and a relatively long shelf life. The second type, soft pretzels, tend to be much larger, up to 30 cm with the baked dough piece diameter  $\sim 2-4$  cm. Moisture content is higher and the texture resembles chewy bread. In addition to the characteristic twisted shape, the feature that sets pretzels apart is the use of caustic lye (usually 1.25% NaOH). The dough pieces are immersed briefly in hot lye solution, baked, and then sprinkled with coarse granular salt.

Pizza crusts in the US are typically made from higher-protein, stronger-gluten flours. English muffins and crumpets are flat breads characterized by a coarse, open grain, grilled top and bottom with light sides, and a flat disk geometry.

Crackers are characterized by thin geometry, low water content, and crisp, crunchy texture. Crackers are popular in North America, Europe, and Australia. Products include soda crackers, cream crackers, water biscuits, graham crackers, sprayed crackers, and savory crackers. Soda crackers, or saltines, are the most definitive cracker product. They are prepared by fermenting a relatively stiff, dry dough. Fermentation is partially accomplished by lactic-acid-producing bacteria. Sodium bicarbonate (hence the name soda cracker) is added to neutralize the drop in pH.

#### Cookies, Cakes, and Other Soft Wheat Products

Soft wheat is used in many diverse food products. In some it is the major ingredient, but in others it ranks second or third. Soft wheat is used in these applications, because (1) the stronger gluten and higher protein levels of hard wheat reduce product quality, (2) soft wheat flours have lower levels of starch damage and consequently lower water absorption and dough viscosity, (3) soft wheat flours generally have a finer texture, or smaller particle-size distribution, and (4) soft wheat is often cheaper than hard wheat and thus reduces ingredient costs. Often a blend of the two types provides an intermediate level of gluten strength. As opposed to hard wheat flour, where the inherent strong viscoelasticity of gluten is the primary reason for its use, soft wheat flour usually has weak, extensible gluten. This lower strength of gluten translates into soft, tender texture of soft wheat products. The major food uses of soft wheat flour are chemically leavened crackers, pie crust, cookies (biscuits), American-style biscuits, scones, Moon Cake, products made from batters (sugar wafer cookies, ice cream cones, pancakes, and waffles), cakes, tempura, breadings, and soup thickeners.

Chemically leavened crackers are similar to fermented crackers except for their method of leavening, which is usually sodium bicarbonate with an acidifying salt. Pie crust has a high fat content and little structure; it is usually sheeted.

Cookies (biscuits) generally have limited threedimensional structure, a high content of sugar and fat, and generally a low moisture content. Most are chemically leavened to reduce product density and impart a desirable texture. The major types of cookies (from lowest to highest water content) are rotary molded, cutting machine, wire-cut, and deposit varieties. In the UK and its former colonies, chemically leavened cookie doughs are classified as shortdough sweet biscuits (sweet biscuits, short doughs, or short sweet doughs) and hard-dough semisweet biscuits. "Marie" and "Rich Tea" are popular examples of hard-dough semisweet biscuits. In these products, gluten is partially developed. Short-dough sweet biscuits may be classified according to the US system.

In rotary-molded cookies the dough is pushed into dies cut on the surface of a smooth cylinder, excess dough is scraped off, and the cookie is released onto a canvas conveyor and moved to the oven. The dies produce three-dimensional raised designs which are maintained after baking. Often, a cream filling is deposited between two cookies (i.e., "sandwich" cookies). Cutting machine cookies are produced from a sheeted dough. Various shapes are cut from the dough sheet and baked. The wire-cut cookie is the most common commercial cookie in the US. A popular variation includes chocolate chips. Wire-cut cookies are produced by passing a taught wire through a stream of extruded dough. Individual dough pieces drop onto a belt and are conveyed to the oven. Deposit cookies are made from higher moisture doughs, some approaching the viscosity of cake batters. Deposit cookies are produced by extruding the dough through nozzles where reciprocating cutters release the dough onto the conveyor, or oven band. Popular variations are the vanilla wafer and Danish butter cookie.

Batter products include some low-moisture, crisp products such as wafer cookies and ice cream cones; griddle cakes such as pancakes and waffles; a vast array of higher volume cakes; and coatings such as tempura. Pancakes are relatively thin, round cakes prepared by cooking batter on a hot griddle or pan. Oil on the griddle surface fries the outer surface of the pancake to a golden brown. Waffles are prepared from similar batters but are cooked in a two-sided griddle which imparts the characteristic threedimensional structure and crisp texture. Where pancakes are fried in a thin layer of oil, doughnuts are completely immersed in hot cooking oil. Cake doughnuts, in contrast to yeast-leavened doughnuts, are made from a formula similar to a standard layer cake which uses both chemical leavening and incorporated air to obtain the desired low product density.

Cakes are baked foams and usually have substantially higher proportions of fat and sugar compared to breads. Cakes derive their light, porous texture through the use of leavening agents such as sodium bicarbonate and/or through the entrapment of minute air bubbles. Like cookies, cakes exhibit little gluten development; gluten development is detrimental to cake quality and imparts an undesirable tough chewy texture. In contrast to cookies that have generally less than 2-3% moisture, cakes are formulated to produce tender, moist textures. Cakes, depending on type, may have amounts of sugar, eggs, and fat in their formula which exceed the quantity of flour. In high-ratio cakes, as the name implies, sugar-to-flour ratios may range from 1 to as high as 1.2. In the case of angel food cake, sugar and egg whites may each exceed flour by a ratio of 2.75. Cakes are made from batters with substantially lower viscosity than cookies or bread doughs. Consequently, the stable entrapment of minute air cells is critical to cake volume and texture. The formation of emulsions with formula fat or egg lipids is crucial for the entrapment of air bubbles and for preventing the establishment of a continuous gluten network. In many cakes, the structure-forming role of egg white or whole egg protein is important. Cakes are often highly flavored with high proportions of chocolate, fruit, and other ingredients. Some of the main classifications of cakes are high-ratio, pound, chocolate, yellow and white layer, sponge, Swiss roll, chiffon, angel food, cup cakes and muffins, and Japanese *castilla* varieties.

Tempura is a Japanese batter used to coat seafood and vegetables prior to deep-fat frying. Breadings, like tempura, are used to coat foods and often to impart a crunchy texture. Soup thickeners are used to provide a high viscosity to soups. Therefore, starch plays the most important role and flours must be free of amylases associated with preharvest sprouting.

#### **Noodles**

In this article, differentiation is made between *noodles* prepared from common, or hexaploid, wheat flour, and *pasta* prepared from durum semolina. Noodles may be generally defined as boiled "strings" of unleavened wheat dough. However, this definition has many exceptions and caveats due to the tremendous diversity of noodle products. Most noodles are produced and consumed in eastern Asia, and as such, their use runs parallel to bread consumption in the West. Noodles are the traditional form in which wheat is consumed and predates recorded history. In addition to rice, noodles form the foundation of the carbohydrate-based diets in that part of the world.

Although tremendously diverse, the vast majority of noodles are prepared from very simple formulas: flour, water, and salts. Because of this, wheat flour quality is particularly critical in determining noodle quality and consumer acceptance. For the purposes of classification and discussion, noodles may be grouped according to formulation (primarily type of salts), noodle geometry, method of preparation, postpreparation processing, method of packaging, and manner in which they are consumed.

Based on formulation, noodles may be classified as white salted, alkaline, "soba," or egg. Additionally, ramen or instant noodles are noteworthy. A number of noodle-like sheeted products include egg roll, pot sticker, and won ton wraps.

The three main features of white salted noodle quality are color, appearance, and texture. Noodle color should be clear and bright, not dull or dark. The leading factor in good color is the absence of discoloring enzymes called polyphenol oxidases. The noodle should appear smooth with a glossy surface and should have square, well-defined edges rather than rounded ones. Texture and biting characteristics vary according to cultural preference: for Japanese udon, the texture should be soft and elastic. For Chinese Mandarin ("gan mian") and Cantonese ("kon mien"), the texture should be firm and chewy. Wheat and flour quality characteristics that contribute to these desired quality traits include good milling characteristics with ease of bran separation, bright endosperm, and flour color. For udon noodle, weak but extensible gluten, relatively high starch paste viscosity, low amylase activity, and lower starch amylose content are desirable. Usually soft white wheat is preferred. For white salted Chinese noodles, high-protein, stronger gluten flours are preferred, similar to those used in bread. In this case a normal level of starch amylose contributes to a firmer texture.

Alkaline noodles, as the name implies, are prepared using alkaline salts. A typical formulation would include 1-3% NaCl and 1-3% alkaline salts (flour weight basis). Alkaline salts, often referred to as kan sui ("can soo-ee," or "can-swee"), may be comprised of combinations of potassium and sodium salts of carbonate (K2CO3 and Na2CO3) and/or sodium hydroxide. Like white salted noodle, the main quality attributes of alkaline noodle are color, appearance, and texture. Color should range from creamy white to yellow. Appearance should be bright, not gray or dull, with as few specks as possible. The color should be stable and should not deteriorate during storage. Texture should be elastic rather than weak and soft, and should not soften or deteriorate during storage. Milling quality, endosperm color, gluten characteristics, and starch pasting quality all contribute to alkaline noodle quality. Alkaline noodles are typically prepared from higher-protein, stronger-gluten hard wheats, similar to those used for bread.

Two popular variations on the basic, simple formula include buckwheat and egg noodles. Buckwheat noodles, called soba, are made from wheat flour and buckwheat (Fagopyrum esculentum Moench.): about three parts wheat flour to one part buckwheat flour. Egg noodles, as the name implies, are prepared using eggs, and may be prepared using whole fresh eggs as the only source of formula liquid. In the US, dry egg noodles contain at least 5.5% egg solids.

Convenience is an important force that drives consumer spending. In the US, Japan, and Korea instant noodles which have been fried in hot oil, cooled, and

packaged provide a quick and easy meal. Some are packed in ready-to-serve cups or bowls and almost all come with a seasoning sachet.

#### "Ready-to-Eat" Cereals and "Breakfast" Foods

Although many rolls, breads, noodles, etc., are eaten at the first meal in the morning, "ready-to-eat" (RTE) cereals and breakfast foods are popular in many parts of the world. RTE foods are cereal-based foods which require no cooking. Generally flaked, extruded, steamed, or shredded processing gelatinizes starch, denatures protein, and develops texture. Most of these foods are very low moisture content and consequently can be stored for long periods of time. Popular brands in the US are produced by General Mills, Kellogg's, Nabisco, Post, and other companies. These and similar products are made from wheat, rice, corn, and other cereals. Soft white wheat grain with large kernels is preferred for easy flaking and better color after roasting. More traditional wheat-based "breakfast" foods include products similar to oat meal-porridge and other cooked cereals.

#### **Pasta and Durum Wheat Products**

Alimentary paste, or "pasta," is prepared from extruded dough pieces of various shapes and sizes, usually dried during production, and later boiled and consumed. A common classification is based on whether the pieces are long or short (e.g., spaghetti versus macaroni). In contrast to the noodles described above, pasta is prepared from durum (Triticum turgidum var. durum) semolina. Although Italian in origin, pastas – such as spaghetti, macaroni, lasagna, and fettuccine – are now a common part of culture and language in the US and worldwide. Some pasta products contain fillings, such as tortellini and ravioli with cheese or meat.

The production of semolina is the primary goal of durum wheat milling. Semolina is simply larger-sized pieces of endosperm (compared to flour), free of adhering bran. Semolina size ranges from about 130 to 550 μm. In addition to the particle size of semolina, color, vitreousness, and protein quantity and quality are key quality traits. Higher levels (>13%) of strong gluten are preferred. The protein must form a continuous matrix to entrap the starch granules so that the pasta surface does not become sticky during cooking. In most cases, the addition of hexaploid wheat to pasta is considered undesirable and is prevented by law in some countries.

As mentioned earlier, pasta is an extruded product. Semolina is first mixed with water in an approximate ratio of 30:100 (water: semolina) into a stiff dough. The dough is forced at high pressure through a die to produce the desired shape and size. The pasta is then collected and dried. Mixing, kneading, and drying are critical steps to successful pasta manufacture.

Other nonpasta durum wheat products include primarily bread, couscous, and bulgur. Durum bread is prepared from durum flour rather than semolina. The salient differences between typical hexaploid and durum wheat flours are coarser particle size, higher starch damage, higher water absorption, strong gluten with low extensibility, high dough stability, and yellow color of durum flour. Due to the high levels of damaged starch and water absorption of durum flour, durum bread stales much more slowly and consequently has an extended shelf life, compared to hexaploid wheat bread.

Couscous and bulgur are precooked, unleavened foods traditionally consumed in North Africa and the Middle East. Couscous is prepared from semolina by hydrating, mixing, steaming, drying, and size-fractionating the resultant particles. Couscous is then rehydrated with oil and meat and/or vegetable sauce when eaten. Bulgur, on the other hand, is prepared from whole or cracked kernels by soaking in water, parboiling, drying, and grinding. Prior to eating, bulgur is boiled or steamed.

#### Starch and Gluten

Wheat starch and gluten each have unique compositions and properties. Consequently, it is useful to isolate each in relatively pure form. Separation is accomplished through one of the three general methods: Martin and batter processes, and differential centrifugation. All three typically employ roller-milled flour, usually harder, higher-protein, stronger gluten wheats. The Martin process mixes flour and water into a dough and then washes out the starch and water-soluble constituents. Usually, NaCl is included to promote gluten agglomeration. Small aggregates of agglomerated gluten are recovered by sieving. In both the Martin and batter processes starch is recovered through centrifugation. Differential centrifugation separates nonaggregated gluten from starch, the main advantage being reduced wash water. Once separated, most gluten is dried and sold as a freeflowing powder.

The primary reason for starch—gluten isolation is to obtain vital gluten. "Vital" refers to the fact that the isolated gluten retains its functional viscoelastic (and therefore bread-improving) properties. The main use of vital gluten is in yeast-leavened bread products, especially variety breads where the load of nonfunctional ingredients is relatively high and flour

protein is diluted. Vital gluten helps maintain product volume and internal structure. During isolation, controlled drying is key to maintaining the functionality of vital gluten. Excessive heat denatures gluten and renders it nonvital. Nonvital gluten, though of little value in baking, does find application in pet foods and other uses.

In the US and other countries with large quantities of maize, wheat starch is of minor importance, gluten being the primary reason for starch—gluten separation. In countries such as Australia, however, starch is an important co-product. After isolation, starch is usually further processed into sugar syrups, ethanol, and specialty modified starches. In this regard, wheat starch use parallels maize.

## **Assessment of Flour Quality**

The main reason for assessing flour quality is to predict its performance in commercial applications – either processing traits or end-product traits. Assessment of flour quality can be classified into two categories: end-product tests and component tests. Both have their own merits and are described below. End-product tests tend to produce a summation of quality – the sum total of all the components of quality as well as their interaction, if any. For this reason, end-product tests are generally considered the best predictors of commercial end-product quality. However, end-product tests usually require more personnel, time, flour, and equipment compared to component tests. Component tests tend to assess one or more fundamental property or component of flour, such that end-product quality may be predicted. The advantage of component tests is that they are generally conservative of resources, quick, and amenable to large numbers of samples. Component tests also have the advantage of more precisely identifying why a particular flour may have better or poorer quality. The main shortcoming of component tests is their limited ability to capture all aspects of commercial end-product quality.

#### **End-Product Tests**

End-product tests typically use a scaled-down, semi-, or nonautomated procedure that mimics the large-scale industrial process. Product formula may match exactly that of the commercial product or may be a simplified, standardized test product. Commonly used standardized end-product tests include pan bread, cookies, and pasta. Additionally, laboratory-scale end-product tests have also been developed for steamed bread, flat breads, bagels, and noodles. Flour quality is assessed on the characteristics of the

prepared product, e.g., the volume and internal crumb appearance of bread loaves, the diameter of cookies, and the cooking loss of noodles and pasta.

#### **Component Tests**

The use of component tests for flour quality assessment is founded on the premise that end-product (or processing) quality can be predicted from one or more attributes of the flour. Beyond predicting end-product quality, there is the practical necessity of determining the processing properties of flour so that plant production runs smoothly and efficiently. End-product quality and processing quality are closely linked.

It is the inherent, fundamental physical and chemical properties of wheat flour that make it the single most important and diverse food in the world's diet. It is also these fundamental properties that are characterized, and by doing so, the processing and end-product quality of a flour is predicted. In this context, these properties, or components, of wheat flour may be grouped as protein, starch, water relations, and color. Each of these will be dealt with following.

The quality and quantity of wheat flour is an important basic property. As discussed earlier, it is the viscoelastic nature of gluten that allows gases produced during fermentation to be trapped in the dough, thereby increasing the volume and producing the appealing texture of bread. Likewise, it is these same gluten proteins that can impart a tough or undesirably chewy texture to many cakes, cookies, and pastries.

The quantity of protein is often important to predicting end-use quality. Two main approaches are most common: determination of elemental nitrogen with an empirical conversion to protein, and an empirically derived prediction of protein based on spectroscopy using specific wavelengths of light in the near-infrared (NIR) region. The techniques include Kjeldahl and combustion methods of elemental nitrogen determination, and NIR reflectance and transmission spectroscopy.

The quantitative assessment of protein "quality" or gluten functionality is substantially more challenging and usually empirical. Standard methods exist for the determination of gluten contents (not all protein is gluten and gluten is not all protein). Two other approaches to gluten quality include dough mixers and machines that record and characterize the elasticity and extensibility of doughs. In the first category are the Mixograph and Farinograph. In the second category are the Extensigraph and Alveograph. Recording dough mixers produce a curve from which assessments of gluten hydration, development, strength, and stability can be derived. Additionally, an assessment of optimum flour water absorption can be made. The extensibility of gluten in doughs is assessed by the Extensigraph by uniaxially stretching a dough piece to the point of failure. The Alveograph biaxially stretches a dough piece by blowing a bubble using air pressure. The bubble volume is likewise increased to the point of failure.

Starch can undergo profound physical-chemical changes. These changes relate primarily to the events known as gelatinization and gelation. During the gelatinization process, starch can interact with several times its own weight in water, and in doing so forms a gel structure. The characteristics of this gel are critical to the structure, texture, and quality of many foods. In many instances such as soup thickeners, the concomitant increase in viscosity associated with gelatinization is the main purpose for including wheat flour as an ingredient. Conversely, the hydrolysis of starch to simple sugars provides a ready source of fermentable carbohydrates for yeast fermentation.

Wheat flour is  $\sim 75-80\%$  starch on a dry weight basis. The starch content of flour can be measured directly using specific starch-degrading enzymes. The functional performance of starch—starch quality, primarily the gelatinization, gelation, and viscosity properties, is usually of much greater interest than the quantity of starch. Two common methods available for assessing starch quality are the Brabender Visco Amylograph and the Newport Scientific Rapid Visco Analyzer (RVA). Both work on starch—water or flour-water slurries and record viscosity as a function of resistance to stirring during gelatinization and gelation.

In wheat, the factor primarily responsible for differences in starch quality is the ratio of amylose to amylopectin. In hexaploid wheat, three genes code for the enzyme responsible for the biosynthesis of amylose (this enzyme is termed "granule-bound starch synthase"). When all three copies of the gene are present, a "normal" amount of amylose is present - $\sim$ 23–27% (starch basis). When all three are absent, amylose is less than 1% and the grain is referred to as "waxy" (Figure 1). The presence of one or two genes produces an amylose content of about  $18 \pm 2\%$  and  $21 \pm 2\%$ , respectively. Very small changes in amylose content are associated with dramatic changes in starch functionality.

Sometimes the functional performance of starch has less to do with starch quality per se as it does with the presence and activity of starch-degrading enzymes. These enzymes may be of two sources: endogenous, the result of preharvest sprouting, or exogenous, in the form of malt flour or commercial fungal sources which are often added to pan bread



**Figure 1** "Waxy" (left) compared to "normal" (right) amylose-content wheat starch gels. Equal amounts of starch of each type were heated (*c*. 95°C) with water under constant stirring in a Rapid Visco Analyzer and then allowed to cool to room temperature. Waxy starch contains no amylose and produces gels which are more translucent, weaker, and viscous.

formulas. The Visco Amylograph and RVA can assess existing starch damage and can also assess potential *in situ* starch damage which might occur during processing. Similarly, they can assess the potential effects of added amylase. The "falling number" test is widely used to estimate sprout damage and is based on viscometry during gelatinization. A weighted plunger is allowed to fall through a gelatinizing sample of ground grain or flour. Aimed primarily at assessing sprout damage, the method also incorporates inherent differences in starch gelatinization properties.

Starch quality and performance are also affected by mechanical starch damage. As noted previously, damaged starch absorbs more water and is more susceptible to enzymatic attack. Although the level of starch damage is highly influenced by grain hardness, milling procedures have a major impact and different mill-streams will differ markedly in damaged starch. Quantitative measures of starch damage rely on the susceptibility of granules to  $\alpha$ -amylase attack or ability to bind  $I_2/KI$ .

The third fundamental aspect of flour functionality relates to water relations. Even though gluten hydration during mixing and starch gelatinization are major contributors to water relations, water relations are often examined independently because (1) there are additional, often poorly characterized sources of water uptake, and (2) often the simple sum total expression of these various components is of immediate interest to the food processor.

Assessment of water relations can be made by observing properties of doughs or from the way in which flour interacts with excess water or various solvents. As noted above, most of the methods used to assess gluten strength also provide an estimate of the optimum hydration level of a dough. "Solvent" tests now include methods which employ water,

ethanol—water, sucrose—water, and Na-carbonate—water. The amount of solvent retained by the flour after hydrating and centrifuging is diagnostic of flour constituents.

Finally, color is an important consideration, because food must be appealing to the eye as well as the other senses. Color in the sense of end-product quality may be considered in the context of the production or maintenance of desirable color or the prevention or reduction of undesirable color. A few examples are the golden-brown color of bread crust which results from Maillard reaction, the bright yellow color of pasta, and the prevention of darkening of Asian noodles.

Although visual assessment of color is often satisfactory, the development of hand-held reflectance colorimeters has made triaxial quantitation of color common (Figure 2). The importance of inherent color systems associated with wheat and flour varies, depending on the particular end product. For example, the whiteness of a soft wheat flour may be crucial to the appeal of an angel food cake, whereas it may matter little in a chocolate cake. Two products where color is especially important are pasta and noodles. Pasta requires high levels of yellow pigments to impart a bright, deep yellow appearance. Loss of color during boiling should be minimized. Noodles, on the other hand, must be bright, not dull and should be creamy white at neutral pH and yellow at alkaline pH. Off-colors are unacceptable and generally result from chemical or enzymatic changes. Due to the importance of noodle color (especially brightness), and due to the tremendous range in noodle brightness produced from different wheats, noodle brightness has received considerable attention. A leading culprit in the loss of noodle brightness is the enzyme polyphenol oxidase (PPO). Small-scale



Figure 2 Hand-held color meter used to objectively measure food product color in the triaxial color space  $(L^*a^*b^*)$  system. When analyzing raw noodle sheets, it is critical to use a standardized color tile for background, as shown.



Figure 3 Small-scale assay for assessing PPO levels in wheat grain. PPO activity is associated with noodle darkening; consequently lower levels of PPO are preferable and selected for. Shown are six small (2 ml) tubes which contain 1.5 ml of buffered L-dopa solution and five wheat kernels. The apparatus rotates the samples slowly for 30 min after which the amount of pink product (produced by PPO) is measured spectrophotometrically or judged visually.

assays exist that quickly assess PPO levels in grain (Figure 3).

Lastly, and beyond these four major categories of component traits, there are specific traits unique to certain foods or processes. Additionally, there are three practical considerations that are highly dependent on milling and on whether the wheat is hard or soft. These are flour moisture content, ash content, and particle-size distribution. Flour in the US is targeted to 14% moisture content and there are several methods for its accurate measurement, similarly ash. Ash is an old but extensively used method

of assessing milling extraction and therefore things such as protein quality and color. The central part of the kernel is low in ash while the bran is high. Flour from the central endosperm generally contains more functional protein (better protein quality) as opposed to flour from near the bran or outer portion of the kernel. Similarly, bran often contributes undesirable colors. Soft wheats produce flours of smaller particle size and lower starch damage; durum wheats are at the other end of the scale, producing coarse semolina. Hard hexaploid wheats are intermediate.

# **Intrinsic Quality of Wheat Grain and Grain Lots**

Grain hardness (kernel texture) is arguably the single most important aspect of wheat utilization (Figure 4). Durum wheats lack the genes responsible for softness and consequently are very hard, whereas hexaploids may be soft or hard, depending on the allelic state of these genes. Also, overlaid on this genetic system is the variation due to the environment. Measures of grain

hardness are empirical but commonly used. A physical method, referred to as particle-size index (PSI), relies on the fact that upon grinding, soft wheats produce finer meals and flours. By sifting and weighing, wheats may be classified as soft or hard, or assigned a quantitative numerical value. More recent developments in the assessment of grain hardness include an NIR spectrophotometric method and a single kernel crushing device, the Perten SKCS 4100 (Figure 5).



Figure 4 Cookies baked from soft (left) and hard (right) wheats which differ only in their Hardness gene. Near-isogenic soft (Ha) and hard (ha) wheat lines were milled and their flours baked using a standard AACC cookie formula.



Figure 5 Perten Single Kernel Characterization System SKCS 4100. This instrument weighs individual wheat kernels before crushing them between a rotor and load cell-mounted crescent. The load cell interprets the crush characteristics as a quantitative measure of kernel hardness. Kernel outer diameter and moisture content are also measured. The computer monitor shows the four different stepped histograms of weight, diameter, hardness index, and moisture. The standard method measures 300 kernels in less than 5 min.

In addition to hardness, kernel mass and morphology affect end-use quality. Generally, millers prefer uniformly large, well-filled (plump) kernels. The measurement of kernel mass is typically expressed in terms of thousand kernel weight, whereas kernel morphology is approximated using bulk density (hectoliter or bushel weight). The Perten SKCS 4100 device provides measures of kernel weight and outer dimension, as well as hardness and moisture.

Protein quantity and quality were discussed above for flour. Since there is a strong and direct relationship between flour protein and grain protein, assessing these traits in grain is often desirable. The quality of protein is primarily controlled by genetics. The quantity of protein, however, is highly influenced by the environment including cultural practices, most notably nitrogen fertilizer. A range of 7-17% protein is possible within one cultivar. The methods of determining grain protein are similar to those employed for flour. Whole-grain NIR methods are particularly convenient and obviate the need for grinding.

Moisture content determines the storability of grain, the relative concentration of other kernel constituents (e.g., protein), and the amount of additional water needed during tempering before milling. For safe storage, wheat must have no more than 12-13% moisture content. The reason grain moisture is critical for safe storage relates to the growth of various molds. In this regard, moisture, time, and temperature all interact to affect grain storage.

An intrinsic quality trait of grain is soundness or sprouting. Preharvest sprouting occurs when rains and high humidity coincide with grain maturation and delayed harvest. The reason sprouted grain is often considered to be of inferior quality relates to the presence of carbohydrases, proteases, and other hydrolytic enzymes normally associated with germination. The two main methods of assessing sprout damage are by visual inspection of kernels (noting pericarp rupture over the embryo or other signs of germination) and the falling number assay (see above). Other methods used for flour or starch, or specific to the detection of individual enzymes are amenable, though not commonly used for the analysis of grain.

Finally, the quality of grain and grain lots may be reduced by the presence or prior activity of molds, insects and rodents, seeds of other species, nonmillable material, stones, etc. The presence of pesticide residues is increasingly an important issue. The presence of mycotoxins produced by Fusarium fungi and proteases resulting from Eurygaster insect are of particular concern to wheat.

#### Rice

Rice is the third-leading cereal in terms of world production and is the staple food for over-half the world's population. The rice kernel, when harvested, has an adhering lemma and palea (husk), and is referred to as "paddy" or "rough" rice. The husk is removed during milling producing what is known as "brown" rice - the "naked" kernel has the brown bran layers present. "Polishing" removes the bran and produces the resultant white "polished rice" that is commonly eaten after boiling.

Botanical classification of rice includes *japonica* (short grain) and *indica* (long grain), although a range of kernel lengths is observed. In the US, short-, medium-, and long-grain rice varieties are developed, each with a specific set of quality criteria. Rice is classified also according to amylose content, gelatinization temperature, and whether it is "aromatic" - indicating that it additionally has desirable aroma components.

Rice quality is assessed in terms of grain appearance, milling quality, and cooking quality. Kernel size and shape, length, length/width ratio, whiteness, gloss, translucency, uniformity, and moisture content are quality criteria applied to rice grain. Rice milling removes the hulls, bran, and germ while minimizing kernel breakage. The majority of rice is consumed in polished form, even though removing the bran greatly decreases the nutritional content of the grain. Milling quality is based primarily on the yield of whole grain polished rice ("head rice"). Broken grain is usually worth only about half as much as whole grain. Breakage results from primarily checking and chalky grain. Checking results when harvesting and threshing are delayed or when drying is too rapid and fissures develop in the kernel.

The preferred texture and flavor and other cooking characteristics of rice vary among consumers. Usually short- and medium-grain varieties become somewhat sticky upon cooking, whereas long-grain rice is harder and maintains the integrity of individual kernels. Generally, cooking quality is evaluated directly with measures of water uptake, optimum cooking time, volume expansion, solids loss to the cook water, etc. Stickiness and other eating quality parameters are evaluated by sensory analysis directly or via instrumental analysis.

Due to the inherent subjectivity and timeconsuming nature of these cooking and eating quality tests, predictive tests have been developed and are extensively used. These often attempt to relate chemical and physical properties of the grain to the desired subjective characteristics of the cooked rice. The two most important physico-chemical properties related to cooking quality in rice are gelatinization

#### Maize

The vast majority of maize produced worldwide is used as animal feed (Table 1). Consequently, that use is reviewed below. Of the remainder, sizable quantities are used in the wet and dry milling industries, which are described below. In addition, some maize is ground directly for human consumption, and some is ground simply as a preparatory step in starch conversion for ethanol production. In the Americas, maize has long been a staple food, and a traditional means of preparing maize is through the production of masa, wherein maize is steeped in lime water, ground, and the majority of bran is separated and discarded. White maize production is estimated at 65-70 mmt and is used mostly for food. For all food uses, common quality criteria include mold and mycotoxin concerns and the general physical health of the grain.

Maize is a single species but has several important kernel types resulting from simple genetic systems. By far the most important commercially is yellow dent. Other types include flint, flour, pop, sweet, white, and pod. Some endosperm mutations which involve alterations in starch biosynthesis include the sweet corns, high amylose, and waxy maize.

# **Wet Milling**

About 10% (or 50 mmt) of the annual worldwide production of maize is used in the wet milling industry, of which about half occurs in the US. The primary aim of wet milling is the recovery of starch which is used to make sweeteners, ethanol, modified and unmodified industrial and food starches, etc. Wet milling, as the name implies, involves steeping maize kernels in water and sulfur dioxide to loosen the bran and germ from the endosperm. The germ is recovered and used to isolate oil; afterwards the remaining germ tissue is approximately one-fifth protein and is valuable as a feed product. Starch is separated from cell wall material (fiber) and endosperm

protein (gluten) through a process of grinding, hydrocyclones, and filtration. Yield of starch is  $\sim 65-68\%$ , fiber 11–13%, germ 7–8%, gluten meal 5–6%, the remainder steep water and loss.

Maize for wet milling should have a high starch content and be low in mycotoxins. Kernels should be well filled; low test weight indicates poor grain filling, whereas high test weight indicates that kernels have a high vitreous to starchy endosperm ratio. Vitreous endosperm, such as that found in flint maize and a portion of yellow dent maize, is undesirable due to the slower steeping and processing time required. Mechanically damaged and broken kernels are also undesirable. Lastly, the drying regimen of maize after harvest is critical to high-quality grain – too high and steeping rates drop, grinding is more difficult, and there is a loss of protease activity and starch release. Endogenous proteases, along with sulfur dioxide, contribute to starch release. Some waxy maize (having 0% starch amylose) is grown on contract for the wet milling industry.

### **Dry Milling**

The primary aim of dry milling is the production of flaking grits - the most valuable product. Flaking grits are large pieces of vitreous endosperm. Naturally, not every maize kernel yields a couple flaking grits and even those that do also produce some quantity of smaller pieces of endosperm. Depending on the size of the particle, it may be classified as coarse or regular grits, or meal or flour. To maximize the yield of flaking grits, the maize kernel is tempered for a short time to loosen the bran and germ (to  $\sim 18$ – 24% moisture and less than 1 h). The first stage of the grinding process is to remove and recover the germ. Oil is generally extracted from the germ as a co-product; the remaining tissue is rich in protein and is used as animal feed. All of the unrecoverable material from dry milling, primarily bran recovered by aspiration and spent germ, becomes "hominy feed."

The primary quality criteria for dry milling is a high proportion of vitreous endosperm in a yellow dent variety, free of mold, and mycotoxins. Stress cracks from poor drying are undesirable because they reduce the recovery of grits. Even though flint maize is entirely vitreous, it is actually less desirable for dry milling because its more spherical kernel morphology is not conducive to producing grits.

#### Popcorn and "Sweetcorn"

All maize kernels have some propensity to "pop" – i.e., explode when superheated steam inside the lumen of each starch granule reaches sufficient pressure to

cause the pericarp to suffer a catastrophic failure. However, specific varieties of popcorn have been bred to maximize these alluded to physical phenomena. Popcorn kernels tend to be nearly spherical and uniformly vitreous endosperm. The pericarp must be sufficiently strong to facilitate a high internal pressure before failing. However, once popped, the pericarp is undesirable from the consumer standpoint and therefore its thickness is held to a minimum.

Quality criteria include the lowest possible levels of mold, mycotoxins and insect damage, hard endosperm with high test weight, and high popping volume with low percentage of nonpopped kernels.

"Sweetcorn," sweet maize, is simply vegetable corn that is eaten prior to full kernel maturity; its flavor is enhanced through a number of endosperm mutations that increase the sugar content at the expense of starch biosynthesis. Sweetcorn may be eaten as "corn on the cob" after boiling or cooking, or as a canned or frozen vegetable. Hominy is a maize product produced from white- or yellow-kernel maize using alkali to remove the bran. It is popular in the southeastern US.

# **Barley**

Barley has three distinct end uses: feed, alcoholic beverages, and human foods. The vast majority is used for feed followed by malt and lastly food. About 75% of the world's barley production is used for feed, whereas only  $\sim$ 5% is consumed as food. Morphologically, there are two major types of barley, two row and six row, which refer to the number of vertical rows of kernels on the spike (ear). Two-row barley tends to have plumper, more uniform kernels, and therefore may have an advantage in malting. Sixrow barley tends to yield better. However, these generalizations have many exceptions depending on the region and the past history of breeding. Speciality types of barley include hull-less, high amylose, and waxy. Feed barley quality is reviewed in the section given below.

Malt quality is related to kernel size uniformity, kernel plumpness, and color. Additionally, protein content and germination rate and uniformity are critically important. Germination should be preferably over 95%, approaching 100%. Like all cereals, there is a concern for microbial contamination including mycotoxins. Naturally, the grain lot should be free of extraneous material, weed seeds, stones, filth, etc.

Generally, barley malt is made from pure varieties, specifically bred for malt production. Occasionally a carefully controlled blend of two malt varieties may be used. The two leading roles of barley malt in brewing are to supply a high quantity of hydrolytic enzymes produced during the germination process, and a high level of "extract" which provides the fermentable substrates during brewing. The hydrolytic enzymes break down the starch, protein, and  $\beta$ -glucan during fermentation. The amount of soluble extract is directly related to the amount of beer that can be produced from a given quantity of malt. A negative correlation exists between barley protein and the amount of extract produced from malt. Malt should additionally have low levels of undesirable compounds such as  $\beta$ -glucan, polyphenols, and dimethylsulfide precursor.

The most common method for testing the potential malting quality of a barley sample remains the malting of the sample. Micro- and pilot malting procedures are used. Afterwards, analyses typically measure the amount of extract that a malt will deliver into solution under standard conditions, amount of solubilized protein, and amount of enzymes. Other useful tests measure moisture and protein contents, germination, kernel size, and plumpness. Varietal purity is also of interest but is technically more challenging. Currently, some major exporters are testing and verifying the varietal composition of barley malt shipments using polymerase chain reaction which can exploit unique DNA sequences.

The leading use of food barley is pearled barley kernels added to soup. Minor uses include barley flour, rice extenders (Japan), and couscous. Barley for pearling should have white endosperm color, colorless aleurone, and should be uniformly floury or vitreous (not a mixture).

#### Sorghum and Millets

The US is the leading producer of grain sorghum, followed by India and Nigeria. In the US essentially all sorghum is used as animal feed, similar to maize. Feed use is covered in the section given below. In other parts of the world, sorghum is an important food. Sorghum is dry-milled into grits and flour for baking, brewing, porridge, snack foods, malt, and other minor uses.

A key quality trait in sorghum is the presence or absence of tannins and pigments in the outer layers of the kernel. Pericarp color is controlled by two genes and confers brown, yellow, or red colors. Testa color is controlled by one gene and can be brown or purple. Yellow endosperm cultivars have a high level of carotenoid pigments. Pericarp thickness affects how these various kernel colors are perceived.

Tannins and other phenolics found in some sorghum cultivars are distinctly antinutritional. However, these compounds afford some protection against insects and birds, features that outweight their disadvantages in some locales. Nevertheless, white sorghums generally produce higher-quality foods. Quality criteria are similar to maize and include kernel hardness, density, and ease of pericarp removal.

Millets include a number of distinct grass species and are generally grown in harsh, dry climates by subsistence farmers. India and Nigeria are the leaders in millet production. Common type names include pearl, foxtail, finger, and proso millet. Most are consumed after decorticating by hand in a large mortar and pestle. Consequently, pericarp removal should be easily achieved and the endosperm should be hard and vitreous; color should be white with low pigment and tannin contents.

#### **Oats**

Oats, like rice, are harvested with an adhering husk (lemma and palea) which is removed during processing. Oats notably have a high oil content (6-8% lipid)in the endosperm compared to  $\sim 1\%$  for wheat), and are rich in  $\beta$ -glucan. Most are used for feed; an estimate in the 1990s placed food usage at  $\sim$ 25% of world production. The primary food uses of oats are rolled oats, steel-cut groats, quick oats, oat flakes, oat bran, and oat flour. Because of the high oil content and the presence of lipid-degrading enzymes (lipase, lipoxygenase, and peroxidase), oats and oat products are heat-treated to inactivate these enzyme systems. Rolled oats are produced by passing whole groats through steel rollers which produce flakes. Steel-cut oats are simply groats cut into two to four pieces. The pieces are in turn generally also flaked, producing quick oats and oat flakes.

The primary food use of oats is a hot porridge. Consumer interest in convenience has generated "instant" forms of this traditional food. Oats are also used to make "breakfast" cereals, perhaps the most notable in the US is the extruded product "Cheerios." The primary quality criteria for oats include presence of other cereals in grain lots, especially barley, because the barley kernel is so similar that it is very difficult to remove. Barley contaminates the oat product with its hull, which is highly objectionable to consumers. Other criteria include test weight, percentage of hulls (groat yield), presence of mold and mycotoxins, "thins" (kernels that pass through a 1.98 × 19.05 mm screen), and free fatty acid content (an indicator of prior kernel damage, poor storage, etc.).

#### **Rve and Triticale**

Most rye is produced in Northern Europe and Russia where it remains a traditional, staple food. Russia,

Poland, and Germany produce over 70% of the world's production of rye. Production of triticale, which is a man-made species derived by crossing wheat and rye, parallels rye. Germany and Poland are the leading producers and account for over 50% of world triticale production.

The unique feature of rve is its high pentosan content. Pentosans impart high water-holding and viscosity to the dough which allow rye to be baked into bread. Rye is essentially the only cereal besides wheat which has traditionally produced a yeastleavened loaf of bread. Rye bread, however, is very dark and very dense (consumers choose rye bread for these specific attributes). Often rye and wheat flours are blended to prepare "light rye" and "dark rye" breads. The primary quality criteria of rye are similar to other cereals, absence of mycotoxins and molds. Additionally, because of rye's biology and area of production, sprouting and ergot are primary concerns. Triticale is essentially an animal feed. When triticale does find its way into human foods, its quality criteria are similar to those of rye and wheat.

#### **Feed Uses of Cereals**

According to recent estimates,  $\sim 16\%$  of world wheat production is used for animal feed. For rice, the percentage used for feed is less than 3%, whereas for maize the figure is 67%. For the other cereals, feed use ranges from 75% for barley, 50% for sorghum, and 7% for millets.

In terms of animal feeds, cereal grains are primarily a source of energy. Since energy is the most expensive component of feeds, cereals play a central role in rations, especially those for monogastric animals such as poultry and swine. For the major cereals, metabolizable energy (ME) ranges from about 11 to 14 MJ kg<sup>-1</sup>. Over 80% of this ME comes from starch.

Cereals also make an important contribution to protein needs as well. Although cereals may contribute over one-third of the protein required in swine and poultry rations, they are all notoriously deficient in the amino acids lysine, methionine, and threonine. Alternative sources of protein, such as soybean meal, which include higher relative quantities of these amino are required.

Various strategies have been developed to address the shortcoming of cereals as feeds. With the exception of maize, cereals are deficient in the essential fatty acid linoleic acid. High oil content maize provides a more concentrated form of energy. Opaque 2 and other types of maize have altered and more balanced protein composition. The starch of waxy maize and barley may have greater digestibility. Generally, with these variants, the cost of production and segregation must be considered against the benefit to the feeder.

With the exception of potassium and phosphorus, cereals are deficient in minerals essential for animal growth and reproduction. Potassium is readily available, phosphorous much less so (to monogastrics). However, researchers now have mutations that free up most of the grain's phosphorous, which is normally "locked up" in the form of phytic acid.

#### **Specific Issues**

Wheat pentosans are considered to be an antinutritional factor, especially for poultry. Similarly for barley, the issue is  $\beta$ -glucan, and is mostly a problem associated with poultry. Tannins are anti-nutritional factors for brown sorghums.

With the exception of white-kernel varieties, maize is high in carotenoids. Maize gluten meal is a concentrated source of carotenoids and is a primary source of these pigments for the poultry industry which often desires a highly yellow yolk or yellow carcass fat. During wet-milling, carotenoids are concentrated 12-fold in this gluten meal.

Barley hulls reduce the ME of rations for monogastrics but are of little concern for ruminants; the hulls may actually be beneficial by reducing the rate of rapid starch breakdown which causes acidosis or "bloat." Hull-less barley has nutritional qualities similar to wheat.

Oats have been traditionally an animal feed. Although they have a good balance of protein, fat, and carbohydrate, they have been largely displaced by cheaper alternatives. The hulls reduce energy concentration by  $\sim 25\%$ . However, oats are still the preferred feed for horses. For race horses, quality criteria of buyers may be more exacting than those of food processors. Opinion varies as to whether hulled or hull-less oats are better.

#### **Quality Criteria**

Cereal grain for feed should be low in mold and mycotoxins, lower for monogastrics than for ruminants. Grain should also be low in smut and bunt (Tilletia sp.) spores.

The primary means of assessing feed quality is through proximate analysis: moisture, protein, and ash. Measures of ME and protein digestibility are substantially more involved (requiring live animals) and not amenable to grain receival stations. Test weight and general appearance of the grain are crude indicators of feed value and can be used to reject individual lots. Naturally, grain lot contaminants pose a variable level of concern based on their nature (e.g., kernels of other cereals versus stones or chaff).

For most poultry and swine rations, cereals are hammer-milled, because smaller particle size increases digestibility. The proportion of vitreous versus nonvitreous yellow dent maize kernels affects grinding energy. Barley and sorghum are often steamflaked for feedlot cattle.

In addition to the use of the entire cereal kernel in feeds, it should be remembered that the processing of all cereals for human foods or industrial products generates by-products which largely become animal feeds. Examples include wheat bran, maize gluten feed, and brewers spent grains.

See also: Animal Feed. Cereals: Overview; Grain Defects; Grain Diseases. Consumer Trends in Consumption. Contaminants of Grain. Cultural Differences in Processing and Consumption. Maize: Foods from Maize. Rice: Overview; Chinese Food Uses. Sorghum: Utilization. Wheat: Dry Milling; Grain Proteins and Flour Quality. Appendix: Test Methods for Grain and Grain-Based Products.

# **Further Reading**

American Oil Chemists' Society (2001) Official Methods and Recommended Practices of the AOCS. Champaign, Illinois: American Oil Chemists' Society.

American Oil Chemists' Society (2002) Standard Methods of the American Oil Chemists' Society. Champaign, Illinois: American Oil Chemists' Society.

AOAC International (2002) AOAC Official Methods. Gaithersbury, Maryland: AOAC International.

Bonjean AP and Angus WJ (eds.) (2001) The World Wheat Book. A History of Wheat Breeding. Secaucus, NJ: Lavoisier and Andover Hampshire: Intercept.

Bushuk W (ed.) (2001) Rye: Production, Chemistry, and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Campbell GM, Webb C, and McKee SL (eds.) (1997) Cereals, Novel Uses and Processes. Proceedings of an International Conference on Cereals: Novel Uses and Processes, 4-6 Jun 1996, Manchester, UK.

CCFRA (2002) Manual of Methods for Wheat and Flour Testing. Guideline No. 3, 3rd edn. Chipping Campden, Gloucestershire: CCFRA Technologies.

Dendy DAV (ed.) (1994) Sorghum and Millets: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Fabriani G and Lintas C (eds.) (1988) Durum Wheat Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Faridi H and Faubion J (eds.) (1995) Wheat End Uses around the World. St. Paul, MN: American Association of Cereal Chemists.

Gooding MJ and Davies WP (1997) Wheat Production and Utilization Systems, Quality and the Environment. Wallingford: CAB International.

Heyne EG (ed.) (1987) Wheat and Wheat Improvement, 2nd edn. Madison, WI: The American Society of Agronomy — Crop Science Society — Soil Science Society of America.

Hill LD (1990) Grain Grades and Standards. Urbana, IL: University of Illinois Press.

Hoseney RC (1994) *Principles of Cereal Science and Technology*, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

International Association for Cereal Science and Technology (2002) *Standard Methods*. Vienna, Austria: International Association for Cereal Science and Technology (ICC).

International Seed Testing Association (1999) International Rules for Seed Testing. Zurich, Switzerland: International Seed Testing Association.

Juliano BO (ed.) (1985) Rice Chemistry and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Juliano BO and Villareal CP (1993) Grain Quality Evaluation of World Rices. Manilla, The Philippines: International Rice Institute.

Kulp K (ed.) (1994) Cookie Chemistry and Technology. Manhattan, Kansas: American Institute of Baking.

MacGregor AW and Bhatty RS (eds.) (1993) *Barley Chemistry and Technology*. St. Paul, MN: American Association of Cereal Chemists.

Marshall HG and Sorrells ME (eds.) (1992) Oat Science and Technology. Madison, WI: The American Society of Agronomy — Crop Science Society — Soil Science Society of America.

Murphy CF and Peterson DM (eds.) (2000) Designing Crops for Added Value. Madison, WI: The American Society of Agronomy – Crop Science Society – Soil Science Society of America.

Olson RA and Frey KJ (eds.) (1987) Nutritional Quality of Cereal Grains: Genetic and Agronomic Improvement.

Madison, WI: The American Society of Agronomy –
Crop Science Society – Soil Science Society of America.

Pomeranz (ed.) (1988) Wheat Chemistry and Technology, 3rd edn. (2 vols.). St. Paul, MN: American Association of Cereal Chemists.

Posner ES and Hibbs AN (2003) Wheat Flour Milling, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Pyler ER (1988) Baking Science and Technology, 3rd edn. Merriam, KS: Sosland.

Rasmusson DC (ed.) (1985) Barley. Madison, WI: The American Society of Agronomy – Crop Science Society – Soil Science Society of America.

Simmonds DH (1989) Wheat and Wheat Quality in Australia. Australia: CSIRO.

Sprague GF and Dudley JW (eds.) (1988) Corn and Corn Improvement, 3rd edn. Madison, WI: The American Society of Agronomy – Crop Science Society – Soil Science Society of America.

Watson SA and Ramstad PE (eds.) (1987) Corn Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Webster FH (ed.) (1986) Oats Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Yamazaki WT and Greenwood CT (eds.) (1981) *Production, Breeding, Milling, and Uses.* St. Paul, MN: American Association of Cereal Chemists.

(1993) Grains and Oilseeds, Handling, Marketing, Processing, 4th edn. Winnipeg, Manitoba: Canadian International Grains Institute.

(2000) Approved Methods of the American Association of Cereal Chemists, 10th edn. St. Paul, MN: American Association of Cereal Chemists.

#### **Relevant Websites**

http://www.aoac.org – Website of the AOAC International.

http://www.aaccnet.org — Website of the American Association of Cereal Chemists.

http://www.aibonline.org — Website of the American Institute of Baking.

http://www.asa-cssa-sssa.org — Website of the American Society of Agronomy, Crop Science Society, and Soil Science Society of America.

http://www.irri.org — Website of the International Rice Research Institute.

http://www.wsu.edu - Website of the USDA ARS Western Wheat Quality Laboratory.

www.icc.or.at – International Association for Cereal Science and Technology.

http://www.seedtest.org — International Seed Testing Association (ISTA).

# **Protein Chemistry**

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#### Introduction

The main source of protein for the human diet comes from the cereal grains (*see* Cereals: Overview). On the one hand, cereal-grain protein is eaten directly in many processed forms as the grains themselves. The second route is indirect, in the form of animal products, such as meat and eggs, following the feeding of cereal grains to animals.

The major cereal grains of importance in the human diet are wheat, barley, rye, rice, maize, and sorghum (see Cereals: Overview). In these cases, the protein part of the grain is important nutritionally and also for functional purposes, relevant to the processing of the grain. Of these, wheat is unique because of the dough-forming properties of its storage proteins, known as "gluten" when formed into dough.

# **Historical Perspective**

Historically, the proteins of the cereal grains have played a significant part in the civilization of mankind, through their role in providing a reliable source of amino acids, the essential dietary compounds that are the building blocks of proteins in the body. This source of dietary proteins could be obtained via agriculture, permitting the establishment of a permanent dwelling place, providing an important change from the nomadic life of the hunter-gatherer way of life. The establishment of permanent towns led, in turn, to specialization of occupation, and to opportunities for mankind to pursue cultural pursuits and scientific enquiry. However, it is only in recent centuries that the chemistry of proteins has been elucidated.

One of the first proteins to be obtained in reasonably pure form was gluten, because it could be prepared so readily (by the washing of dough). This important experiment, first reported by the Italian scientist Beccari in 1729, is simply performed in the home kitchen. To do so, a dough is mixed by adding water, a little at a time, to wheat flour until a cohesive mass is formed. This dough is kneaded between the fingers under a gentle stream of water from the tap. If a glass is placed underneath, a milky-white stream of starch can be caught in the glass, whilst the dough in the fingers becomes smaller and tougher. The gluten ball held in the fingers is mainly protein, separated very simply from the starch that settles in the bottom of the glass.

This gluten-washing procedure has since become very big business internationally, because the gluten thus isolated is needed for bolstering the protein content of a wide range of food products – bread especially, but also breakfast cereals, meats, cheeses, snack foods, and texturized products. Nonfood applications include pet and aquaculture feeds, biodegradable plastics, films, coatings, adhesives, inks, cosmetics, and pharmaceuticals (see Gluten and Modified Gluten).

The word "protein" was proposed by the French scientist Berzelius, writing to the German scientist Mulder, with the appropriate meaning of "primary substance," as recently as 1838, long after the acceptance of the terms for specific types of proteins, such as "gluten," "zymom," and "fibrin." Early concepts of protein chemistry imagined it to have the complex formula C<sub>20</sub>H<sub>31</sub>N<sub>5</sub>O<sub>6</sub>, meaning that 20 molecules of carbon (C) were compounded with 31 of hydrogen, five of nitrogen, and six of oxygen. The dogma of those early days also maintained (incorrectly) that this complex was absorbed (unchanged) directly by the intestine. It is now known that dietary proteins are hydrolyzed to the component amino acids for absorption into the blood stream.

Late in the nineteenth century, the American scientist Thomas Osborne studied the proteins of many edible grains, using a succession of solvents to extract diverse classes of proteins from the crushed grain. As a result, he proposed the generic names of "albumin" (extracted with water), "globulin" (extracted by salt solution), "prolamin" (extracted by 70% aqueous ethanol), and "glutelin" (extracted by dilute acid). These major classes of protein have continued in use, but their definitions have been refined, and it has become clear that any one of these protein classes contains a large number of distinct but related proteins.

#### What Is a Protein?

It is now known that protein molecules are linear polymers of amino acids. There are  $\sim 20$  different amino acids, each of them having the combination of an acidic group (-COOH) and a basic group (-NH<sub>2</sub>), with a side chain that provides the essential difference between all of them. The amino acids are joined together by peptide bonds to form a polypeptide chain, by the formula (for two amino acids, 1 and 2)

$$R_1$$
-COOH +  $H_2$ N- $R_2$   $\rightarrow$   $R_1$ -CO-NH- $R_2$  +  $H_2$ O

The sequence and specificity of the amino acids are "spelt out" exactly in the genetic code of the genes, thereby ensuring that the protein will be made in the same manner each time in the specific species and tissue (see Genomics and Proteomics). For this reason, the determination of protein composition is a valuable means of establishing variety identity (see Variety Identification of Cereal Grains).

It is amazing that the relatively simple chemical structure of the protein can produce a great diversity of properties that are found in proteins, such as enzymes and epidermis, hair and horn, feathers and flagella, silk and sinew, and even spider's web. The diversity depends on the selection and sequence of the amino acids, plus the length of the protein (polypeptide) chain. Further diversity is provided by crosslinking between polypeptide chains, and from linking to accompanying molecules (lipids, carbohydrates, or metal ions). The resulting protein molecules are large compared to many compounds, with molecular weights often in the range 10-100 kDa (1 Da being equivalent to the mass of a hydrogen atom). Some proteins are much larger, the largest glutenin proteins of wheat gluten having sizes extending up to tens of millions of Daltons.

# Why Is Protein Important?

First, the protein component of the grain is critical to the grain's role as the beginning of a new plant. The grain protein has the natural purpose to serve as a reserve of amino acids for the embryo (germ) to draw upon during germination. On the other hand, the grain protein is important for the grainprocessing industry, because it is often the most important part of the grain in determining processing quality.

Protein's importance cannot be attributed to it being the component present in the highest proportion of the grain (Figure 1). In fact, starch is the most prolific component of the cereal grain. After starch come protein, lipids (fat), and nonstarch polysaccharides in relative abundance (see Cereals: Overview). The protein content of cereal grains ranges from  $\sim$ 8% to 15% of the grain weight (Table 1). Even for certain grain species, the actual protein content covers a range of values, depending on the genetic potential of a specific variety of that grain species, and on the nutritional status of the plant (see Barley: Agronomy and Wheat: Agronomy).

The protein components may be considered as the most important of the range of chemical components in the cereal grain. The protein fraction contains the many enzymes that are essential for the synthesis of all the other chemical components of the grain, and also for their breakdown during germination. In the mature grain, a large proportion of the protein fraction serves as a storage for amino acids (polymerized in linear chains as proteins), which in turn are supplied to the growing plant during and after germination.

Most of the protein is in the endosperm of the grain (the part that becomes white flour after the milling of wheat), because the endosperm is the largest part by mass. However, the proportion of protein may be higher in some of the nonendosperm tissues of the grain, especially in the germ (the embryo, which will become the new plant after germination). The barley grain is taken as an example of this in Figure 2. Figure 3 shows the chemical composition of wheat grain, with respect to the distribution of protein (and other components) in its various anatomical parts (see Grain, Morphology of Internal Structure).

The importance of protein content is seen in the valuation of grain shipments. The "single figure" of

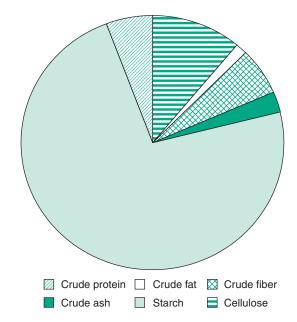


Figure 1 Chemical composition of whole cereal grain.

Table 1 Approximate protein content of the more common cereal species, as percentages of the "as is" weight of whole grain, except for rice

Cereal species	Protein content (range as % of "as is" weight of whole grain)		
Barley	9–12		
Maize (corn)	10-12		
Oats (as groats)	12-15		
Rice (milled)	8–11		
Rye	12-15		
Wheat	9-16		

the protein content of a grain sample is an important specification in the marketing of most cereal grains. In general, grain of higher protein content commands a higher value. For wheat, a higher protein content indicates better baking quality, and a higher protein content for feed grains provides a richer supply of essential amino acids (irrespective of the proportion of essential amino acids in their storage protein) (see Animal Feed).

However, malting barley is an exception to the rule that high protein content is desirable, because of the need to have a high starch content to provide a correspondingly high extraction of fermentable sugars in the malting process. Nevertheless, even for malting barley, there is a minimum level of protein ( $\sim$ 8%), to ensure that there is an adequate supply of the hydrolytic enzymes needed throughout the malting process.

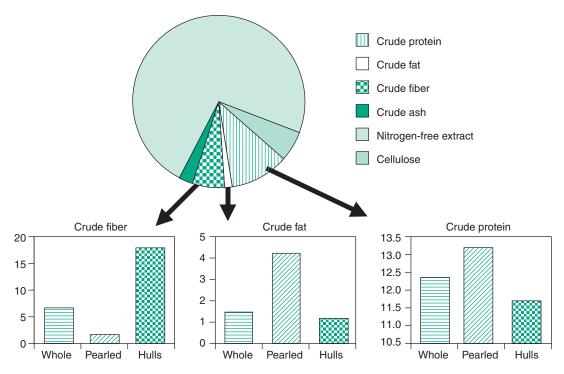


Figure 2 Chemical composition of barley seed and its distribution in its different parts.

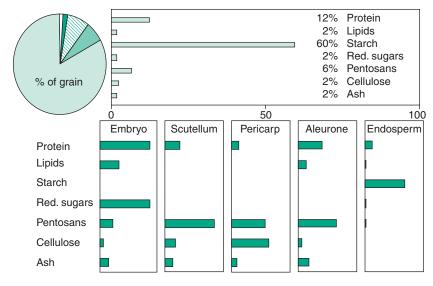


Figure 3 Chemical composition of wheat grain; distribution between anatomical parts. "Red. sugars" means reducing sugars, such as glucose.

# **Analysis of Protein Content**

Today, the routine determination of protein content of all grain species generally involves the use of near infrared (NIR) spectroscopy, applied to whole grain, milled grain, or flour (Table 2). In the case of wheat only, protein content can also be determined by washing out the gluten protein (*see* Gluten and Modified Gluten) and weighing its wet mass. Even though gluten protein is less than 80% of the total grain

protein, this approach can be used if appropriate correlations are established between the level of washed gluten and "true" grain protein content.

Further possibilities include colorimetric methods (e.g., the biuret reaction) or the amide distillation procedure (Table 2). The standard ("true") method for protein determination involves the complete digestion of the grain or flour to its elements, and determination of the proportion of the element

nitrogen content.

The total-nitrogen content is used as an absolute value in establishing correlations for the routine method of NIR spectroscopy to determine the protein content of whole grain or of milled products (Table 2).

**Table 2** Methods used to determine the protein content of cereal grain, especially wheat

Process	Material assayed	Assay method	
Mill	Grain Flour/meal	NIR NIR	
Wash (for wheat only)	Gluten	Weight (wet or dry)	
Extract	Protein in solution	Biuret or dye-binding reaction	
Hydrolyze	Amino acids	Gas or liquid chromatography	
Alkaline digestion	Amide N	Titrate ammonia released	
Acid digestion	Ammonia or nitrogen	Kjeldahl or Dumas methods	

# **Protein Quality**

The "single figure" of protein content, however, does not take into account the quality of the protein. This quality is indicated for nutritional purposes by analysis of the levels of essential amino acids after hydrolysis of the peptide bonds. For wheat, for example, the quality of the protein relates to the dough strength provided by the particular combination of gluten proteins present, determined partly by the genotype (the variety) and also by the growth conditions.

For the animal-feed industry, the amino-acid composition is especially important, due to the need to ensure an adequate supply of essential amino acids (see Animal Feed). The protein of cereal grains is a good source of most of the essential amino acids, with the exception of lysine and possibly tryptophan, which are lower in the cereal grains than in most animal proteins. The amino-acid composition for wheat is shown in Figure 4, with respect to the major classes of wheat proteins.

# **Comparing the Proteins of Cereal Species**

The various cereal species differ in the composition of their grain proteins. To a large extent, these differences explain the differences between the cereals with respect to their food uses. The most obvious example is wheat, whose gluten-forming storage protein is unique amongst the cereals in suiting wheat flour for bread making. Furthermore, the proteins of the rice grain contribute to its eating quality. The differences in the protein composition of the

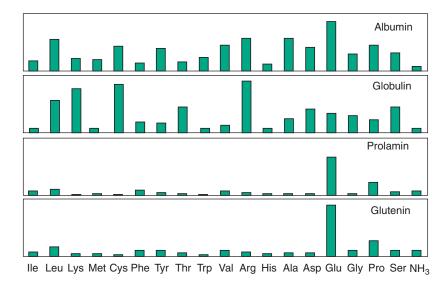


Figure 4 Amino-acid composition of the Osborne fractions of wheat.

cereal grains reflect the taxonomic relationships between them, as is illustrated in Figure 5. The storage proteins of the cereal endosperm are broadly termed "prolamins," with specific names for the prolamin proteins of each cereal genus, as listed in Table 3.

# The Proteins of Wheat, Rye, and Triticale

Wheat and rye are closely related, and triticale is a manmade hybrid between wheat and rye (*see* Taxonomic Classification of Grain Species). Their protein compositions are thus similar to a limited extent, but the dough-forming quality of rye and triticale is much poorer than that of wheat. The roles of wheat-grain proteins are described in greater detail in relation to wheat quality in Wheat: Grain Proteins and Flour Quality.

The popularity of rye-based foods is not so widespread as that of foods made from wheat. Rye is especially popular in eastern European Scandanavian countries. Baked products are commonly crispbread and varieties of bread, including pumpernickel. Whereas "true" rye bread is made from 100% rye, it is common for rye bread to be made from a blend of rye and wheat flours, the contribution of the wheat gluten being needed to provide the baking quality that is lacking in the rye gluten. The poorer dough-forming characteristics of rye gluten is largely due to the lower level of the

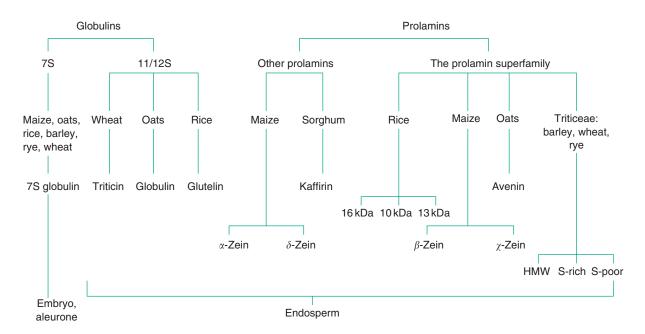
glutelin-type protein, combined with the more hydrophilic (water loving) properties of the rye secalins, compared to the corresponding classes of protein in wheat (*see* **Wheat**: Grain Proteins and Flour Quality.

The water-soluble proteins of rye contain the albumin and globulin classes of proteins, similar to those in wheat, but for rye there is generally a higher level of amylase enzymes than in wheat because of the greater susceptibility of rye to preharvest sprouting and the consequent production of starch-degrading enzymes.

The essential amino-acid composition of rye is slightly better than that of wheat, because of the lower content of prolamin-type proteins in rye (Figure 5). The properties of triticale are intermediate between those of rye and wheat, reflecting the genetic origins of triticale.

**Table 3** Names for the storage prolamins of the cereal grains

Cereal species (common name)	Genus name	Name of storage prolamin
Barley Maize (corn) Oats (as groats) Rice (milled) Rye Sorghum	Hordeum Zea Avena Oryza Secale Sorghum	Hordein Zein Avenin Oryzin Secalin Kafirin



**Figure 5** Storage-protein classes in cereals. (Adapted from Shewry PR, Tatham AS, and Halford NG (1999) The prolamins of the Triticeae. In: Shewry PR and Casey R (ed.) *Seed Proteins*, pp. 35–78. The Netherlands: Kluwer Academic.)

# The Proteins of the Barley Grain

The protein content of cultivated barley ranges from  $\sim 9\%$  to 12%. For barley, the protein content is generally calculated as total nitrogen content multiplied by the factor of 6.25. The protein content of hull-less (naked) barley genotypes is generally higher (up to 18%) because of the absence of the outer layers, which contain relatively little protein (Figure 2). The production of the enzymes of germination is an important part of a good malting barley, but paradoxically, high protein content (e.g., over 11%) is not necessarily desirable for best malting quality. This is because the ultimate test of malting quality is the extent of "extract" produced during the mashing process. The amount of extract determines how much beer can be produced from a given amount of malt. "Extract" represents the amount of soluble fermentable sugars from the hydrolysis of starch, resulting from the combination of very active amylases and high starch content. For this reason, a low protein content (i.e., less than 8%) is also undesirable, as it may have the consequence of inadequate enzyme production.

Consumption for human food is a traditional use of barley, but this now accounts for only  $\sim$ 5% worldwide. Feed use predominates, amounting to  $\sim 75\%$  of barley use. The amino-acid composition of barley does not provide a consistently better balance nutritionally than other cereal grains, still being deficient in lysine, methionine, and threonine. Nevertheless, it is a popular source of protein and energy in feedstuffs, in combination with other sources of energy. High lysine barley genotypes have been developed, analogous to those in maize, the first of these being the Hyproly line, screened from the world barley collection. The hordein polypeptides show considerable polymorphism, like the prolamins of wheat and rye. A large proportion of the barley prolamins are disulfide linked with naturally occurring polymers of high molecular weight. Unlike the glutenin polymers of wheat, these are not desirable for the processing of barley. Because they generate problems in the malting process and downstream, they are likely to produce haze in the final product, i.e., (beer) if they have not been adequately hydrolyzed during processing.

The hordein polypeptides are classified into groups (A, B, C, and D hordeins) according to their decreasing mobilities during SDS gel electrophoresis. The C hordeins have analogy to the omega-gliadins of wheat, having a general absence of the sulfurcontaining amino acids. They are coded by genes (locus *Hor 1*) on the short arm of barley chromosome 1H, as indicated in Table 4. The genes (*Hor2* and 5) for the sulfur-rich B and gamma hordeins are also

**Table 4** Classes of barley proteins (hordeins) and corresponding loci

Hordein class	Loci	Gene copy number	Proteins present in cv. Carlsberg.	MW (kDa)	Corresponding wheat prolamin
B C D		•	10 9 1 7	50-60 105	LMW glutenins $\omega$ -Gliadins HMW glutenins $\gamma$ -Gliadins

coded on chromosome 1H. The D hordeins, which occur as disulfide-linked polymers, are analogous to the high-molecular-weight subunits of wheat glutenin; like them, their genes (*Hor 3*) are located on the long arm of the corresponding barley chromosome (1H).

#### The Proteins of the Rice Grain

The proteins of rice are a contrast to those of wheat and barley, because the rice-grain proteins are predominantly of the globulin class. As a protein source, milled rice contains the lowest amount of protein  $(\sim 5\%)$  among the major cereals. Moreover, this protein is not easily digestible by human and monogastric animals. However, compared to that of other cereal proteins, the overall amino-acid composition of rice protein is significantly more balanced due to the relatively higher level of lysine content. This unusual amino-acid composition results because rice is one of the few cultivated plants in which there are significant levels of globulins and prolamins. These are the two major classes of storage proteins in the seeds of higher plants. Unlike other cereals that accumulate prolamins as their primary nitrogen reserve, the major storage proteins in rice are the glutelins, which are homologous at the primary sequence level to the 11S globulin proteins (Figure 5), a class that is the dominant form of nitrogen deposition in legumes. Rice prolamin proteins have a number of characteristics that are different from the prolamins present in most other cereals (see Wheat: Grain proteins and Flour quality).

The most important characteristic of the deposition of nitrogen in the rice kernel is that there are three kinds of protein bodies in the rice endosperm, namely, large spherical, small spherical, and crystalline protein bodies. Each of these is surrounded by a single continuous membrane. Although the spherical protein bodies form within vacuoles, the proteins are synthesized in the endoplasmic reticulum and in the Golgi apparatus, and are then transported to the

vacuoles via vesicles. Removal of the husk (hull) from rough (paddy) rice yields the kernel. This is composed of the pericarp, seedcoat, aleurone, endosperm, and the germ, and is known as "brown rice." It has a protein content of  $\sim 9-10\%$ , with a significantly higher nutritional value than the most commonly utilized rice product - white polished (milled) rice, whose protein content is  $\sim 8\%$ .

The milling process for paddy rice results in 40-55% white milled rice, together with three major by-products, namely, husks (20%), bran (10%), and brokens (10-22%). These by-products have protein contents of 3%, 17%, and 9%, respectively. The aleurone layer, the tissue with the highest level of proteins and nutritionally important minor components, is removed during the rice-milling process.

The subaleurone region of the rice grain plays an important part because of its nutritional value. It is a globulin-rich layer, being several cell layers thick. Its lysine content is much higher than that of the proteins located in the rice endosperm. Thus, rice should be milled as lightly as possible to retain as much as possible of the subaleurone layer.

The albumin fraction of rice is highly heterogeneous, containing biologically important components. It can be separated into four subfractions, based on the molecular size of its proteins. More than 50 individual polypeptides were observed in the albumin fraction, using isoelectric focusing. As for cereal grains in general, the albumins are mainly enzymes and enzyme inhibitors.

Like the prolamins from other cereals, rice prolamins are readily soluble only in alcohol/water mixtures. However, the classical extraction procedure had not been useful in early studies. More recently, these estimates of prolamin content have been shown to be low. Using 55% propan-l-ol and reducing agents, higher yields of prolamins (20-25% of the total protein) have been obtained. Rice prolamins are  $\sim 10-16$  kDa. Thus, their molecular size range is significantly smaller than the prolamins from other cereals. They are highly variable between rice cultivars, based on their electrophoretic and isoelectric focusing fingerprints.

According to the Osborne classification system, the glutelins comprise the major protein fraction of the rice grain, representing up to 80% of the total protein fraction (Figure 5). The rice glutelins have been difficult to study because of their general insolubility in all solvents except dilute alkali, due to their high molecular weight and their heterogeneity. When analyzed by gel filtration chromatography, the purified glutelin fractions were resolved into three subunits, linked by disulfide bonds, which varied in stoichiometry depending on the report and on the rice variety used.

When subjected to cation-exchange chromatography, the glutelins separate into a heterogeneous set of acidic and basic subunits. The acidic subunits are 28.5-30.8 kDa in size, with pIs between 6.5 and 7.5, while the basic subunits are smaller (20.6–21.6 kDa) with pls from 9.4 to 10.3. The acidic subunits contain nearly twice as much glutamic acid/glutamine and more serine and glycine, whereas the basic subunits contain more alanine, lysine, aspartic acid/asparagine, and isoleucine.

Despite their general insolubility, rice glutelins are homologous in structure to 11S globulins (Figure 5). The structural relationship is evident when comparing the N-terminal amino-acid sequences and by using immunological methods. The extent of homology between the rice glutelin and legume 11S globulins is  $\sim 30-35\%$ . Structural similarity is evident in the primary sequences of homology to a motif common in wheat, rye, barley, and maize prolamins.

#### The Proteins of Maize

Maize (or corn), often classed in world trade as one of the "coarse" grains (see Cereals: Overview), is an important source of protein in the diets of many people in countries such as Africa and South America (see Grain Production and Consumption: Africa; South America). In addition, maize is used extensively as a feed grain and in a range of industrial processes (see Maize: Dry Milling; Wet Milling; Foods from Maize). The process of cornstarch manufacture produces the grain protein as a by-product. It is sometimes referred to as "corn gluten" in the trade, but this name is inappropriate, because maize protein has no relationship to the gluten of wheat (see Nutrition: Beriberi, A Deficiency Related to Grains and Celiac Disease).

Like the other cereal grains, maize protein is slightly deficient in lysine. This limits its utilization as an animal feed. This nutritional imbalance is largely due to the low lysine content of "zein," the major group of maize proteins (Figure 5). The development of the "opaque-2" and "floury-2" mutations has led to the development of maize genotypes with higher levels of lysine, due to a lower content of zein and more of the nutritionally superior albumin type of protein. Although these new maize types offer nutritional advantages, they carry a yield penalty.

The zein protein of maize can be isolated by extraction of the crushed endosperm (the starchy part of the grain) with 70% aqueous ethanol, after breaking disulfide bonds. Maize zein has been further fractionated into subclasses of proteins, designated alpha-, beta-, gamma-, and delta-zeins. The genes for their synthesis have been mapped to maize chromosomes 6 and 7 (see Maize: Genetics).

# The Proteins of Sorghum

The major storage proteins of sorghum, termed "kafirins" (Figure 5), are fractionated by a procedure similar to that for the zeins of maize. Alpha-kafirin, comprising ~80% of the overall kafirin content, can be further subdivided into two proteins with molecular weights of ~23 and 25 kDa. Three beta-kafirins and one gamma-kafirin have been identified, all of them having molecular weights in the range 16–28 kDa. Like maize, high lysine mutants of sorghum have been developed.

# **Future Prospects**

Recent decades have seen enormous increases in the understanding of the chemical and functional properties of the cereal proteins, permitting more efficient selection for improved processing quality in the breeding of new varieties. This knowledge is also being applied to testing for protein quality after the grain is harvested, so that grains of specific quality can be segregated for appropriate marketing and processing. Knowledge at the chemical level is now complementing new genetic insights, so that breeders and molecular geneticists can employ conventional and novel methods to create quality types that have previously not been possible – varieties with new protein functionality – so that the future holds great promise for grains that will be processed more efficiently with nutritional advantages.

See also: Cereals: Chemistry of Nonstarch Polysaccharides. Lipid Chemistry. Nitrogen in Grain Production Systems. Nitrogen Metabolism. Protein Synthesis and Deposition. Wheat: Grain Proteins and Flour Quality.

#### **Further Reading**

Bailey HC (1941) A translation of Beccari's lecture "Concerning Grain" (1729). Cereal Chemistry 18: 555.
Bushuk W (ed.) (2001) Rye: Production, Chemistry and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Dendy DAV (ed.) (1995) Sorghum and Millets: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Henry RJ and Kettlewell PS (eds.) (1996) Cereal Grain Quality. London: Chapman and Hall.

Juliano BO (ed.) (1985) Rice: Chemistry and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists. Kent NL (ed.) (1975) Technology of Cereals, with Special Reference to Wheat, 2nd edn. Oxford, UK: Pergamon.

Lasztity R (1999) Cereal Chemistry. Budapest, Hungary: Akadémiai Könyvkiadó.

McGregor AW and Bhatty RS (eds.) (1993) *Barley: Chemistry and Technology*. St. Paul, MN: American Association of Cereal Chemists.

Pomeranz Y (ed.) (1988) Wheat: Chemistry and Technology, 3rd edn. St. Paul, MN: American Association of Cereal Chemists.

Shewry PR and Casey R (eds.) (1999) *Seed Proteins*. The Netherlands: Kluwer Academic.

Shewry PR and Miflin BO (1985) Seed storage proteins. In: Pomeranz Y (ed.) *Advances in Cereal Science and Technology*, vol. 7, pp. 1–83. St. Paul, MN: American Association of Cereal Chemists.

Sikorski ZE (ed.) (2001) The Chemical and Functional Properties of Food Proteins. Lancaster, PA: Technomic Publishing.

Watson SA and Ramsted PE (eds.) (1987) Corn: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Webster FH (ed.) (1985) Oats: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

#### **Relevant Websites**

http://www.aaccnet.org – American Association of Cereal Chemists.

http://www.campden.co.uk - Campden & Chorleywood Food Research Association.

http://www.wheat.pw.usda.gov — Grain genes.

http://www.icc.or.at — International Association for Cereal Science and Technology.

http://www.usda.gov — United States Department of Agriculture.

# **Evolution of Species**

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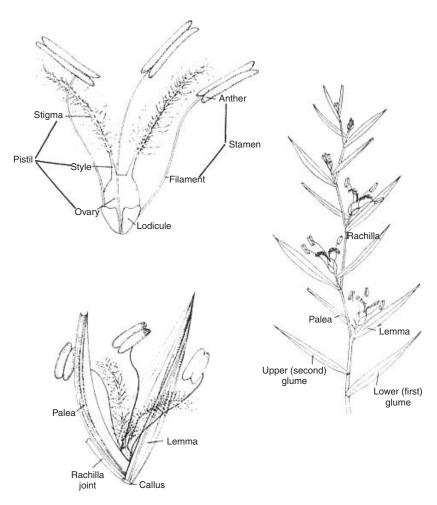
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#### Introduction

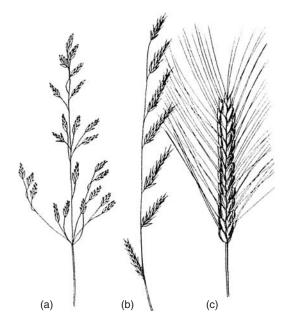
Cereals comprise a diverse collection of species, all belonging to the grass family (Poaceae). As a group, they are named for the Roman goddess Ceres who, along with her Greek counterpart Demeter, was worshipped as the mother of agriculture. Among Ceres' symbols were spikes of wheat and thus her designation as goddess of grain. Cereal evolution is a complex subject given the vast array of species adopted into agriculture by cultures across five continents. Most researchers and consumers think in terms of a relatively narrow selection of cereal species that now dominate international commerce as dietary staples or constituents in processed food products. In this select group are barley, maize, millet, oat, rice, rye, sorghum, and wheat. In actuality, cereals comprise a much larger group with many lesser-known species that serve as dietary staples on a local basis, particularly in subsistence farming cultures.

# **Reproductive Biology**

Members of the grass family all have inconspicuous flowers that have been reduced to the essential reproductive structures. There are no showy bracts, nectaries, and other features to attract pollinators, for these species rely on wind pollination to distribute their pollen. Although grass flowers all have the same basic design (Figure 1), there is variation in the development of the female and male reproductive structures in terms of their presence or functionality. The flower, when perfect, contains three or six stamens, one pistil, and two lodicules, which are scalelike appendages positioned on either side of the ovary base. Enclosed by two outer leaf-like bracts (lemma and palea), these structures form a floral unit or floret (Figure 1). The floret is attached to a stem axis, the rachilla. Subtending the rachilla are two empty bracts, the glumes. One or more florets, packaged within the glumes, form a *spikelet* (Figure 1). Spikelets are alternately positioned at attachment points (nodes) located along a main inflorescence axis (rachis) or on branches extending off the rachis. The interval between two nodes on the rachis is the internode. Within the spikelets, florets are similarly arranged, alternately along a secondary axis (rachilla). Although



**Figure 1** Stylized drawings of a grass flower (upper left), floret with adjacent rachilla internode (lower left), and spikelet with subtending rachis internode (right). (Reproduced with permission from Clark LG and Pohl RW (1996) Agnes Chase's *First Book of Grasses*. Smithsonian Press.)



**Figure 2** Illustrations of the three general inflorescence types found in grasses: (a) panicle, (b) raceme, and (c) spike. (Reproduced with permission from Clark LG and Pohl RW (1996) Agnes Chase's *First Book of Grasses*. Smithsonian Press.)

inflorescence morphology is very complex, a simplistic classification designates three basic inflorescence types (Figure 2), each differing in branch development and spikelet attachment: (1) *panicle* with long, open branches (rice) or short, dense branches (pearl millet), each bearing a pediceled spikelet; (2) *raceme* with spikelets borne on short pedicels along the rachis (barley); and (3) *spike* with spikelets sessile along the rachis (wheat).

#### **Pollination and Fertilization**

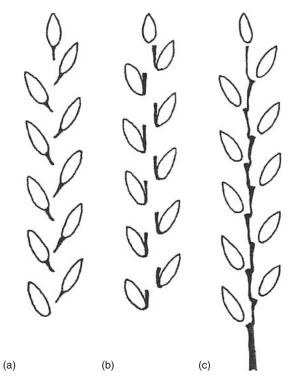
Cereal species can be either self- or cross-pollinating. Wind and air currents serve as the mechanism by which the pollen of cross-pollinating cereals is distributed. In cross-pollinating species, the functional role of the lodicules is evident at anthesis when they swell, forcing open the florets to expose the stigmatic surfaces and allowing the anthers to shed their pollen into the air. Elongation of the stem-like filaments on the stamens pushes the anthers upwards and out of the floret. The glumes can bend outward at their bases, allowing more room for the florets to open. For selfpollinating cereal species whose florets do not stay tightly closed at anthesis, cross-pollination can occur at low levels. Given the hybrid speciation events that have shaped the formation of polyploid species such as oats and wheat, it is clear that the functional morphology of the floret and spikelet serves an important role in cereal evolution.

The stigma, a collection of sticky feather-like branches, serves as the pollen trap (Figure 1). Upon pollination, a pollen tube grows downward through the stigma and style into the ovary which holds a single ovule. Fertilization occurs when the pollen sperm nuclei unite with the egg and two female polar nuclei. From this event, a grain develops with a 2n embryo and 3n endosperm. When fully developed, the cereal grain is the fruit and seed combined, i.e., a specialized fruit known as a caryopsis, whose outermost layer is the dried remains of the ovary (pericarp) now adherent to the seed within. In wild grass species, the mature caryopsis can be protected within a hardened, shell-like package formed by the lemma and palea or encased as one of several caryopses within a spikelet unit formed by tough, tightly clasping glumes. Another means of protection are adherent lemmas and paleas, which, while not forming a hardened barrier, do serve a protective function. These wild-type strategies serve to protect the caryopsis during dispersal and early seedling establishment. In domesticated cereals, the protective features of the bracts have been lost or, to some extent, reduced. Cereals with naked grains have lost all the protective features of the lemmas, paleas, and glumes.

#### **Dispersal**

In wild grasses, survival depends upon natural dispersal strategies that move the mature grain off the mother plant and spread it to new habitats, expanding opportunities for success into the next generation. By contrast, the domesticated cereal grain is retained on the plant rather than dispersed and in many cases, no longer protected by the bracts. As a consequence, it is dependent upon humans to move it away from the mother plant (i.e., to disperse the grain) and to protect it from predation and environmental threats. These different strategies illustrate variations on the evolutionary theme of dispersal. Cereal researchers often overlook the connection between the wild dispersal mechanisms and the domesticated traits of grain retention on the plant and grain release from the bracts during threshing. With respect to the latter, free-threshing grains are those, like wheat, which can be easily freed from the brittle glumes, lemma, and palea by threshing action.

Dispersal units (*diaspore*) are created by a spontaneous shattering (*disarticulation*) in abscission zones located at the nodal points along the rachis or rachilla (**Figure 3**). Disarticulation is typically described as above (rachilla disarticulates into floret units) or below the glumes (rachis disarticulates into spikelet, branch, or inflorescence units). The rachilla



**Figure 3** Disarticulation points along the rachis illustrating the location of the break and the internode piece attached to the diaspore: (a) arrow-shaped diaspore with subtending rachis piece; (b) cylindrical diaspore with adjacent rachis (rachilla) piece; (c) tough rachis with no disarticulation. Disarticulation points along the rachilla follow the same pattern illustrated in (a) and (b). (Reproduced with permission from Peterson RF (1965) Wheat: botany, cultivation, and utilization. In: Polunin N (ed.) *World Crop Books*. New York: Interscience.)

and rachis internodal pieces that remain attached may either subtend or lie adjacent to the diaspore (Figures 1 and 3). Rudimentary or sterile inflorescence structures may also form part of the diaspore as is the case with the two sterile awn-like lateral spikelets on the wild barley diaspore and the reduced inflorescence branches that form a bristly fascicle on the wild pearl millet diaspore.

In some species, toughened glumes completely enclose the spikelet in a protective package that secures the grain from predation and environmental damage (wild wheat). In other species, the lemma and palea adhere to the grain (wild barley) or form a hard shell around the grain (wild pearl millet). Shape and awning of the diaspore determines its mode of movement once reaching the ground. For example, cylindrically shaped diaspores move by rolling on the ground, floating on water, or getting caught in mud that sticks to the hides or in the hooves of animals (wild wheat); arrow-shaped units fall or are moved point downward into soil cracks (wild rye). Awns, which are extensions of the vascular nerves of glumes and

lemmas, can move the diaspore downward into the soil in several ways. Long, straight awns vibrate in the wind to push the diaspore downward (wild wheat). Hygroscopic awns twist in response to moisture changes, thereby drilling the diaspore into the ground (wild oat). Awns and bristles also function to move dispersal units away from the mother plant to a new site. Tiny barbs on awns can attach the diaspore to the fur of animals or clothing of humans (wild wheat and barley). Lightweight, feathery bristles can move a diaspore in the wind (wild pearl millet).

#### **Evolution and Domestication**

Acting as evolutionary selection agents, early humans channeled the inherent genetic variation of wild grasses in the direction of agricultural traits that eased work to cultivate, harvest, and extricate grain. Domesticated cereals have continued to intercross with their wild progenitors and relatives, with the result of blurred boundaries between wild and domesticated species. As a consequence of the variation that has developed under human hands and the intermixing of wild and domesticated forms, cereals are problematic in their taxonomy and phylogenetic treatment (see Taxonomic Classification of Grain Species). Alternative generic and species concepts abound in cereal species taxonomy. They reflect divisions within the research community as how to best interpret the relationship between wild and domesticated forms and how to categorize the extensive phenotypic, yet minor genetic, variation within a given cereal species. Often missing from these debates is the recognition that domestication is representative of the larger process of evolutionary change and speciation.

# **Wild Progenitors and Weedy Species**

Wild progenitors of domesticated cereals are often species that adapted well to human-made habitats, i.e., they prospered in the disturbed environments created by human activities and proved amenable to cultivation. With domestication, these habitats expanded to agricultural fields where wild progenitors or their close relative species often became weeds. When domesticated forms and their wild, weedy progenitors hybridized, crop-weed complexes developed, and continue to develop. For these species, the line between the domesticated form and its wild relatives is not always well drawn. On the other hand, the resultant gene flow has played a significant role in shaping their genetic variation. Development of weed races is the natural consequence of the interdependent evolutionary relationship between humans and the wild grasses that they domesticated.

#### **Traits of Domestication**

Under domestication, features of dispersal, reproduction, plant growth, and survival of wild grasses were transformed into desirable agricultural traits for improved cultivation and grain yield. Among the more critical transitions were: (1) suppression of dispersal mechanisms; (2) reduction of seed dormancy either by loss of physiological dormancy or by loss or reduction of enclosing bracts that contained germination inhibitors; (3) increase in seed production by development of reproductively functional florets and spikelets from sterile or reduced inflorescence structures; (4) increase in seed size; (5) synchronous development of tillers and ripening of the grain; and (6) greater agricultural fitness for soil and climatic conditions and pest resistance.

While all of these traits have played significant roles in domestication, the transition of dispersal traits is the critical event by which the effort expended on gathering and processing grains was made easier. Development of a tough, non-disarticulating rachis enabled humans to harvest mature inflorescences directly from the plant. Reduction or loss of tough, tightly clasping bracts led to free-threshing cereals. In a separate evolutionary development, loss of adherent lemmas and paleas produced a naked grain easily released in threshing. The term *hulled* cereal collectively refers to different protective features of the bracts in

domesticated cereals that have not completely lost wild-type features. A *hulled grain* is one that is either encased within the lemma and palea, which either form a hard shell around the grain (pearl millet) or are adherent to the grain surface (barley). A *hulled spikelet* has hardened, tough glumes that tightly enclose one or more grains in a spikelet package (spelt wheat).

# **Major Cereal Species**

Major cereal species (Table 1) are those that are either in international commerce or serve as a dietary staple beyond a localized region. Wheat and barley are temperate cereal crops of the Fertile Crescent and along with oats and rye formed the major grain complex for Western Asia, North Africa, and Europe. Maize and rice are the tropical cereals upon which civilizations in the New World and Eastern Asia developed. Although sorghum, millet, and African rice, all tropical African species, have historically been given a lesser role, they are equally important in the establishment of early human civilizations in Africa and South Asia.

#### **Barley**

Barley was domesticated along with wheat as one of the founder crops of Old World Neolithic agriculture. Unlike the wheats, barley consists of only one domesticated diploid species (*Hordeum vulgare*) and its wild relative counterpart (*H. spontaneum*), which is found in both wild and weedy forms (*see* Barley: Genetics and Breeding). Although barley has a lengthy agricultural history and development of an extensive

Table 1 Major cereals and their wild and weedy relatives

Common name	Domesticated species	Wild progenitor species	Weed race	Region of domestication	Date of domestication <sup>a</sup>
Barley	Hordeum vulgare	H. spontaneum	H. spontaneum	West Asia	9500-8400 BP
Common oat	Avena sativa	A. fatua A. sterilis	A. fatua A. sterilis	Europe	4000-3000 BP
Maize	Zea mays	Z. mays (teosinte) Tripsacum dactyloides	Z. mays (teosinte)	MesoAmerica	7000 вр
Pearl millet	Pennisetum glaucum	P. violaceum	P. sieberanum	West Africa	4500 BP
Rice	Oryza sativa	O. rufipogon O. nivara	O. sativa f. spontanea (O. fatua)	Southeast Asia	5500-4000 вр
Rye	Secale cereale	S. montanum ( = S. strictum)	S. cereale ssp. ancestrale ssp. dighoricum ssp. segetale ssp. afghanicum	West Asia	4000 вр
Sorghum Bread wheat Durum wheat	Sorghum bicolor Triticum aestivum Triticum durum	S. arundinaceum unknown T. dicoccoides	S. halepense (4×) Aegilops spp. Aegilops spp.	Central Africa Central Asia Western Asia	2000 BP <sup>b</sup> 8000-7000 BP 9000-8000 BP <sup>c</sup>

<sup>&</sup>lt;sup>a</sup>Based on reports in the current literature dealing with the archeological record, approximate date of known cultivation of the domesticated species.

<sup>&</sup>lt;sup>b</sup>Early archeological sites in Africa are unknown. These dates reflect sorghum's later movement into India.

<sup>&</sup>lt;sup>c</sup>Dates represent first appearance of free-threshing tetraploid wheats in the archeological record.

number of cultivars and land races, its morphological diversity is relatively limited. Its unique feature is the presence of three spikelets at each rachis node. Development of these spikelets forms the basis for dividing barley into two groups: (1) two-rowed barley with only the central spikelet fertile (primitive form) and (2) six-rowed barley with all three spikelets fertile (advanced form). Most domesticated barley has a hulled grain, although there are naked six-rowed cultivars given the botanical ranking of var. nudum (= var. coeleste). A fragile-rachis, six-rowed domesticated barley discovered in Tibet was originally proposed as a distinct wild, progenitor species and named H. agriocrithon. It has proven to be an introgressed form arising from hybridization between domesticated barley and weedy forms of H. spontaneum.

#### Maize

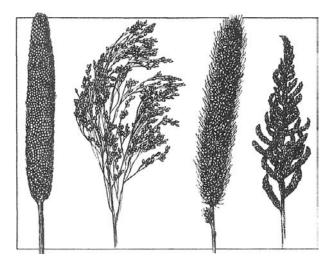
Among the domesticated cereals, maize (Zea mays) is unusual as a monoecious species whose cob, a highly compressed female raceme, bears naked grains. Wild maize relatives include various infraspecific forms of Z. mays and other wild and weedy annual and perennial Zea species (teosinte) and gamagrass, a member of the closely related genus Tripsacum. Theories explaining maize evolution have had to contend both with the absence of an identifiable wild progenitor as well as provide an explanation for the development of the highly specialized female inflorescence. Although researchers generally agree that maize evolved from teosinte, there is no universal agreement for explaining the steps from wild to domesticated forms. Current theories explaining maize evolution include the following: (1) a gradual change toward the domesticated form by mutation; (2) feminization of male flowers by a catastrophic mutation event, (3) rapid change by human selection of maize-like mutants, and (4) human selection of teosinte × *Tripsacum* hybrids (see Maize: Genetics).

The possibility of a teosinte × *Tripsacum* hybrid was originally proposed as the "tripartate hypothesis" whereby teosinte evolved from hybridizations between a maize progenitor, identified from the archeological record as pod corn, and a *Tripsacum* species. A more recently proposed scenario suggests human selection of maize-like forms that originated from natural hybridization between teosinte and *Tripsacum* and subsequent introgression. This hypothesis is supported by experimental hybridizations and offers the advantage of tying together biological and archeological evidence. Whatever the final elucidation of the evolutionary steps leading to maize, the role of humans in promoting the conditions by which speciation events (e.g., creating hybrid habitats)

occurred is as equally important as was their role as selection agents in directing the naturally occurring variation toward domesticated maize.

#### Millet

Millet in a broad sense actually comprises a loose grouping of small-seeded, drought-tolerant species that can be cultivated on poor soils (Figure 4). They belong to nine genera in the grass subfamilies: Paniceae (eight genera) and Chloridoideae (one genus) (Table 2). Due to a small size and hardened lemma and palea, millet grains are insect resistant, a feature that adds to their value for the African and Asian cultures that cultivate them. Only pearl millet (Pennisetum glaucum) is a major cereal grain on an international scale (see Millet: Minor). It was domesticated in tropical West Africa and still forms an integral component of décrue (flood stage to dry) agriculture in the Central African river deltas where it is cultivated along with African rice (Oryza glaberrima) and sorghum (Sorghum bicolor). Low genetic variation suggests a relatively limited region of origin, although the diversity of morphological forms has led to the classification of as many as 18 domesticated species (see Millet: Pearl). Pearl millet is a largely cross-pollinating species due to the unsynchronized maturity of the respective female and male flower structures. Although originally native to West Africa, pearl millet spread into India in prehistoric times where it became a major food staple. Active introgression makes it difficult to draw clear phylogenetic lines between pearl millet, its wild progenitor *P. violaceum*,



**Figure 4** Diversity and form in the millets. From left to right: panicles of pearl millet, proso millet, foxtail millet, and Japanese millet. (Reproduced with permission from Hancock JF (2004) *Plant Evolution and the Origin of Crop Species*, 2nd edn. Portland, OR: Book News Inc.)

Table 2 Minor cereal grains either cultivated or harvested as uncultivated wild species

Cereal	Species	Native region	Domestication status <sup>a</sup>
Adlay	Coix lacryma-jobi	South Asia	Domesticated
Fonio	Digitaria		
Raishan	D. cruciata	India, Vietnam	Domesticated
Fonio	D. exilis	West Africa	Domesticated
Black fonio	D. iburua	Nigeria	Domesticated
Sanguin panic	D. sanguinalis	Transcaucasus, Kashmir	Domesticated
Wild fonio	D. fuscescens	West Africa	Wild
Wild fonio	D. longiflora	West Africa	Wild
Mango	Bromus mango	South America	Domesticated
Millets			
Guinea millet	Brachiaria deflexa	West Africa	Wild
Browntop millet	Brachiaria ramosa	India	Domesticated
Shama millet	Echinochloa colona	India	Domesticated
Japanese millet	Echinochloa esculenta	East Asia	Domesticated
Finger millet	Eleusine coracana <sup>b</sup>	Ethiopia	Domesticated
Proso millet	Panicum miliaceum	Asia	Domesticated
Sowi millet	Panicum sonorum	Mexico	Domesticated
Little millet	Panicum sumatrense	Burma	Domesticated
Kodo millet	Paspalum scrobiculatum	India	Domesticated
Yellow foxtail	Setaria glaucum	India	Domesticated
Foxtail millet	Setaria italica	China, Europe	Domesticated
Oats	Avena		
Abyssinian oat		East Africa	Wild
Sand oat	A. abyssinica A. hispanica	Spain Spain	Domesticated
	A. Hispariica	Spain	Domesticated
Rice			
Wild rice	Oryza barthii	West Africa	Wild
African rice	Oryza glaberrima	West Africa	Domesticated
Southern wild rice	Zizania aquatica	North America	Wild
Northern wild rice	Zizania palustris	North America	Wild, domesticated
Sand Bur	Cenchrus biflorus	Central Asia	Wild
Tef	Eragrostis tef	Ethiopia	Domesticated
Wheat $(2\times)$	Triticum		
Small spelt	T. monococcum	Eurasia	Domesticated
Wheat $(4 \times)$	Triticum		
Emmer	T. dicoccum	Eurasia	Domesticated
Isphahan wheat	T. ispahanicum	Iran	Domesticated
Georgian emmer	T. paleocolchicum	Georgia	Domesticated
Ethiopian wheat	T. aethiopicum	Ethiopia	Domesticated
Khorasan wheat	T. turanicum	Iran .	Domesticated
Persian wheat	T. carthlicum	Southwest Asia	Domesticated
Polish wheat	T. polonicum	Southwest Asia, Mediterranean Basin	Domesticated
Poulard wheat	T. turgidum	Eurasia	Domesticated
Timopheev's wheat	T. timopheevii	West Asia	Domesticated
Wheat (6×)	Triticum		
Spelt wheat	T. spelta	Eurasia	Domesticated
Macha wheat	T. macha	Georgia	Domesticated
Vavilov's wheat	T. vavilovii	Georgia	Domesticated
Shot wheat	T. sphaerococcum	India	Domesticated
Zhukovsky's wheat	T. zhukovskyi	Georgia	Domesticated
	Znako vokyi	5.001gia	

<sup>&</sup>lt;sup>a</sup>Wild species are undomesticated and are weedy species that usually inhabit disturbed sites or agricultural fields. Some domesticated species listed here are recognized in other regions.

and the weed species *P. sieberianum*. Crop mimicry is a problem in African fields of pearl millet where weed races of *P. sieberianum*, known as shibras, are almost indistinguishable from the domesticated species.

# Oats and Rye

Common oat (*Avena sativa*) and rye (*Secale cereale*) developed as secondary cereal crops, beginning their

<sup>&</sup>lt;sup>b</sup>Member of the subfamily Chloridoideae. All other millet species belong to the subfamily Paniceae.

evolutionary routes toward domestication as weeds of wheat and barley fields. The early diploid progenitor species of A. sativa, a hexaploid, are unknown. The two weedy hexaploid species, A. sterilis and A. fatua, are considered to be the immediate wild ancestors, with A. sterilis believed to have played the principal role (see Oats). Differentiated by their disarticulation from the mature panicle, the A. sterilis form breaks as spikelet dispersal units containing all florets, whereas the A. fatua form disarticulates as individual floret units. According to current theories, oats were domesticated in Europe after they arrived there as weed contaminants in emmer, a primitive tetraploid wheat species (Table 2). Common oat has two hulled-grain types grouped by the location of the rachilla break (Figures 1 and 3) during threshing: (1) sativa form (sensu stricto) with the rachilla piece attached adjacent to the floret and (2) byzantina form with the rachilla piece attached below the floret. The naked-grain common oat cultivars are found in the byzantina group.

Rye is a complex of wild, weedy, and domesticated forms classified as S. cereale. The weedy rye forms that first infested wheat and barley fields in Western Asia are believed to have evolved from the perennial species S. montanum. Intermediate forms derived from hybridizations among the wild S. montanum and weedy and domesticated forms of S. cereale complicate the taxonomy. The weed races - segetale, afghanicum, dighoricum, and ancestrale - from which the domesticated form evolved are included in the S. cereale group (see Rye). This conspecific group includes non-shattering or semi-shattering forms with regional distributions across Western and Central Asia. A true wild race, vavilovii, whose spikes disarticulate, is presumed to have evolved from S. montanum. Although interfertile with domesticated rye, it is not believed to be a direct progenitor. Rye is a free-threshing, naked-grain species.

#### Rice

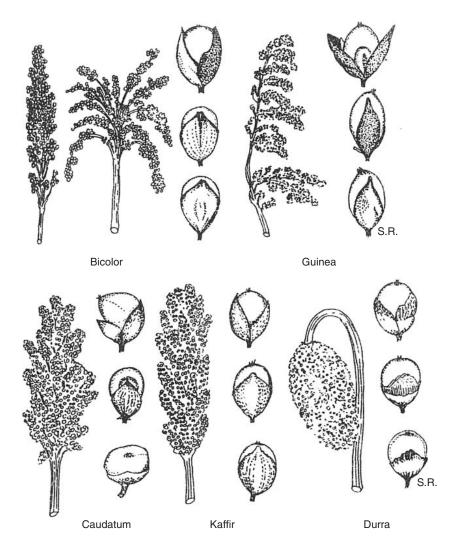
Although there are two species of rice, only one of these,  $Oryza\ sativa$ , which evolved in tropical Asia, has become a major cereal staple in international agriculture. In contrast,  $O.\ glaberrima$  is a relatively minor rice whose cultivation is confined to the equatorial belt of Western Africa where it is native. Both domesticated species and their wild relatives are diploid. Their high chromosome number (2n=24) suggests that they may be evolutionarily derived from ancient polyploid species whose genomes became diploidized. Both species are semiaquatic plants whose premodern cultivation was tied to seasonal flooding and rains. Of the two,  $O.\ sativa$  has a greater climatic and latitudinal diversity with

a range of forms found from tropical to temperate zones and cultivable in semiaquatic (lowland rice) versus dry land conditions (upland rice). It is a highly diverse species with three major races — the tropical races indica and javanica, and the temperate race japonica — each with a correspondingly wide range of cultivar diversity. The javanica race may be a derivative of hybridization between indica and japonica cultivars.

Most experts agree that O. sativa was domesticated in independent events in South and Southeast Asia. The wild progenitor species O. rufipogon is a perennial and it is likely that the steps toward domestication actually began with the annual derivative species, O. nivara (see Rice: Genetics). A weed race of intergrading forms, designated as O. sativa f. spontanea (= O. fatua), has developed from hybridization between domesticated rice and its wild and weedy relatives. The story with O. glaberrima is similar. Its direct wild progenitor is the wild annual species, O. barthii, which evolved from the perennial species, O. longistimata. Oryza barthii populates savanna water holes and has weedy forms that infest rice fields located in the high rainfall belt of the African savannah. For both rice species, genetic and morphological differences are sufficiently blurred as a consequence of introgression between the wild and domesticated taxa to prevent any well-defined phylogenetic distinctions.

#### Sorghum

Sorghum (S. bicolor) constitutes a polymorphic species complex of taxa evolved from introgression between domesticated, wild, and weedy forms (see Sorghum: Breeding and Agronomy). Knowledge of sorghum's domestication history is limited due to the poor archeological record for sub-Saharan Africa. Although the oldest archeological finds are from sites in India, they are interpreted as representing previously domesticated African sorghums brought to the Indian subcontinent by early human migrations. Five sorghum races – bicolor, guinea, durra, caudatum, and kafir – are identified, each with a distinctive eco-geographic range and place in indigenous African and Asian agriculture as well as morphological and use differences (Figure 5). Bicolor is the first evolved race, and along with the durra and kafir races form the stocks from which modern Western sorghum cultivars are developed. Relationships among the various infraspecific forms of sorghum are complicated due to active introgression between and among the domesticated and weed races. Four types of weed races form the sorghum crop—weed complex, each illustrating a different evolutionary route to weed status: (1) weedy wild race, (2) weedy hybrid form



**Figure 5** Panicles and mature grains still enclosed within their glumes for each of the five sorghum races. (Reproduced with permission from Luven P (1988) *Traditional Food Plants*. FAO Food and Nutrition Paper 42. FAO.)

derived from a cross of domesticated sorghum × wild sorghum, (3) weedy domesticated forms with a secondary shattering trait, and (4) weedy hybrid forms derived by crosses between domesticated forms. Sorghum also hybridizes with the wild, perennial Asian species, *S. propinquum*, and is believed to be one of the hybrid parents of *S. halepense*, whose weedy form is known as Johnson grass.

#### Wheat

The wheats comprise a large polyploid complex of wild and domesticated species belonging to the genera *Triticum* (wild and domesticated) and *Aegilops* (wild). For the domesticated species, two distinct evolutionary lines trace to the wild diploid species *T. urartu* and *T. boeoticum*, whose respective genomes are designated as "A," a cause for considerable confusion when not distinguished as either A<sup>u</sup> or

A<sup>b</sup> (see Wheat: Genetics). The two major wheats – T. durum (macaroni wheat) and T. aestivum (bread wheat) – are in the A<sup>u</sup>-genome line. Primitive tetraploid wheat (A<sup>u</sup>B-genomes) evolved directly from the wild tetraploid species T. dicoccoides, a derivative of a hybridization between *T. urartu* and a species as yet not definitively identified, but likely Ae. speltoides (B-genome). First domesticated in Western Asia, tetraploid wheats (Figure 6) spread inland to Central Asia, westward across Europe, and southward to Northern Africa, differentiating into a diverse array of minor cereals unique for their regional distribution and morphological and agronomic features (Table 2). The hexaploid group, which includes T. aestivum (A<sup>u</sup>BD-genomes) and several minor hulled and freethreshing forms (Figure 6 and Table 2), has no known wild progenitor. It is believed to have evolved in the continental regions of Central Asia as a consequence of a hybrid speciation event between a domesticated



Figure 6 Diversity within the wheats. From left to right: T. monococcum, T. dicoccum, and T. spelta (all hulled wheats), T. polonicum, T. turgidum, T. aestivum (compactum form), T. durum, T. aestivum (awned: "Turkey"), and T. aestivum (awnless: "Wilhelmina"). (Adapted from The Field Museum, negative B79676.)

tetraploid and the wild diploid species, Aegilops tauschii (D-genome). By comparison with the A<sup>u</sup>Bgenome tetraploids, diversity in the hexaploid group is relatively low.

The T. boeoticum line of domesticated wheat evolution has produced fewer and less important wheats, all hulled – T. monococcum (diploid; Figure 6), Triticum timopheevii (tetraploid), and T. zhukovskyi (hexaploid). T. timopheevii evolved from the wild species T. araraticum, which formed from a hybridization event between T. boeoticum and Ae. speltoides, here designated as donating the G-genome. Triticum araraticum is almost indistinguishable morphologically from T. dicoccoides, understandable given the similarities in their genome constitutions. T. timopheevii, and the related hexaploid T. zhukovskyi (A<sup>m</sup>A<sup>m</sup>G-genomes), have a limited distribution in the Transcaucasus region and display little infraspecific diversity.

Although the wheats are distinctive among the major cereals for the absence of intergrading cropweed complexes, there is evidence of gene flow between wild and domesticated species. The wild

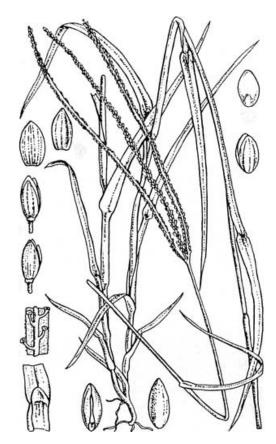


Figure 7 Vegetative and reproductive structures of fonio (D. exilis). The inflorescence is a digitate raceme with a winged rachis. (Reproduced with permission from Hag N (1995) A neglected cereal and two promising ones. In: Willams JT (ed.) Cereals and Pseudocereals. Chapman and Hall.)

tetraploid Ae. cylindrica (jointed goatgrass) is spread across Eurasia and has been introduced into North America where it has become a serious wheat-field weed. Recent studies suggest that hybridization between jointed goatgrass and bread wheat may involve gene flow of domesticated genes into the wild species on the basis of their shared D-genomes. It is not yet clear whether more aggressive, weedy forms of jointed goatgrass have actually arisen as a consequence. Gene flow between weedy forms of T. dicoccoides and durum wheat is also suspected.

#### **Minor Cereals**

The major cereals discussed above actually represent only a small sampling of the diverse collection of grass species that have been domesticated. Table 2 provides a partial listing of lesser-known cereals that are currently, or at one time were, important crops. Some of these cereals are grown on an extensive scale or across large regions (foxtail millet); others are locally restricted (fonio, Figure 7). Their importance is measured by their place as a dietary staple for the cultures

that cultivate them. Many of these species have been supplanted by the major cereals. For example, proso millet (Panicum miliaceum), once cultivated widely in Europe and the United States, has ceased to have importance as a cereal in these regions. As discussed above, African rice (O. glaberrima), an important species of décrue agriculture on the river deltas of Central Africa, is gradually being replaced by O. *sativa*. In the case of mango (Bromus mango), a regional cereal of Chile and Argentina, expansion of wheat and barley cultivation in these countries led to its disappearance in the nineteenth century. Mango is now under development as a promising new cereal. Some minor grains are being rediscovered, as is the case with the hulled hexaploid wheat, T. spelta, which is now a major player in the specialty USA cereal market.

Wild grasses and partially domesticated grasses still play an important role in the diet for some subsistence agricultural cultures. In famine times, West African peoples gather the wild fonio species *D. longiflora* and *D. fuscescens*. The weedy oat species, *A. abysinnica*, forms an integral component of the seed mixture for wheat and barley fields in Ethiopia. Subsistence farmers on the Anatolian plateau of Turkey allow weedy forms of *S. cereale*, which are more cold- and drought tolerant than the wheat crop that they infest. In bad years, this harvestable weed crop provides the "Wheat of Allah" for this traditional culture.

#### **Research Trends**

Evolution of the cereal species continues under plant breeding programs and the application of biotechnology to traditional breeding methods. Two new cereal species, Triticale (× Triticosecale) and intermediate wheatgrass (Thinopyrum intermedium), are products of modern breeding programs. Triticale, now grown on a commercial scale in Europe and North America, was synthesized from an intergeneric hybridization between wheat and rye. Intermediate wheatgrass, a species reported to have been cultivated as an ancient grain crop in Western Asia, is under development as a promising perennial cereal crop.

Traditional breeding methods are now being combined with the more sophisticated tools of biotechnology to broaden the base of usable germplasm sources for interspecific and intergeneric crosses through which new genes for pest resistance, salt tolerance, improved protein quality, and other agronomic traits can be introduced into cereal species. Genetically engineered cultivars, with alien genes, are being developed to enhance nutritive value (e.g., vitamin A in the O. sativa "Golden rice" cultivar) or to improve agronomic quality (e.g., insect resistance with Bt-maize; herbicide resistance in

wheat). Whether by traditional methods or with biotechnology, the same fundamental mechanisms are at play, with humans acting as selection agents in furthering the evolution of cereals.

See also: Barley: Genetics and Breeding. Cereals: Overview. Maize: Genetics. Millet: Pearl; Minor. Grain and Plants, Morphology. Oats. Rice: Genetics. Rye. Sorghum: Breeding and Agronomy. Triticale. Wheat: Genetics.

# **Further Reading**

Clark LG and Pohl RW (1996) Agnes Chase's *First Book of Grasses*. Washington, DC: Smithsonian Press.

Clayton WD and Renvoize SA (1986) Genera Graminum: Grasses of the World. Kew Bulletin Additional Series XIII. Kew: Royal Botanic Gardens.

Eubanks MW (2001) The mysterious origin of maize *Economic Botany* 55: 492–514.

Hancock JF (2004) Plant Evolution and the Origin of Crop Species, 2nd edn. Portland, OR: Book News Inc.

Harlan JR (1992) Crops and Man, 2nd edn. Madison, WI: American Society of Agronomy.

Harlan JR (1995) The Living Fields: Our Agricultural Heritage. Cambridge: Cambridge University Press.

Luven P (1988) *Traditional Food Plants*. FAO Food and Nutrition Paper 42. FAO.

Norman MJT, Pearson CJ, and Searle PGE (1995) *The Ecology of Tropical Food Crops*, 2nd edn. Cambridge: Cambridge University Press.

Peterson RF (1965) Wheat: botany, cultivation, and utilization. In: Polunin N (ed.) World Crop Books. New York: Interscience.

Schery RW (1972) *Plants for Man*, 2nd edn. Englewood Cliffs, NJ: Prentice-Hall.

Smartt J and Simmonds NW (eds.) (1995) *Evolution of Crop Plants*, 2nd edn. Essex: Longman Scientific and Technical.

Vietmeyer ND (Director) (1996) Lost Crops of Africa: Grains, vol. 1. Board on Science and Technology for International Development. National Research Council. Washington, DC: National Academy Press.

Williams JT (ed.) (1995) Cereals and Pseudocereals. London: Chapman and Hall.

Zohary D and Hopf M (2000) Domestication of Plants in the Old World: The Origin and Spread of Cultivated Plants in West Asia, Europe and the Nile Valley, 3rd edn. Oxford: Clarendon Press.

#### **Relevant Websites**

http://singer.cgiar.org — A searchable crop germ plasm database (System-wide Information Network for Genetic Resources) with links to the international agricultural research center members of the Consultative Group on International Agricultural Research (CGIAR).

http://www.ars-grin.gov — A searchable United States Department of Agriculture crop germplasm database designed around *World Economic Plants: A Standard Reference*, which is set up in three language versions — English, Portugese, and Spanish. http://mansfeld.ipk-gatersleben.de — An internet-searchable, version of *Mansfeld's World Database of Agricultural and Horticultural Crops*, which is set up for queries on the taxonomy, common names, wild distribution, and other miscellaneous information about crop plants.

http://wheat.pw.usda.gov — The United States Department of Agriculture's searchable database for a variety of topics dealing with the genomics, mapping, germplasm, pathology, and taxonomy of the cereals barley, rye, triticale, wheat, and oats.

http://www.gramene.org — Gramene is a comparative mapping site for cereal species funded by the United States Department of Agriculture. It operates as a curated, open-source on-line data resource for comparative genome analysis.

# CHEMICALS FOR GRAIN PRODUCTION AND PROTECTION

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#### Introduction

Chemicals have a critical role in modern cereal production and are used at all points in the grain production and storage cycle, from seed dressing, through to soil preparation, to crop, and stored-product protection. There are several classes of agro-chemicals associated with grain production and protection. These include seed dressings, trace elements, fertilizers, herbicides, crop-protection pesticides, stored-product pesticides, structural treatments, and rodenticides. Trace elements, fertilizers, herbicides, and crop-protection pesticides are often very specific for particular biogeographical and agro-geographical situations and represent a diverse range of chemicals.

Chemicals used for structural treatments of storages, stored products, seed storage, and rodenticides tend to be more universal and cover a much smaller range.

In most jurisdictions, there are specific regulations concerning the use, supply, labeling, transportation, storage, and importation of pesticidal chemicals (insecticide, acaricide, rodenticide, fungicide, nematicides, molluscicide, and herbicide). The administration of these regulations may be associated with the

authorities concerned with environment, public health, workers' safety, and agriculture. The specifics of the regulations may differ from place to place but the general rule is that a chemical may only be used in compliance with the label accompanying the product. This label will be specific to a particular jurisdiction. The label will contain specific information about what crops the material can be used on, what pests it is intended for, how to apply and rates of application, withholding times for treated crops, disposal of containers, personal/environmental safety, and first aid.

Registration is normally granted when it is shown that a pesticidal chemical can be used in compliance with all regulations, and there is a label that, when followed, will ensure that the user will meet regulatory requirements. The registration of agricultural chemicals is usually handled by a government agency. In the United States of America, this agency is the Environmental Protection Agency (EPA); in Australia, it is the Australian Pesticides and Veterinary Medicines Authority (APVMA); in the United Kingdom, it is The Pesticides Safety Directorate (PSD); other countries have equivalent bodies.

Trace elements and fertilizers are not generally subject to specific government registration. Application rates and methods of use will usually depend on local agronomic requirements and practice.

There have been many concerns expressed regarding the use of all chemicals in agriculture over recent years. However, their use continues because their role

remains an important part of food production, handling, and storage. Considerable effort has been directed since the 1980s to finding ways of using agro-chemicals (biocides and fertilizers) in a way that is sustainable, environmentally safe, and with minimal threat to the health of agricultural workers or consumers.

Although many agricultural chemicals are potentially dangerous, they should pose little threat if used strictly in accordance to the manufacturers' instructions. Risks are further reduced if the user reads and understands a "material safety data sheet" (MSDS) for the chemical concerned. In many jurisdictions, an MSDS must be available to people that handle, store, or use potentially dangerous goods.

The aim of this article is to provide an overview of the diverse range of chemicals used in grain production and storage, and to give a brief insight into their use and composition. Any detailed discussion of this subject would entail a review of large portions of the subject matter contained in the sciences of agronomy, economic entomology, plant pathology, and stored-product technology.

#### **Seed Treatments**

#### **Fungicides**

Fungicidal seed dressings are sometimes applied to stored seed to prevent disease development, which would impair the seed's ability to germinate, or the seedling's ability to grow in a normal manner. Fungicides are not generally used to prevent the development of storage fungi, which are better controlled by maintaining the seed in cool dry storage conditions. Germination is best maintained under conditions where fungal growth is not a problem. The optimum conditions for seed storage are well documented and have been known for many years.

Recommendations for the use of fungicidal seed dressings will vary with the prevalence of particular disease risks, national and state regulations, and availability of chemicals. Fungicidal seed dressings are particularly useful in cereal crops for controlling bunt and smut, and can assist in reducing the rate of development of some rusts. The following is a synopsis of the recommendations for bunt control in the State of Queensland, Australia. It is shown in order to give an idea of the role of preplant fungicides.

- Seed that is sown to provide the following season's seed needs to be treated with a fungicidal seed dressing.
- Seed from plants grown from untreated seed should be treated with a fungicidal seed dressing before planting.

- All seed entering Queensland should be treated with an appropriate fungicidal seed dressing.
- Grain from a crop with bunt should not be used for seed.
- Wheat seed from farms where a crop has been affected by bunt should be treated with fungicidal seed dressing for at least 6 years.

In Australia, fungicidal products containing the following active compounds are available, either singly or as components of a mixture, for seed dressings to control bunt (and some other seed-borne fungal diseases): carboxin, fluquinconazole, flutriafol, tebuconazole, thiram, triadimenol, and triticonazole.

Almost all chemical treatments have some disadvantages as well as the obvious advantages. The use of a fungicidal seed dressing, in general, renders the seed unfit for animal or human consumption, while a number of fungicidal seed dressings may reduce or retard seedling emergence. This is a problem that may be aggravated where the seed is sown in suboptimal conditions.

#### **Fertilizers**

There are many parts of world where additional phosphorus and/or nitrogen is essential to maintain a viable cereal cropping industry. The requirements for the other nontrace nutrient elements – potassium, calcium, magnesium, and sulfur – are less common and more localized.

#### Nitrogen

Cereal cropping removes nitrogen from the soil and, in almost all cases, that nitrogen will need to be replaced in order to allow sustainable cropping. Crop rotation with nitrogen-fixing plants and animal grazing has been used traditionally to replace soil nitrogen. Application of nitrogen fertilizer has now become standard practice for cereal grain producers in many parts of the world. The major nitrogen (N) fertilizers are: ammonium nitrate (34% N), urea (46% N), anhydrous ammonia (82% N), and urea—ammonia nitrate mixtures (ammonium nitrate and urea dissolved in water containing 28–32% N).

The cost and availability of nitrogen fertilizers varies greatly depending on location. Liquid urea—ammonium nitrate is often the most expensive treatment. The dry forms are intermediate and anhydrous ammonia is the most economical. Ammonia can, in some places, cost as much as 30–40% less for an equal amount of nitrogen, compared to the dry and liquid nitrogen forms. However, the complexity and costs associated with logistics and supply of ammonia make its use impractical in some regions.

#### **Phosphorus**

Phosphorus-containing fertilizers, like nitrogencontaining ones, are widely used in cereal growing. In places where the soil is low in phosphate, fertilizer addition is essential to give and maintain viable cereal yields. In recent years, concerns have been expressed about the effects of phosphate fertilizers on the surrounding environment associated with soluble phosphate ending up in waterways. This has led to several jurisdictions formulating guidelines for the application of phosphate fertilizers (also other fertilizers).

Phosphorus is applied as ammonium phosphates or superphosphate, and phosphorus fertilizers are often characterized by their (N-P-K) composition. Common phosphate fertilizers include liquid ammonium polyphosphate (10-34-0), dry mono- or di-ammonium phosphate (11-48-0, 16-48-0, or 18-46-0), superphosphate (0-20-0), or concentrated superphosphate (0-45-0).

#### **Sulfur**

Sulfur-deficient growing conditions are much less common than is the case for nitrogen or phosphorus. The most common sulfur-supplying fertilizers are ammonium sulfate or gypsum. These are best applied as top dressing where they are immediately taken up by wheat plants. Elemental sulfur may have some role as a preplant sulfur source. However, it is chemically unavailable to the plant when used as top dressing for growing plants.

#### **Potassium**

Potassium deficiency can limit wheat crop growth but the deficiency is not common. There are some restricted localities around the world where the use of a potassium-supplying fertilizer is useful. Potassium is usually applied as either potassium chloride (~60% K) or potassium sulfate (50% K). Potassium nitrate (37% K), another potential fertilizer, is not commonly used for cereal crops.

#### **Calcium and Magnesium**

There may be a need for additional calcium and magnesium in leached soils in high rainfall and acid-rainfall areas. Calcium is frequently obtained coincident with liming as part of soil pH control. Both calcium and magnesium can also be supplied by adding crushed limestone.

#### **Trace Elements**

Deficiency of trace minerals such as boron, iron, zinc, manganese, molybdenum, and copper can be limiting

to crop growth and health. Seed dressings along with foliar sprays are often effective in correcting nutrient deficiencies, particularly when used in combination with other ameliorating techniques (e.g., soil sprays, nutrient-enriched granular fertilizers). Seed dressing with trace minerals is rarely adequate on its own. Where no nutrient deficiency exists, the application of trace element seed dressings and foliar sprays may be of little benefit. Trace element deficiencies can be localized to very small areas, and are rarely general over wide regions or countries. Trace element deficiency is usually first recognized as a possibility by defects in plant growth. However, the visual symptoms of a particular deficiency can be confused with those produced by a range of other crop growth problems. Diagnosis of a trace element deficiency should be verified by plant and/or soil analysis where this is possible.

#### **Herbicides**

Herbicides are used both as a component of zero- or low-till agriculture and for weed control in the growing crop. Herbicides are best used when the weeds are young and actively growing. Both weed and crop growth stages are critical to the successful use of postemergence herbicides. Cereal species and varieties can vary widely in their herbicide tolerance. As is the case with the use of all bioactive chemicals, it is important to read labels carefully for details on crop tolerance and information on optimum and safe application conditions.

Many broad-leaf weeds can be economically controlled using a strategic application of a post-emergence herbicide. Some grassy weeds such as wild oats, annual ryegrass, and phalaris can also be controlled. This can be achieved by carefully controlled pre- or postsowing spraying in some cereals but not all. There are other grassy weeds in cereals that cannot be controlled with herbicides.

Some weed species, particularly grasses, germinate throughout the growing season, especially in grazed crops, when ground cover is reduced and soil temperatures rise. In this case, two herbicide applications at different growth stages could be warranted. For example, an early postemergence spray may not completely control weeds such as wild radish and a late application can be justified to minimize seed set and seed harvested with the grain.

In Australia, the following active compounds are registered as herbicidal preparations for various phases in the cereal growing cycle; they may either be registered as components of a mixture or singly or both.

- Presowing and seedbed preparation. Chlorsulfuron, oyzalin, pendimethalin, sulfosulfuron, triallate, trifluralin, and triasulfuron.
- Salvage seedbed preparation. 2,4-D, dicamba, diquat, glyphosate, glyphosate trimesium, and paraquat.
- Early postemergent. 2,4-DB, bromoxynil, chlorsulfuron, clodinafop-propargyl, clopyralid, dicamba, diclofop-methyl, diflufenican, diuron, fenoxapropp-ethyl, flamprop-M-methyl, flumetsulam, MCPA, metasulfuron, metosulam, picloram, sulfosulfuron, terbutryn, thifensulfuron, and tralkoxydim.
- Late postemergent. 2,4-D amine, 2,4-DB, 2,4-D ester, dicamba, flamprop-M-methyl, MCPA, flumetsulam, picloram, and triasulfuron.
- Fallow and stubble weed management. 2,4-D, 2,4-D amine, 2,4-D ester, diquat, dicamba, glyphosate, glyphosate trimesium, imazameth paraquat, tribenuron methyl, and triclopyr.

The label instructions for use of these materials are complex and specific and take into account a wide range of parameters, which include the occurrence of resistance, stages in the planting/growing cycle of the cereal, cereal variety, target weed species, and its stage in the growing cycle, crop stress, and local regulations.

#### **Crop-Protection Chemicals**

Insect pests of growing cereal crops will vary widely on a geographical basis. The situation in Australia gives an indication of the role for crop-protection chemicals, but each region will have its own suite of pests and its own range of allowable pesticides.

In Australia, lepidopteran larvae, a few mite species, and aphids pose the most common and widespread invertebrate threat to growing cereal crops. There are also occasional regional and severe outbreaks of Australian plague locusts, and there are some areas where white Mediterranean snails can cause problems in harvesting and marketing grain. There are times when mouse plagues are so severe that action is needed to prevent economic consequences. Some soil insects such as wireworms, false wireworms, wingless cockroaches, black field earwigs, and seed harvesting ants may cause economic levels of damage. Additional groups of pests in other parts of the world include Hemiptera, dipteran larvae, and thrips.

The precise chemical to use for a particular control need is too complex to consider here. In Australia, the United Kingdom, Canada, the United States, and many other countries, there are a series of electronic resources that make the selection of appropriate

chemicals relatively simple. These assist in the decision process and help match the permitted use of the chemical to the target pest, the target crop, stage of crop growth, and withholding periods. The list that follows shows the suite of active compounds available in Australia for use on growing cereal crops for various broad taxonomic groups of pests. The complexities of registration mean that a chemical may not be appropriate for every species in the indicated group or indeed for every cereal crop.

- For Lepidoptera. α-Cypermethrin, Bacillus thuringensis preparations, β-cyfluthrin, carbaryl, chlorpyrifos, cypermethrin, deltamethrin, diazinon, esfenvalerate, malathion, methidathion, permethrin, trichlorfon, and ζ-cypermethrin.
- For aphids.  $\alpha$ -Cypermethrin,  $\beta$ -cyfluthrin, dimethoate, and esfenvalerate.
- For locusts and grasshoppers. Carbaryl, chlorpyrifos, dimethoate, fenitrothion, and maldison.
- For mites. α-Cypermethrin, β-cyfluthrin, bifenthrin, chlorpyrifos, cypermethrin, dimethoate, λ-cyhalothrin, maldison, methidathion, omethoate, and phosmet.
- For snails. Methiocarb.
- For mice. Zinc phosphide.

# **Foliar Fungicides**

The use of foliar fungicides is highly dependent on the suite of fungal pathogens present and the degree to which they present a risk to economic crop production. The nature of this risk will vary considerably between crops, from region to region, and in many cases from year to year. Region-based advice is usually required to determine the specific requirements for foliar fungicide application. This advice is available, depending on the country concerned, through a range of commercial, government, or university agencies. In recent times, many of these agencies have set up websites addressing this type of regional advice. A sample of these websites is included in the references at the end of this article.

While there is a wide range of fungicidal preparations, the number of active chemicals involved is relatively small. For example, in Australia the following are registered for application to growing cereal crops: benomyl, flutriafol, propiconazole, tebuconazole, terbufos, triadimefon, and tridemorph.

#### **Stored-Product Protection**

Chemical treatment of stored grains can have a profound effect in maintaining cereal stocks by reducing (almost eliminating) losses caused by animal pests. In many climatic zones, long-term storage of grain without loss of biomass or quality would be almost impossible without some form of postharvest chemical treatment. Despite this, there is considerable pressure to reduce chemical treatments of grain in storage. This is largely based on the complexities of dealing with the residues that may be associated with the treatment.

In stored products, compared to field crops, these residues are much closer to the final consumer in the consumption chain. Unlike horticultural products that also have a short path to the consumer, grains have many end uses including: direct consumption without preprocessing by animals (and to a small extent humans), milling into multiple physical fractions, a raw product for extraction of chemical fractions (starch and gluten), a source of carbohydrate for the fermentation industry, and the base product for a whole range of manufactured human and animal foods. This means that treated cereals have multiple entry points for residues into food for human consumption, either directly or via animal products such as meat and milk. This complicates the pathway to registration to such an extent that it may become too hard to achieve within financial constraints.

#### **Chemical Control**

For the purpose of convenience, chemical use in grain storage may be divided into several broad groups: fumigants, where the active agents are gases or vapors; grain protectants, where the active agents are applied directly to the grain as liquids or powders; structural treatments, where they are applied within the structure but not directly to the grain; and low risk use chemicals, where the toxic agent has a low mammalian toxicity and limited environmental risk.

**Fumigation** As of 2003, phosphine and methyl bromide are the only two fumigants in widespread use for fumigation of grain and grain-storage structures.

Phosphine Phosphine is applied in two physical forms: as a metallic phosphide (usually aluminum phosphide) that reacts with ambient moisture to release phosphine, or as gaseous phosphine from pressurized cylinders. Phosphine is a relatively slow-acting fumigant and depending on concentration and target species takes from 5 to 25 days for complete disinfestation.

Phosphine is the more commonly used of the two major fumigants and is used under a wide range of conditions, many of which are well below those associated with good fumigation practice. A consequence of this widespread use is that the continued use of phosphine is threatened by the world-wide occurrence of resistant insect populations and concerns about risks to human safety associated with its application.

Methyl bromide Methyl bromide is a rapidly acting fumigant typically giving complete disinfestation in 12–48 h. This role is not sustainable, as it has been shown to be a potent stratospheric ozone-depleting substance. This means that its use has been severely curtailed, and under international treaty after 2005 it will only be available for genuine quarantine and related preshipment uses.

Fumigants under development Because of the importance of fumigation there has been considerable research into replacements for methyl bromide and alternatives to phosphine. Carbonyl sulfide, ethyl formate, and sulfuryl fluoride all show some promise at the research level. They are not yet available for commercial use on grain and are unlikely to be commercially available for a further 2–3 years, even if the process of commercialization proceeds without problems.

Grain protectants Grain-protectant chemicals are designed to protect (as opposed to disinfest) grain. Early grain protectants included DDT and dieldrin in the 1940s and 1950s. Newer, less persistent chemicals replaced them in the 1960s. By the mid-1980s a suite of  $\sim$ 21 grain-protectant chemicals were either available or close to being commercialized. The number has fallen considerably since then and, by 2003, in Australia only carbaryl, chlorpyrifos, cypermethrin, deltamethrin, dichlorvos, fenitrothion, malathion, methoprene, and pirimiphos-methyl remain. Of these, some are not available for grains destined for some specific end uses. For example: carbaryl can only be used on feed grain; cypermethrin can only be used on seed grain; only low doses of methoprene and no chlorpyrifos methyl can be used on malting barley; and no deltamethrin can be used on export grain for some destinations. Other chemicals, such as malathion, have considerable resistance problems and finally a few like dichlorvos are under active regulatory review.

A further complication is that many of these materials are required to be part of a mixture to ensure a complete kill of all insect pest species. In some parts of the world, the list of registered materials is even smaller; for instance, in the UK only chlorpyrifos and pirimiphos-methyl are listed for use on stored grain.

It appears that grain-protectant chemicals only have a limited future in many parts of the world and alternatives such as fumigation, controlled atmosphere, and physical methods will have to become much more common.

Controlled atmospheres Controlled atmospheres can be thought of as a special case of fumigation or as low-risk chemical treatments. The gases used are normal components of the atmosphere and biological systems. The gases most commonly used are nitrogen to displace oxygen, and carbon dioxide as a toxic agent in its own right. Costs and logistics limit the use of these treatments. Long exposure times (typically 15 days or longer), high level of sealing, and the large volumes of gas required to establish or maintain concentration of >40% for carbon dioxide and 99% for nitrogen (<1% oxygen) are the main constraints. However, both types of controlled atmospheres are used where these factors are not limiting, or where special requirements exist.

Structural treatments There is a range of chemicals that can be used to treat empty grain storages, grain handling, and harvesting equipment. This is a slightly larger range of chemicals than that available for direct application to grain. It is generally recognized that insect pests associated with residual grain and other plant residues/dust are best dealt with by removing the residues rather than chemical treatment. Nevertheless, there are occasions where a somewhat persistent surface treatment or time-release chemical space treatment may be useful as an adjunct to but not a replacement for good hygiene.

Registration of chemicals for structural treatments vary greatly, but in Australia the following are registered for use in structures where there is no chance of direct application to grain (however, some do have separate registration for direct application to grain): amorphous silica, azamethiphos, deltamethrin, dichlorvos, phosphine, methyl bromide, and pyrethrins plus piperonyl butoxide.

#### **Rodenticides**

Chemical control of rodents is an integral part of stored-grain management. In grain storages, unlike most industrial and domestic situations, it is almost impossible to control populations by isolating the rodents from their food. In grain storages, there is an ever-present, easily available food resource. Physical isolation of that resource from the outside environment is often impractical. There are also obvious risks associated with using a chemical designed to kill

mammals in close proximity to a major human and domestic animal food.

The range of behavioral and physiological adaptations available for the target organisms means that rodent control strategies have to be well-planned to be successful. There is a fairly wide range of active materials that allow a flexible range of control to fit with the circumstances surrounding the need for rodent control. Factors influencing choice include the severity of infestation, the proximity of the stored grain, the previous history of treatment, the phase in the rodent control cycle (disinfestation or maintenance), the species present, physiological resistance, bait shyness, and the risks to nontarget species including humans.

The following active compounds are available in Australia for use in and around grain stores: brodifacoum, bromadiolone, cholecalciferol (vitamin  $D_3$ ), coumatetralyl, flocoumafen, strychnine, warfarin, and zinc phosphide. In the UK, the list is similar but does not include strychnine. A similar range of materials is permitted in most other countries.

See also: Barley: Harvesting, Storage, and Transport. Canola: Harvest, Transport, and Storage. Cereals: Grain Diseases. Contaminants of Grain. Food Safety through the Production Chain. Plants: Diseases and Pests. Sorghum: Harvest, Storage, and Transport. Stored Grain: Handling from Farm to Storage Terminal; Physico-Chemical Treatment. Wheat: Harvesting, Transport, and Storage. Appendix: Test Methods for Grain and Grain-Based Products.

#### Further Reading

Engelstad OP (ed.) (1985) Fertilizer Technology and Use. Madison: Soil Science Society of America.

Lægreid M, Bøckman OC, and Kaarstad O (1999) Agriculture, Fertilizers and the Environment. Farnham: CABI Publishing.

Lazenby A and Matheson EM (eds.) (1987) Australian field crops, vol. 1. Wheat and Other Temperate Cereals. North Ryde: Angus and Roberson.

Mullen CL, Dellow JJ, and Tonkin CJ (2000) Weed Control in Winter Crops 2000. NSW: NSW Agriculture.

Owen EB (1956) The Storage of Seeds for Maintenance of Viability. Farnham Royal: Commonwealth Agricultural Bureaux.

Rowland P and Bradford D (eds.) (1998) Proceedings of the Australian National Pesticide Risk Reduction Workshop. Canberra, Australia: Bureau of Rural Sciences, Agriculture, Fisheries and Forestry.

Snelson JT (1987) Grain Protectants. ACIAR Monograph No 3. Canberra, Australia: Australian Centre for International Agricultural Research. The e-UK Pesticide Guide 2003 gives details of pesticides actively marketed in the UK and is designed to ensure the correct pesticide for the job in hand. Wallingford: CABI Publishing.

Chemical Safety CD-ROM International Occupational Safety and Health Centre CIS International Labour Office 4 Route des Morellos, CH-1211 Geneva 22. Switzerland.

Tomlin C (ed.) (1995) *The Pesticide Manual*. Farnham: British Crop Protection Council.

#### **Relevant Websites**

Much of the information on chemical use is appropriate to small geographic regions; this type of information is best obtained by specific searches of the Internet, where this is available. Search terms should include the crop, the region, the broad generic nature of the chemical, and, where the chemical is a biocide, the target organism. Where the Internet is not available, the information should be available from traditional agricultural extension sources such as local offices of government departments responsible for agriculture, regional universities, independent farm advisors, and chemical suppliers.

http://www.fao.org — The FAO Inpho website deals with postharvest matters. Using the built-in search engine to find "Pesticides," provides a variety of topics around the subject of grain protectants.

http://www.pesticides.gov.uk — Provides information on registered pesticides in the United Kingdom. http://www.cdpr.ca.gov — A website maintained by the California Enviornmental Protection Authority that provides convenient access to the USEPA/OPP database. This database contains comprehensive information about pesticides registered in the United States of America.

http://www.apvma.gov.au — This website from the Australian Pesticides and Veterinary Medicines Authority contains information on all registered pesticides available for use in Australia.

http://www.defra.gov.uk — Department for Environment Food and Rural Affairs; Fertilizer recommendations for agricultural and horticultural crops: Section 1: Principles of nutrient management and fertilizer use. http://www.hse.gov.uk — A UK website on the safe and efficacious use of rodenticides in agricultural enterprises. http://www.grdc.com.au — Seed dressings and trace-element-deficient conditions under Australian conditions.

http://www.teagasc.ie — The site of Teagasc, which is the national body responsible for research, training, and advice for the Agri-Food industry in Ireland. It provides an example of an extension source that addresses advice on appropriate use for various agro-chemicals on a constantly updated basis tailoring the advice to the time of year and contemporary seasonal conditions.

http://www.defra.gov.uk - Considers effective trace elements and fertilizers use in the UK from an environmental point of view.

http://www.ianr.unl.edu — General discussion on major fertilizers from Cooperative Extension, University of Nebraska, Institute of Agriculture and Natural Resources.

http://www.dpi.qld.gov.au — Soil Insect Pests in Leucaena, Pasture and in Summer and Winter Field Crops. Discusses the identification, importance, and control of a wide range is soil inhabiting insect pests.

http://apps.fao.org — Site of the Codex Alimentarius Commission of the Food and Agricultural Organisation that explains and defines a whole range of terms associated with pesticide residues in foods. It is also an interactive database of maximum residue limits for pesticides of food materials (including grains) in international trade.

http://www.dpi.qld.gov.au — Infopest AGVET (updated three times a year). The complete reference of Australian registered insecticides and miticide chemicals and their uses. Available from Animal and Plant Health Services, Department of Primary Industries, GPO Box 46 Brisbane Queensland 4001 Australia.

# **CHICKPEA**

Contents

**Overview** Agronomy

#### Overview

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#### Introduction

Chickpea was one of the first legume crops domesticated. Today it is a key component of cropping systems in many parts of Asia and Africa, providing families of resource-poor farmers with a valuable source of dietary protein. Chickpea is also becoming established in some agriculturally advanced nations in response to a growing world demand. This article describes the species, its uses, and role in farming systems around the world.

# **Biology**

Chickpea (Cicer arietinum L.) is a herbaceous, annual legume. Plants are typically short (20-50 cm) with a semi-erect to erect growth habit and a variable number of primary and secondary branches. An imparipinnate leaf type, producing 11–18 small leaflets, gives the foliage its distinct fern-like appearance (Figure 1). Chickpea is a long day plant; however, time to flowering is accelerated by increasing temperature. Flowering is indeterminate with single (rarely double), small purple or white flowers produced on racemes arising from the leaf axil. Pods have a characteristic oval to rhomboid shape and inflate rapidly after (self-) fertilization (Figure 1). All aerial plant parts excepting flowers have a dense covering of fine hairs which secrete a mixture of organic acids. Roots are colonized by Mesorhizobium ciceri, a nitrogen-fixing bacterium specific to the genus Cicer. Nodules formed by these bacteria vary in size, the largest approaching 3 cm in diameter.

The seed is highly variable in appearance but invariably possesses the characteristic chickpea "beak" protruding over the embryo. Seed shape, color, and, to a lesser extent, size separate the species into three main groups: "desi," "kabuli," and "pea" (Figure 2).



Figure 1 Chickpea branch showing typical leaf and pod shape. (Chickpea normally produces single, axillary flowers; the "doublepodded" variant shown here may confer some yield advantage in low productivity environments.)



Figure 2 Seed type in chickpea showing, clockwise from top of picture: kabuli, desi, and pea (intermediate) forms.

Desi (derived from the Hindi and Urdu word for "native" or "local") seeds have an angular shape and thick, colored (mostly brown) seedcoat. In contrast, kabuli seeds have a more rounded shape and thin, white to cream seedcoat. There is considerable overlap between these two seed types in seed size; however, desi seeds are generally smaller (weighing  $80-350 \,\mathrm{mg}$ ) than kabuli seeds ( $100-750 \,\mathrm{mg}$ ). The pea-type seed has little commercial significance.

# **Origin and Spread**

The genus Cicer contains 43 species, 34 perennial, and 9 annual. All annual species are diploids and have 16 chromosomes (2N = 16). Amongst these, C. reticulatum is the most closely related to the cultivated chickpea and the presumed progenitor. Domestication occurred about 10 000 years ago in the region centered on south-eastern Turkey, part of the "Fertile Crescent." This would have involved a number of steps, including selection for more erect plant type, reduced pod shattering, and palatable, nondormant seeds. About 4000-5000 years ago the domesticated forms, initially desi types, commenced a westward movement along established trade routes toward the Mediterranean. Subsequent spread to southern Europe and North Africa, including Ethiopia, occurred by land and/or sea routes. Differentiation of the kabuli type is thought to have occurred in the Mediterranean region in comparatively recent times. The eastward movement of desi types to India commenced about 4000 years ago, whereas kabuli types did not arrive there until about 300 years ago. (The prominence of Kabul on the "Silk Road" suggests both an overland introduction and

the origin of the word "kabuli.") In the sixteenth century Spanish and Portuguese travelers carried kabuli types to South and Central America where they came to be known by their Spanish name "garbanzo." Chickpea industries have comparatively recent histories in the USA (1950s), Australia (1970s), and Canada (1990s).

#### **Production**

During the 1990s world chickpea production has averaged 8 million tons (Mt) from 10.5 million hectares (Mha). Crop area now is similar to that in the early 1960s; increased production since then has been generated by higher yields (Figure 3). Historically, Asia has been the major chickpea producer. For the five years 1997–2001, it accounted for 87.4% of all production, followed by the Americas (5.5%), Africa (3.9%), Australia (2.5%), and Europe (0.8%). Desi types contribute ~80% of production worldwide.

Chickpea is cropped in  $\sim 50$  countries. India still produces two-thirds of the world crop (Table 1) despite a steady decline in the area sown. A similar trend has occurred in Pakistan, the world's second largest producer and, more dramatically, in European countries where chickpea area has fallen by 60-97% since the 1960s. On the other hand, production has risen significantly in some countries. In Turkey, the world's third largest producer, chickpea area increased rapidly during the 1980s as fields previously left fallow after cereals were sown to chickpeas. New chickpea industries in Australia and Canada have also helped stabilize or even increase world production in recent years.

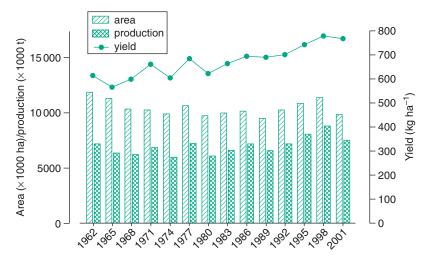


Figure 3 World area, production, and yield of chickpea, 1961–2001. Each datum is the mean for a three-year period. (Source: FAO.)

Table 1 Mean area, production, and yield of chickpea in major chickpea-producing countries (1997-2001)

Country	Area (ha)	Production (t) <sup>a</sup>	Yield (kgha <sup>-1</sup> ,
India	6 783 000	5 427 840 (66.4)	796
Pakistan	1 031 240	604 180 (7.4)	581
Turkey	658 400	597 600 (7.3)	905
Iran	649 480	238 236 (2.9)	366
Canada	162 080	219 880 (2.7)	1351
Mexico	136 167	219 947 (2.7)	1593
Australia	245 400	204 168 (2.5)	869
Ethiopia	180 901	164 917 (2.0)	917
Myanmar	127 607	89 880 (1.1)	705
Syria	88 349	59 400 (0.7)	660
Spain	88 320	52 600 (0.6)	591

<sup>&</sup>lt;sup>a</sup>Percentage of world production in brackets. Data from FAO.

Average world yield over the period 1997-2001 was 773 kg ha<sup>-1</sup>. Central and North America had the highest yields  $(1483 \text{ kg ha}^{-1})$  followed by Australia (869 kg ha<sup>-1</sup>), Asia (750 kg ha<sup>-1</sup>), Africa  $(689 \text{ kg ha}^{-1})$ , and Europe  $(625 \text{ kg ha}^{-1})$ . Yields were generally highest in countries where all or most of the crop was irrigated, such as Lebanon  $(2367 \text{ kg ha}^{-1})$ , Israel  $(1886 \text{ kg ha}^{-1})$ , and Egypt  $(1792 \text{ kg ha}^{-1}).$ 

#### **Consumption and Trade**

Chickpea is an important part of the diet in most producing countries and is gaining in popularity elsewhere. Per capita consumption is generally much higher in producing countries, such as India (5.1 kg pc) and Pakistan (4.4 kg pc), than in developed, nonproducing countries such as the United Kingdom (0.2 kg pc). Consumption is also comparatively high in some wealthier, producing countries such as Turkey (6.3 kg pc) and Spain (2.3 kg pc).

Historically, most chickpeas were consumed in their country of origin. Until the late 1970s, international trade accounted for only 2% of world production. This percentage rose to 8% in the following two decades as demand exceeded supply in major producing countries, especially in east Asia. During the period 1996–2000, India was the largest importer (138 000 t) followed by Pakistan (60 000 t) and Spain (56 000 t). For the same period, Australia was the largest exporter (240 000 t), followed by Turkey (153 000 t) and Mexico (132 000 t). The volumes of desi and kabuli types now traded are similar. Prices fluctuate according to supply and demand; however, there is generally a premium for kabuli types and particularly for those with large seed (diameter > 10 mm).

## **Role in Farming Systems**

Chickpea is grown in a range of environments encompassing extensive variation in latitude (53° N to 39°S), altitude, soil type, photoperiod, temperature, and rainfall. Seven broadly different regions can be distinguished.

#### Indian Subcontinent (India, Pakistan, Nepal, Bangladesh, and Myanmar)

Desi types predominate in this region, accounting for more than 90% of production. Chickpea is typically grown as a "winter" crop with sowing from October to December and harvest from February to April. The crop is mostly rainfed, relying essentially on soil moisture conserved from monsoon rains; there is little effective growing season rainfall. Soil types vary from the heavy, black "cotton" soils of central and southern India to the alluvial soils of the Indo-Gangetic Plain and the much lighter textured sands of Rajasthan in India and the Thal area of Pakistan.

Chickpea is grown mainly as a sole crop in a range of rotations. Where sufficient rainfall or irrigation allows, e.g., in north western India or in the ricebased systems of north eastern India, Bangladesh, and Myanmar, chickpea follows rainy season crops such as rice, millet, sorghum, maize, sugar cane, cotton, guar, or sesame. However, in lower rainfall zones where only one crop per year is possible, chickpea is grown as a fallow crop. Less frequently, it is intercropped in varying proportions with *Brassica* spp., linseed, safflower, or sorghum, or sown as a mixed crop with wheat, barley, linseed, or sorghum.

Fertilizer and pesticide use is generally low and there is minimal mechanization of sowing, harvest, or weed, insect, and disease control. These operations are performed by hand or with the assistance of animal drawn implements. Mature plants are cut at the base by hand and left to dry in the field. They are later transported to a central area and threshed.

#### West Asia, North Africa, and Southern Europe

Production in this region is almost exclusively of kabuli types. The crop is grown at high altitudes in west Asia (Afghanistan, Iran, and Turkey) but at comparatively lower altitudes in the Middle East (Syria, Yemen, Jordan, Israel, and Iraq), North Africa (Egypt, Sudan, Algeria, Tunisia, and Morocco), and southern Europe (Greece, Italy, Spain, and Portugal). It is grown as a "spring" crop in this Mediterraneantype environment, relying mainly on growing season rainfall. Sowing is usually delayed until late February to May to minimize losses from cold and/or ascochyta

blight. Rapidly increasing temperatures and moisture stress in summer hasten maturity and limit crop duration to 90–120 days.

Chickpea is generally confined to areas having an annual rainfall above 400 mm. It is grown as a sole crop in rotation with wheat (bread or durum) or barley; a fallow phase or summer or forage crop may be included in drier areas. There is some mechanization of production, particularly sowing and weeding, but harvest is still largely manual. High labor costs have contributed to the decline in chickpea production in parts of the region, especially in Europe.

# East Africa (Ethiopia, Eritrea, Tanzania, Malawi, and Uganda)

Mostly desi and some kabuli chickpeas are grown in this region, usually at high altitudes (up to 2400 m). Crops are mainly sown as pure stands in rotation with wheat, barley, and tef, but to a lesser extent as mixtures with sorghum, safflower, and maize. Crop growth relies mainly on residual soil moisture. Sowing follows the wet season, from July to early September in Ethiopia and from February to April further south. Inputs of fertilizers and pesticides are minimal, and although there is some use of tractors in sowing, harvest and threshing remain manual operations.

#### **Central America (Mexico)**

Desi chickpeas are produced under rainfed conditions in the west-central parts of the country and kabuli types under irrigation in the drier northwest. Chickpea is grown as a "winter" crop following maize in the westcentral region and following soybean or sesame in the northwest. Sowing is from October to December and harvest from March to May. There has been some recent transition to mechanized production, particularly for kabuli types.

#### North America (Canada and USA)

The North American chickpea industry began in coastal districts of California where spring-sown kabuli crops were grown on residual soil moisture. Later, winter crops were grown under irrigation in the drier San Jaoquin Valley. During the 1980s, both desi and kabuli chickpeas were introduced to the Palouse region of northwestern USA and, in the following decade, to the brown and dark brown soil zones of Saskatchewan and Alberta in Canada. The threat of cold and ascochyta blight force chickpeas to be grown as a "spring crop" in both the Palouse and Canada. Sowing commences in May and harvest, particularly in Canada, needs to be completed by September to avoid freezing injury to immature seeds.

In the USA and Canada all stages of chickpea production, including seed inoculation, sowing, weed, disease and insect control, harvest, and seed drying are highly mechanized. In most cases the machinery and processes have been modified from those used in cereal production.

#### **Australia**

Production is mainly (>90%) of desi types and occurs in two distinctly different environments in Australia: the summer rainfall-dominant regions in the northeast and the winter rainfall-dominant Mediterraneantype environments in the southeast and southwest. Chickpea is grown as a "winter" crop, sown from May to July and harvested from October to January. Most crops are produced under rainfed conditions; incrop rainfall is a major yield determinant in most regions, especially in the southeast and southwest. As in North America, production is highly mechanized. Control of ascochyta blight, the major industry problem in most regions, relies heavily on ground rig application of foliar fungicides. Application of insecticides by plane to control pod borers is also widely practiced.

#### **Production Constraints**

There is a large discrepancy between the yield in farmers' fields and that commonly obtained under experimental conditions. The low yields generally obtained by farmers reflect a plethora of problems that beset the crop. The major production constraints and their impacts are described below.

#### **Physical Constraints**

**Drought** Chickpea is mostly grown in low rainfall environments. Inadequate soil moisture is therefore a critical production constraint and may occur throughout the growth cycle. Winter/spring rains in west Asia, or soil moisture remaining from the wet season in the Indian subcontinent and east Africa, may not be sufficient for farmers to sow; for many regions variability in main season rainfall explains much of the year-to-year variation in chickpea area. Receding soil moisture, particularly in low rainfall areas in India and Pakistan, may also be inadequate to sustain seedlings. Intermittent moisture stress of established crops can result from breaks in winter and early spring rainfall, but a more serious problem is terminal moisture stress suffered by springsown crops (and winter crops in the Indian subcontinent) as podding coincides with rapidly declining soil moisture.

**Cold** Low temperatures affect the plant in two main ways: tissue damage through disruption of cell membranes and abortion of flowers. Sensitivity to low temperatures ( $<-5^{\circ}$ C) is one reason for spring sowing in higher elevation areas of west Asia. However, genotypes have now been identified which can survive temperatures as low as  $-25^{\circ}$ C. Winter sowing of these cold tolerant genotypes can increase yield potential by ~70% through increased biomass production and improved water-use efficiency. Where the crop is sown in autumn/winter, such as in northern India, Pakistan, and Australia, chilling damage in the early flowering phase narrows the "window" for pod setting. Sowing is normally delayed so that commencement of flowering coincides with mean daily temperatures favorable for podding (15°C).

Hostile soils A range of soil chemical and physical problems limit chickpea growth. The most important of these is salinity, since chickpea is comparatively intolerant of the ionic imbalances characteristic of "saline" soils. "Sodic" soils, in which high levels of exchangeable sodium are associated with increased bulk density, low water infiltration, and poor aeration, are also common in northern India and in other regions where soil pH is very high. Retarded root growth and poor nodulation inhibit chickpea growth and productivity under such conditions. Other locally important soil factors are acidity (poor nodulation), deficiencies of iron and zinc, and either a deficiency or toxicity of boron.

#### **Diseases**

Ascochyta blight Ascochyta is the most important disease of chickpea worldwide and has been recorded in nearly all producing countries. Recent epidemics in the USA (1980s) and Australia (1990s) caused major industry disruptions, reflected by a sharp decline in the area sown. Elsewhere, for example, in west Asia and the Mediterranean region, the threat of ascochyta causes sowing to be delayed, thereby significantly reducing yield potential. The causal agent of ascochyta is Ascochyta rabiei (Passerini) Labrousse, a fungus spread by infected seed and crop residues, longrange dispersal of sexually produced ascospores, or, within the crop, short-range dispersal of asexually produced pycnidiospores. Lesions are formed on leaves, stems, pods (Figure 4), and seeds. The disease progresses quickly under favorable wet conditions and can cause total crop failure. Ascochyta can be controlled by an integrated disease management approach based on host resistance and, depending on location, including removal of infected plant debris, delayed sowing, seed dressing, and foliar fungicides.



**Figure 4** Ascochyta blight on pods. The concentric rings of dark colored pycnidia (fruiting bodies) are a useful diagnostic feature for this disease in chickpea.

Other foliar diseases Botrytis gray mold (Botrytis cinerea Persoon: Fries) is the most important of the foliar diseases after ascochyta. The pathogen has a very broad host range; therefore, the disease is widely distributed. Most damage occurs under high humidity and generally at temperatures higher than optimum for ascochyta. Botrytis has contributed to the decline of the Bangladesh chickpea industry and has also caused significant or total crop losses in the higher rainfall zones of northeastern India and in Australia. The following diseases are also regarded as locally significant: sclerotinia stem rot (Sclerotinia sclerotiorum (Lib.) de Bary), phoma blight (Phoma medicaginis Malbr. and Roumeguère), alternaria blight (Alternaria alternata (Fries:Fries) Keissler), stemphylium blight (Stemphylium sarciniforme (Cavara) Wiltshire), and rust (Uromyces cicerisarietini Jaczewski in Boyer & Jaczewski).

Fusarium wilt Fusarium is the other major disease of chickpea, and like ascochyta, causes economic damage in a large number of producing countries. It is a soil and seed-borne disease caused by the fungus Fusarium oxysporum Schlecht:Fries emend. Snyder and Hansenf. sp. ciceris (Padwick) Matuo & Sato. Characteristic wilt symptoms appear from the seedling stage, in highly susceptible varieties, through to the podding stage. Fusarium is more frequently expressed under warm, dry growing conditions and is therefore a greater problem in the Indian subcontinent and in regions where the crop is spring sown. Fungicidal seed dressings provide protection against seed-borne infection, but host resistance is emerging as the most successful way of combating the disease.

Root and collar rots A number of fungal root or collar diseases have the potential to inflict significant yield losses. Black root rot (Rhizoctonia bataticola (Taubenhaus) Butler) is the most important of these, especially on the Indian subcontinent. The disease generally presents as a sudden drying of scattered plants at the podding stage and is favored by hot, dry conditions. Other diseases include wet root rot (Rhizoctonia solani Kühn), black root rot (Fusarium solani (Maartius) Saccardo), collar rot (Sclerotium rolfsii Saccardo), and phytophthora root rot (Phytophthora medicaginis Hansen and Maxwell). The latter has been recorded in a number of countries, but economically significant damage has only been observed in northeastern Australia where it is the major production problem.

**Viruses** Considerable crop losses have been attributed to virus disease, especially in India, Pakistan, Iran, the USA, and Australia. In most cases a complex of viruses has been implicated. Aphids, particularly Aphis craccifora Koch, are almost always the vector responsible for disease transmission. In India chickpea stunt is the name given to a syndrome characterized by foliage discoloration (red in desi, yellow in kabuli), stunting, phloem browning, and plant death. The disease has been ascribed to the leafhopper-transmitted chickpea chlorotic dwarf virus (CCDV) and some aphid-transmitted luteoviruses, including bean leafroll luteovirus (BLRV). Other viruses known to cause disease are alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), beet western yellows virus, and subterranean clover redleaf virus (SCRV).

Nematodes Nematodes reduce yield by colonizing and damaging roots, reducing the efficiency of nitrogen fixation and in some cases acting synergistically with fungal diseases such as fusarium wilt. Many nematode species are known to infect chickpea although only three are considered economically important. Rootknot nematodes (Meloidogyne spp.) cause abundant formation of galls, whereas both cyst nematode (Heterodera ciceri Vovlas, Greco & di Vito) and root-lesion nematodes (mainly Pratylenchus thornei Sher and Allenand and P. neglectus (Rensch) Chitwood and Oteifa) damage root cellular tissue causing necrotic regions. Root-knot nematodes and root-lesion nematodes have an extensive host range and are widely distributed; cyst nematode has a very limited host range and its distribution is confined to the Middle East.

#### Insects

The small number of insect pests causing economic damage to chickpea is attributed to the antibiotic

effect of the plant's acidic (mainly malic acid) exudate. The most widespread and damaging pest is the pod borer (Helicoverpa spp.). Various larval instars feed on leaves, flowers, and pods. Most yield loss is caused when late instar larvae enter the pod and partly or fully consume the developing seed. There is little effective host resistance within chickpea so control is reliant on a combination of chemical and bio-pesticides (such as nuclear polyhedrosis virus). The other major field pest is leaf miner (Lyriomyza cicerina (Rondani)) whose distribution is confined to the Mediterranean region. Larvae tunnel through the leaf parenchyma causing loss of photosynthetic tissue and premature leaf drop. Infestation of stored seed is mainly by small bruchid beetles (Callosobruchus spp.). Larvae emerge from eggs laid on the (stored) seed surface and tunnel beneath the seedcoat where they feed and pupate. Adults emerge from the seed, ready for the next life cycle and leaving a substantial cavity.

#### Weeds

Chickpea's slow early growth and low total biomass predispose the crop to severe weed competition. In most countries weed control is done by manual weeding or cultivation by animal- or tractor-drawn implements. These practices are feasible where crop area is small, although yield potential is often compromised where wide rows are employed to facilitate cultivation. Options for pre- and post-emergent control of both grass and broadleaf weeds are now available and used extensively in Australia, the USA, and Canada. Grass herbicides are very effective, have no toxicity to chickpea, and contribute to the productivity of ensuing cereal crops by eliminating graminaceous diseases that can otherwise survive on grass weeds.

#### Utilization

#### **Nutritional Status**

Desi chickpea seeds are comprised of the embryo (1.5%), seedcoat (15.5%), and cotyledons (83%); the seedcoat fraction of kabuli seeds is much lower (6.5%), resulting in a higher cotyledon fraction (92%). Protein averages 23% and is deficient in the sulfur-containing amino acids methionine and cystine. Carbohydrate, the main constituent, averages 63.5% of total seed and is mainly starch. Soluble sugars comprise  $\sim 10\%$  of the starch and include oligosaccharides such as stachyose and raffinose that cannot be broken down by human digestive enzymes. Gases produced by bacterial degradation of these oligosaccharides cause flatulence and intestinal discomfit. Oils comprise  $\sim 5\%$  of the seed but are well below commercially extractable levels. Chickpea is

Table 2 Main uses of chickpea for human consumption

Plant/seed part used	Process/product	Country/region
Green leaves	Boiled as vegetable	Indian subcontinent (ISC)
Immature plant	Roasted, pods shelled	ISC, Middle East, Ethiopia
Unripened pods	Pods shelled, seeds raw or boiled	ISC, Ethiopia, Turkey
Whole seed – kabuli	Soaked, boiled alone or with vegetables, rice, meat, spices	Most countries
	Soaked, boiled, canned	North America, Europe, Australia, Middle East
	Soaked (briefly), roasted	Middle East, North Africa
	Soaked, boiled, pureed (Hommos)	Middle East, North Africa
Whole seed - desi	Soaked, boiled with vegetables, rice, spices	ISC
	Soaked (briefly), roasted	ISC
	Germinated	ISC
Dhal	Soaked, boiled with spices	ISC
Flour (besan)	Batter for fried vegetables	ISC
. ,	Batter with rice, fermented	ISC
	Thickener for gravies	ISC
	Fried extrusion products	ISC
	Mixed with wheat flour, bread	ISC, Middle East

generally low in antinutritional factors such as protease and amylase inhibitors, whose activities are further reduced by cooking, and protein-binding phenolics, which are largely removed with processing.

#### **Uses and Processing**

Chickpea is a key dietary component in many countries where animal protein is too expensive, or in other countries where the crop has a long history of cultivation and consumption. The complementarity of pulse and cereal proteins has given rise to many traditional dishes in which chickpea is combined with wheat, rice, or some locally important cereal, such as tef, to provide the bulk of dietary protein and calories.

The use of chickpea as human food is hugely varied, reflecting both the crop's antiquity and its broad cultural base (Table 2). The mature seed, either whole or processed, accounts for nearly all consumption. However, there are specialty uses for the green, immature plant. Fully podded plants are commonly sold in street markets in India and the Middle East. The seeds are either roasted in the pod, consumed raw as a snack or boiled; fresh leaves are also used as a vegetable. Ripe seeds (desi and kabuli) are presoaked and boiled, with or without the addition of vegetables or meat, to produce a range of traditional dishes. Kabuli seeds are also canned after cooking, but a more important use is as hommos bitihneh (pureed and mixed with oil). Both desi and kabuli seeds are also roasted. This process generally requires a quick soaking followed by high-temperature treatment (e.g., at 250°C) for 2-3 min in preheated sand or an oven. Variations to the soaking, roasting, and final processing (e.g., decortication, polishing, sugar coating) give this snack food its regional characteristics.



**Figure 5** Dhal produced from desi seeds following removal of the seedcoat and separation of the cotyledons.

Most desi seeds are milled to produce dhal, a process that involves removal of the seedcoat and cleaving of the cotyledons (Figure 5). Milling of chickpeas is a major industry in the Indian subcontinent and the newer high-throughput mills achieve a dhal yield close to the theoretical maximum of 83–85%. Dhal is the main form of consumption in the Indian subcontinent; the prepared dish, also called "dhal," is almost always part of the main meal. The third main use of chickpea is as "besan," the flour milled from dhal. (Small kabuli seeds have recently been substituted to produce a cheaper, but inferior product.) Besan is used for a range of purposes, most commonly as a batter or to produce fried, extrusion products.

Chickpea also plays an important, if secondary, role in animal nutrition. In Mexico desi types are grown specifically for inclusion in pig rations. Elsewhere residues from processing (seedcoat and kibble

from milling, damaged or undersized seeds from grading) are routinely used in intensive livestock production. Residues from harvest (stems, leaves, pods, and seeds) are also a valuable feed source for ruminant animals.

## **Genetic Improvement**

Worldwide there has only been a limited breeding effort in chickpea compared to the major cereals and oilseeds. Primitive landraces still account for much of the crop area in developing countries despite the new varieties being released by national programs and the major international centers ICRISAT (India) and ICARDA (Syria). Major breakthroughs have been achieved in developing resistance to the major diseases ascochyta and fusarium. Cold tolerance, coupled with ascochyta resistance, has been deployed in new varieties that enable winter sowing in traditional spring-sowing areas and early maturing varieties, providing some escape from drought, have facilitated the wider adoption of chickpeas in central and southern India. Mechanization of harvest has been facilitated by the development of erect plant types which grow up to 1 m tall and have stronger, more lodging resistant branches than the traditional semi-erect forms.

There is a paucity of genes for many economically important traits in C. arietinum, presumably as a consequence of the genetic "bottleneck" caused by domestication. However, genes conferring improved resistance to many biotic stresses, such as bruchids, cyst nematode, and phytophthora root rot, can be found in either or both the closely related "wild" species C. reticulatum and C. echinospermum. Breeding programs are now employing backcross programs to incorporate a range of attributes from these species into adapted backgrounds. Introgression of genes from unrelated taxa has also been achieved by Agrobacterium- mediated transformation. "Transformed" plants containing genes with the potential to confer improved protein quality and insect and disease resistance have been developed and await field evaluation.

See also: Celiac Disease. Chickpea: Agronomy. Extrusion Technologies. Grain Production and Consumption: Overview. Plants: Diseases and Pests. Pulses. Overview. Starch: Chemistry.

## **Further Reading**

Knight R (ed.) (2000) Linking Research and Marketing Opportunities for Pulses in the 21st Century. Dordrecht: Kluwer Academic.

Loss S, Brandon N, and Siddique KHM (eds.) (1998) The Chickpea Book: A Technical Guide to Chickpea Production. Bulletin 1326, Agriculture Western Australia.

Maiti R and Wesche-Ebeling P (eds.) (2001) Advances in Chickpea Science. Enfield: Science Publishers.

Saxena MC and Singh KB (eds.) (1987) The Chickpea. Wallingford: C.A.B International.

Saxena NP (1984) Chickpea. In: Goldsworthy PR and Fisher NM (eds.) The Physiology of Tropical Field Crops, pp. 419–452. Chichester: Wiley.

Sharma RN, Shrivastava GK, Rathore AL, Sharma ML, and Khan MA (eds.) Chickpea Research for the Millennium. Raipur: Indira Gandhi Agricultural University.

Singh KB and Saxena MC (eds.) (1999) The Tropical Agriculturalist: Chickpeas. London: MacMillan.

Smithson JB, Thompson JA, and Summerfield RJ (1985) Chickpea (Cicer arietinum L.). In: Summerfield RJ and Roberts EH (eds.) Grain Legume Crops, pp. 312-390. London: Collins.

van der Maesen LJG (1972) Cicer L., A Monograph of the Genus, with Special Reference to the Chickpea (Cicer arietinum L.), its Ecology and Cultivation. Wageningen: H Veenman and Zonen NV.

Van Rheenen HA and Saxena MC (eds.) (1990) Chickpea in the Nineties. Patancheru: ICRISAT.

### **Relevant Websites**

http://www.icrisat.org - Information on chickpeas (mainly desi) in semiarid areas: role in cropping systems, management, utilization, new varieties.

http://www.cgiar.org - General information on chickpea R&D conducted by international centers funded by the Consultative Group on International Agricultural Research.

http://www.icarda.org - Information on kabuli chickpeas, mainly in Mediterranean-type environments: role in cropping systems, management, utilization, new varieties.

http://www.ars.usda.gov - R&D undertaken on chickpea in the USA.

## Agronomy

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#### Introduction

Chickpea (Cicer arietinum L.) is an ancient crop first taken into cultivation by Neolithic farmers. Today, chickpea continues to play an important role in agricultural systems in the world, ranking third

behind dry bean (*Phaseolus vulgaris* L.) and field pea (Pisum sativum L.) in terms of grain legume production. World chickpea production has remained relatively stable since the 1960s, but recently there have been improvements in productivity, and changes in the regions sown to chickpea. World chickpea production was 6.1 million tons (Mt) in 2001 from an area of 8.6 million hectares (Mha). Traditionally, chickpea was grown in India, Iran, Bangladesh, Pakistan, Mexico, and Ethiopia with limited mechanization. Improvements in varieties, agronomy, and production technology and new export market opportunities have seen the expansion of chickpea production in countries such as Turkey, Canada, and Australia. This article summarizes the key constraints to chickpea production and the agronomy of the crop in major production environments.

## **Adaptation and Production Constraints**

Chickpea has broad adaptation, hence the wide geographical distribution of its production. It is considered to be the most drought-resistant, cool-season grain legume and is commonly grown on stored soil moisture or under rainfed conditions, but also responds well to supplemental irrigation in many environments. Chickpea also exhibits a considerable degree of heat tolerance provided there is sufficient soil moisture. Despite its broad adaptation, chickpea production is restricted by several biotic and abiotic stress factors, depending on the environmental conditions under which the crop is grown. Drought, cold, transient waterlogging, soil salinity/sodicity, and high boron in the subsoil are the main abiotic stresses constraining chickpea production. Among the key biotic stresses, a number of diseases and pests can cause serious yield losses. In addition to this, competition from weeds can also result in considerable yield reduction. In recent years, improved varieties and specific agronomic practices have been developed to manage some of the above constraints.

#### Chickpea Agronomy

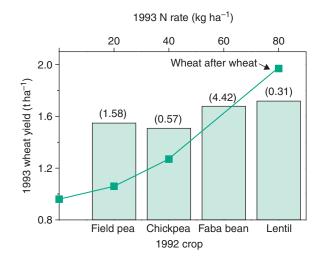
#### **Rotational Benefits**

Chickpea is often grown in rotation with other crops, mainly cereals, because it reduces the risk of pests and diseases associated with monocropping of cereals and hence increases the production of the entire rotation. An additional benefit is the ability of chickpea to fix atmospheric nitrogen via a symbiotic relationship with rhizobium. Some of this fixed nitrogen and organic matter from roots and nodules are left in the soil in an accessible form, hence reducing the

requirement for nitrogen fertilizer in the following crop. In southern Australia, chickpea crops get 37-86% of their total nitrogen through fixation. The amount of nitrogen remaining in the soil after harvest is in the range of 41-56 kg ha<sup>-1</sup>. Legumes derive only part of their nitrogen through fixation and tend to prefer using soil nitrogen, especially when there is a large amount available. In many instances, more nitrogen is removed than the crop actually fixes, hence the negative values. In these situations, the subsequent cereal crop will still need additional nitrogen fertilizer. However, the non-nitrogen benefits of having a chickpea crop in the rotation may still contribute to increased grain yield and protein contents in wheat (Figure 1).

#### Soil Type and Land Preparation

Chickpea is successfully grown on a wide range of soil types throughout the world, ranging from coarse-textured sands to fine-textured black soils. Ideally, chickpea is most suited to deep, neutral to alkaline, fine-textured soils (sandy loams, clay loams, and well-drained clays) with a pH of 5.5–9.0 (measured in calcium chloride – CaCl<sub>2</sub>) and good water-holding capacity. Chickpea is sensitive to waterlogging and sodicity; therefore, soils must have a good structure or a slope that allows drainage. Moreover, chickpea is relatively sensitive to salinity and boron toxicity, and



**Figure 1** Grain yield of wheat grown in 1993 without nitrogen (N) fertilizer after various grain legume crops at Pingaring, Western Australia. Figures (bars) in parentheses are grain legume yields in 1992. Squares show the yield of wheat in 1993 when grown after wheat with various rates of N fertilizer (top axis). (Reproduced with permission from Loss S, Brandon N, and Siddique KHM (eds.) (1998) *The Chickpea Book: A Technical Guide to Chickpea Production.* Bulletin 1326, Agriculture Western Australia.)

areas where these conditions occur also need to be avoided.

Chickpea has a slow early growth and competes poorly with weeds. Because options for the control of especially broad-leafed weeds in chickpea crops remain limited, land with low weed burden is desirable. These occur generally after a well-managed cereal crop. Choosing a relatively even land surface with few obstacles is desirable for improving the efficiency of mechanical harvest.

#### **Time of Sowing**

Moisture availability, temperature, and photoperiod are the main environmental factors affecting chickpea germination, growth, flowering, podding, and seed set. Chickpea is a long day plant, which is susceptible to extreme temperatures and moisture stress, especially at flowering. Flowering is generally more affected by temperature than by day-length. As a result, the optimal time of sowing will vary depending on the geographical region (Table 1) and sowing too early or too late will lead to reduced crop yields.

In the West Asia and North Africa (WANA) region (except in Pakistan, Egypt, and Sudan where chickpea is sown in winter), chickpea is traditionally sown in spring and grown on stored soil moisture from winter rains. The productivity of this type of cropping system is constrained by drought and heat stress during the grain-filling stage, partly because a large amount of stored soil moisture is lost before sowing. One way to deal with this problem is to develop genotypes that are drought-resistant. Alternatively, these areas need to change their cropping system from spring-sowing to winter-sowing. However, a move to winter sowing requires improved disease resistance and winterhardiness, because fungal infections are likely to increase and the cold temperatures lead to seedling death or during flowering may result in flower and pod abortion. Advancing the chickpea-sowing date has led to vield increases of more than 100% in some regions due to longer crop-growth periods and increased water use (Table 2 and Figure 2). In the Mediterranean-type environments of southern Australia, chickpeas are generally sown after the first autumn rains and grow on winter rainfall. The autumn-sown chickpea crops yield more seed than the winter-grown ones. In the semiarid Canadian prairies, chickpea is sown early in the spring growing season when soil moisture is still high.

In the northern hemisphere tropics, including South Asia, Africa, and Central America, the best crop performance is realized when chickpea is sown in the cooler part of the year after the rain season on stored soil moisture. An early sowing can lead to seedling mortality as a result of high soil temperature whereas sowing too late may inhibit germination because of insufficient soil moisture. In the northeastern part of Australia, chickpea is sown in May or June on stored soil moisture from summer rainfall.

Table 1 Time of sowing and harvest of chickpea in various producing regions

Country	Season	Sowing time	Harvest
Morocco	Spring	Mid-Feb./mid-Mar.	June to early July
Tunisia	Spring	Mid-Mar./mid-Apr. small areas of winter sowing	June to early July
Iraq	Spring	Mid-Feb./mid-Mar.	June
Iran	Spring	Mid-Mar./mid-Apr. small areas of winter sowing	JulAug.
Israel	Winter	DecFeb.	June
Jordan	Spring	Mar.	July
	Winter	Nov./Dec.	Mid-June
Turkey	Spring	Feb./Mar.	June
		Highlands sown later to avoid Ascochyta blight	
Algeria	Spring	Mid-Feb./end Mar.	June to early July
Egypt	Winter	Nov. (under irrigation)	April
Ethiopia	Spring-autumn	Sept./Nov.	Jan./Feb.
Sudan	Winter	OctNov.	June
Syria	Spring	Late Febearly May	June to early July
	Winter	Dec.	
Indian subcontinent	Winter	Late SeptNov.	March/April
Canada	Spring	Apr./May	July to early August
USA	Spring	Apr./May	July to early August
Australia			
Southern	Autumn	May/June	Oct./Dec.
ORIA <sup>a</sup>	Autumn	May	Sept.
Northeastern	Autumn	May/June	Oct./Dec.

<sup>&</sup>lt;sup>a</sup>ORIA: Ord River Irrigation Area, Western Australia.

	Early sowing	Late sowing	Reference
North Syria	3.4 (Nov.)	1.0 (Mar.)	Singh and Saxena (1999) <sup>a</sup>
India	1.6 (Sept.)	0.97 (Nov.)	Singh and Singh (1997) <sup>b</sup>
Southwestern Australia	1.35 (May)	0.94 (Jul.)	Siddique and Sedgley (1986) <sup>c</sup>
Canada	2.35 (Apr.)	1.99 (May)	Gan <i>et al</i> . (2002) <sup>d</sup>

<sup>&</sup>lt;sup>a</sup> Singh KB and Saxena MC (1999) The Tropical Agriculturalist: Chickpeas. London, UK: MacMillan Education Ltd.

in a semiarid environment. Canadian Journal of Plant Science 82: 531-537.

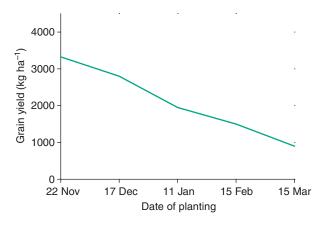


Figure 2 Average grain yield of 20 chickpea genotypes as affected by sowing date at Tel Hadya in northern Syria. (Reproduced with permission from Singh KB and Saxena MC (eds.) (1996) Winter Chickpea in Mediterranean-Type Environments. A Technical Bulletin. Aleppo, Syria: ICARDA.)

#### **Table 3** Plant density, sowing rate, and row spacing of chickpea in various regions

Country	Plant densities (plants m <sup>-2</sup> )	Sowing rate (kg ha <sup>-1</sup> )	Row spacing (cm)
Algeria	20-30	< 100	50-300
Jordan	25-33	80-100	30-40
Morocco	25-35	80-120	40-70
Southern Australia	25-50	80-120	18–36
Northeastern Australia	30-40	80-120	18–70
Syria	40-50	120-180	17.5-35
Indian subcontinent	33	40-65	30-45
Canada	45	120-150	25
USA	40	90-125	30
Turkey	35	90-120	25
Tunisia	20	70-90	70-100

#### Sowing Rate, Depth, and Method

Plant density controls establishment success, yield, and ultimately, the profitability of a crop and is determined by the sowing rate and germination percentage. Inadequate plant stand is one of the most common yield retardants in many production areas in the world.

Chickpea sowing rates vary between 40 and 200 kg ha<sup>-1</sup>, depending on the genotype used, seed size, and the environmental conditions (Table 3). On an average, a plant density of 33 plants m<sup>-2</sup> has been found to produce optimum seed yields across a range of environments. However, this density can be increased in favorable soil conditions and should be reduced under marginal conditions. Sowing rates are higher for "kabuli" type than for "desi" type chickpea because they have larger seeds. In addition to this, the thin seedcoat of kabuli makes the seeds more susceptible to mechanical damage during harvest and handling which results in a lower germination percentage. In addition to this, the seedcoat of kabuli lacks the phenolic compounds that reduce fungal attack, which can lead to reduced germination in the absence of seed-dressing fungicide. The plant density producing the maximum yield is not always the most profitable and hence an economically optimum plant density needs to be determined (Figure 3).

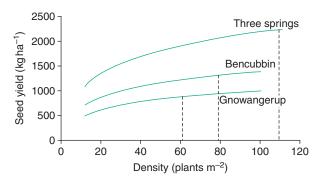
In India and Africa, chickpeas are traditionally sown in wide rows up to 150 cm to facilitate manual weed control and also, in developed countries, a row spacing of 70-100 cm are used to allow mechanical inter-row cultivation. However, this wide spacing often leads to low plant densities and low seed yields and in Mediterranean-type environments it can lead to excessive soil moisture loss through evaporation. Generally, a close row spacing of 18–35 cm has been found to be most productive.

The optimum sowing depth for irrigated chickpea or chickpea grown in high soil-moisture conditions

<sup>&</sup>lt;sup>b</sup> Singh KB and Singh O (1997) Prospects of creating higher yield potential in chickpea. In: Asthana AN and Masood Ali (eds.) Recent Advances in Pulses Research, pp. 65-88. Kanpur, India: Indian Society of Pulses Research and Development, IIPR.

<sup>&</sup>lt;sup>c</sup>Siddigue KHM and Sedgley RH (1986) Chickpea (Cicer arietinum L.), a potential grain legume for south-western Australia: seasonal growth and yield. Australian Journal of Agricultural Research 37: 245–261.

<sup>d</sup>Gan YT, Miller PR, Liu PH, Stevenson FC, and McDonald CL (2002) Seedling emergence, pod development, and seed yields of chickpea and dry pea



**Figure 3** Relationship between plant density and grain yield at typical high, medium, and low yielding sites in Western Australia. Dotted lines indicate the estimated optimum plant density. (Reproduced with permission from Jettner RJ, Siddique KHM, Loss SP, and French RJ (1999) Optimum plant density of desi chickpea (*Cicer arietinum* L.) increases with increasing yield potential in south-western Australia. *Australian Journal of Agricultural Research* 50: 1017–1025.)

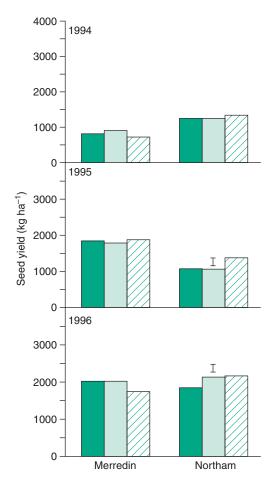
varies between 5 and 8 cm but can increase to 10–15 cm in moisture-deficient soils without affecting emergence and yield (Figure 4). Deep sowing is beneficial for crops grown on stored soil moisture in dry areas with high temperatures and soil-evaporation rates. Moreover, deep sowing protects the crop from pre-emergence herbicide damage, frost, wind and insect attacks, and improves survival of rhizobium bacteria inoculated on the seed. It is likely that an interaction exists between seed size and sowing depth in chickpea, but this deserves further research.

Greater seedling emergence can be realized with mechanical sowing methods (drills, precision seeders) than seeding by hand. The sowing process is fully mechanized in North America, Australia, and most parts of Turkey and Europe. It is still broadcast or drilled using local implements in many regions of WANA and the Indian subcontinent.

#### **Inoculation and Nitrogen**

Being a legume, chickpea is capable of fixing atmospheric nitrogen through its symbiotic nodules formed upon infection with the nitrogen-fixing rhizobium bacteria. These bacteria, however, are species-specific and survive poorly on acidic coarse-textured soils. Therefore, inoculation of seed with rhizobium is needed for successful establishment of chickpea in marginal soil conditions and on fields that have not grown chickpea in the past.

Due to the symbiotic relationship with nitrogenfixing bacteria, chickpea is able to survive on soils with low nitrogen levels. However, the rhizobium bacteria need 6-8 weeks to form the root nodules



**Figure 4** Seed yield of chickpea sown at 2.5 (solid green), 5.0 (light green), and 10.0 cm (hatched) at Merredin and Northam, Western Australia. Vertical bars denote I.s.d. (P=0.05) where differences are significant. (Reproduced with permission from Siddique KHM and Loss SP (1999) Studies on sowing depth for chickpea ( $Cicer\ arietinum\ L.$ ), faba bean ( $Vicia\ faba\ L.$ ) and lentil ( $Lens\ culinaris\ Medik$ ) in a Mediterranean-type environment of south-western Australia.  $Journal\ of\ Agronomy\ and\ Crop\ Science$  182: 105–112.)

and commence nitrogen fixation. The seedling growth depends upon seed-source nitrogen in the initial periods and seed reserves of nitrogen tend to run out before the commencement of nitrogen fixation. In nitrogen-poor soils, therefore, a small starter dose of nitrogen (10–25 kg N ha<sup>-1</sup>) can stimulate root and shoot growth during early crop development and lead to increased seed yields. Once the root nodules are active, no yield increase is observed upon addition of nitrogen fertilizer.

#### **Other Nutrient Requirements**

In contrast to cereal plants that take up almost all the phosphorus in their early growth stages, grain legumes require a continuous supply of phosphorus throughout their entire growing season. Phosphorus deficiency is a widespread problem in South Asia and Africa and application of  $60 \text{ kg ha}^{-1}$  of  $P_2O_5$  has been shown to increase chickpea yield. However, the response to phosphorus tends to be less in chickpea than in other cool-season food legumes and cereals because chickpeas are able to exploit sources of phosphorus unavailable to most plants. Chickpea roots excrete organic acids that dissolve insoluble phosphorus compounds in the soil. Secretion of organic acids also dissolves insoluble copper, zinc, iron, and manganese, thereby avoiding deficiency of all these nutrients and hence few of these deficiencies have been reported. Iron deficiency, however, is common on high-pH calcareous soils in South Asia and chickpeas respond positively to foliar spray of 0.5–2% FeSO<sub>4</sub> solution. More recently, the considerable genetic variation among chickpea genotypes has made it possible to develop cultivars which are tolerant to iron deficiency. The critical zinc concentration in dried shoots is 17-21 mg kg<sup>-1</sup> and zinc deficiency has been observed in India and southern Australia. Sulfur deficiency is a problem in light textured soils in India and addition of 20 kg ha -1 sulfur can increase yield by 0.3-0.6 t ha<sup>-1</sup>. Application of boron in chickpea leads to increased seed yield in boron-deficient soils of Nepal, Bangladesh, and parts of eastern India. Since soils of most chickpea-growing regions have a high potassium status, potassium deficiencies in chickpea are rare.

#### **Weed Management**

As a slow grower, chickpea competes poorly with weeds and therefore good weed-management is critical for high yields and quality. Because there are only limited options for chemical weed-control in chickpea crops after emergence, it is essential to deal with these weeds in the previous crop and before sowing. In developing countries, weeds are controlled mainly through cultural, manual, and mechanical techniques, whereas chemical weed-control methods are mainly used in North America, Canada, and Australia. The most effective chemical weed-control is generally achieved by a presowing application, a postsowing, pre-emergence application, and a post-emergence application of herbicides. There are numerous presowing and postsowing pre-emergence herbicides available for controlling broad-leafed and grass weeds. The most effective and commonly used presowing herbicides are Simazine and Cyanazine at rates of 1-21ha<sup>-1</sup>, whereas Metribuzin<sup>TM</sup> and Spinnaker<sup>TM</sup> at 200 ml ha<sup>-1</sup> are used after sowing and before emergence. Although there are several grass selective post-emergence herbicides, the options for broad-leafed weeds are limited.

## **Disease and Pest Management**

Over 50 diseases are known to affect chickpea worldwide, including *Ascochyta* blight, *Fusarium* wilt, root rots, rust, *Botrytis* gray mold, and viral diseases. From these, *Ascochyta* blight and *Fusarium* wilt are the most important and devastating diseases worldwide, whereas the others are only important on a more regional level. In addition, numerous pest species have been associated with chickpea, including more than 46 species of nematode.

Ascochyta blight is caused by the fungus Ascochyta rabiei Pass. (Labr.). The disease was first reported in 1911 in the Indian subcontinent, but since then has been reported in 32 different countries. Changing from spring to winter sowing in Mediterranean region has increased the occurrence of the disease in these areas. The fungus attacks all the aerial parts of the chickpea plant, making it the most destructive of the chickpea diseases and causing yield losses up to 100%. Severe infections with Ascochyta blight cannot be controlled by chlorothalonil fungicide and disease management through resistant cultivars is essential. In addition to the use of fungicides (seed dressing and foliar application) and resistant cultivars, cultivation practices such as crop rotations with cereal crops and managing infested debris have contributed to successfully managing Ascochyta blight.

The most devastating soil-borne disease of chickpea is *Fusarium* wilt, caused by the fungus *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. f. sp. *ciceri* [Padwick] Snyd. & Hans. Most chickpea-growing countries have reported the disease, which affects both roots and aerial plant parts and can cause yield losses of 10–50%. Although the disease can be minimized by seed dressings with fungicide (Benlate T at 0.15% rate), it is more economical to use resistant cultivars. Several resistant cultivars have been developed and released but these have not shown resistance across all locations due to area-specific races of the pathogen. Therefore, supplemental management practices like crop rotation are needed to control *Fusarium* wilt infestations.

Only very few of the more than 46 reported nematode species found in association with chickpea cause a severe yield loss in chickpea. The root-knot (*Meloidogyne* spp.), cyst (*Heterodera* spp.), and root-lesion nematodes (*Pratylenchus* spp.) are most damaging to the crop and nematode infections have been reported from all over the world. Proper crop rotations are the most effective and economical ways to control nematode infections, as opposed to the use of

nematicides. The search for nematode-resistance in cultivated chickpea has been unsuccessful and efforts are being made to find nematode-resistance in wild relatives of chickpea and ways to transfer resistance to the cultigen.

Insect pests generally cause less damage and yield loss to chickpea crops than diseases for two reasons. First, chickpea plants excrete organic acids from hairs on their leaves, stems, and pods, which are unpalatable to most crop pests. And second, chickpea is generally grown around winter in most countries when insect activity and populations are very low. This is not the case, however, in the subtropical chickpeagrowing regions, where some insect pests pose a real treat to chickpea production. The most damaging insect pests are pod borers (Helicoverpa spp.) and leaf miners (Liriomyza cicerina). Pod borers occur in all chickpea-growing countries whereas leaf miners are restricted to the WANA region and eastern Europe. Both insects can cause significant reductions in yield and quality, especially pod borer. Spraying with insecticides is economical only when the infestation is serious and when a high yield (>1 t ha<sup>-1</sup>) is expected. In Australia, pheromone traps specific to pod-borer moths are used to monitor moth flights and adjust timing of insecticide application to minimize damage to crop yield and quality. Moreover, the use of conventional insecticides has declined because insects have developed resistance to these insecticides, and due to possible health and environmental hazards. Alternatives to spraying are biological control (e.g., nuclear polyhedrosis virus kills Helicoverpa spp.), cultural control (e.g., advancing sowing date), and host-plant resistance. Efforts are being made to develop chickpea cultivars that combine insectresistance, disease-resistance, and high yield.

Bruchids (Callosobruchus spp.) are the main insects attacking stored chickpea seed and have been reported from all chickpea-producing countries, particularly from the Indian subcontinent. Such infestations can damage up to 70% of the seeds. An effective way to avoid bruchid damage is harvesting soon after the crop is mature and storing seed in insect-proof containers. This method is preferred to treating the stored seed with fumigants (e.g., phostoxin) or insecticides (e.g., malathion). No useful bruchid-resistant chickpea genotypes have been identified so far.

#### **Water Use**

Chickpea is considered to be the most drought-tolerant, cool-season grain legume. It has the greatest ability to tolerate intermittent drought and respond to subsequent rainfall due to its more indeterminate growth habit. However, seed-yield loss due to

terminal drought can be as high as 60%. To avoid drought conditions, chickpea is generally grown during or just after the rain period on stored soil moisture. However, the crop sometimes faces mild to severe terminal drought during flowering and seed filling, which are the most drought-sensitive stages. Low soil moisture can cause abortion of flowers, immature pods, and developing seeds and hence considerably reduce seed yield. Recent studies in southern Australia have shown water-use efficiencies for dry-matter production between 11 and 18 kg ha<sup>-1</sup> mm<sup>-1</sup> and wateruse efficiencies for grain yield between 2.6 and 7.7 kg ha<sup>-1</sup> mm<sup>-1</sup>. This study also showed chickpea to be less water-use efficient than the high-yielding faba bean and field pea (WUE $_{dm}$ :  $19-39\,kg\,ha^{-1}$  mm $^{-1}$  and WUE $_{gr}$ :  $6-16\,kg\,ha^{-1}$  mm $^{-1}$ ) crops that escape terminal drought by vigorous early growth, early flowering and pod set, and rapid seed fill at maturity. Increasing early growth for rapid ground cover and reduced soil evaporation, and tolerance to cold temperatures during flowering and pod setting are essential to improve water use efficiency of chickpea in Mediterranean-type environments. Selecting genotypes with larger root systems, early flowering and pod setting, increased osmoregulation or greater translocation of biomass from stems and leaves to seed are other strategies explored by chickpea breeders for improving yield potential.

In areas where annual rainfall is less than 400 mm or where there is a risk of drought during the late vegetative and reproductive stages, chickpea responds positively to supplemental irrigation. Supplemental irrigation in Egypt, Israel, Lebanon, Mexico, and Central Queensland (Australia) has helped the realization of higher yields. Mexico has the highest yield in the world, a reflection of the irrigated conditions in which the crop is grown in that country. In the Ord River Irrigation District in northern Western Australia, chickpea is grown during the dry winter and relies fully on irrigation.

#### **Harvesting and Storage**

Harvest and storage have a significant impact on the quality of chickpea grain. Chickpea is ready to harvest when stems and pods start yellowing and the seed moisture content is  $\sim$ 15%. When harvest is delayed beyond this point, the seed moisture content decreases and the seed becomes susceptible to cracking. Moreover, it increases crop lodging and pod shedding. Early harvesting of the chickpea crop has been shown to increase yield up to 30% when suitable drying facilities are available.

In developing countries, most of the harvesting and threshing is still done by hand, a time-consuming and

expensive process. In Australia, Canada, the USA, and most parts of Europe, harvesting is completely mechanized. Generally, a maximum harvesting speed of 8 kmph and a low cutting height of 10–15 cm is used, but harvester settings vary depending on the conditions. Crop desiccation before harvesting is a valuable tool for easier harvesting when a lot of maturing weeds are present. However, desiccant can only be applied when more than 90% of the crop reached physiological maturity to prevent yield reductions.

Seed quality deteriorates rapidly with storage and reducing moisture and temperature increases longevity of the seed. Storing seed at less than 13% moisture, however, has adverse effects on the viability because it makes the seed shrink away from the seedcoat. Therefore, reducing the storage temperature to 20°C is the best option for increasing seed longevity.

#### **Marketing and Quality**

Being a good source of carbohydrate and protein, chickpea is traded for human consumption in both developing and developed countries, and to a limited extent as stock feed. Most of the world's chickpea seed is consumed close to the place where it is grown. However, a large number of chickpea-producing countries are unable to meet local demand and need to import. India is the world's largest producer, consumer, and importer of chickpea. The main exporters of chickpea are Turkey, Canada, Australia, and Mexico with main markets in India, Pakistan, Bangladesh, Europe, USA, Middle East, and the former USSR.

Visual grain quality is becoming increasingly important when marketing chickpea and is affected by characteristics like seed size and shape, seed color, insect damage, and amount of split, chipped, broken, and foreign seeds. Other quality parameters like cooking time, and splitting and milling recoveries are also important to chickpea consumers and processors. Good crop-management practices are essential to produce high-quality seed and at the same time result in high yields.

#### **Future Prospects**

New chickpea varieties with greater resistance to key biotic and abiotic stresses are currently being developed by various national and international agricultural research organizations. Development of variety-specific agronomy packages and immediate transfer of such production technology to farmers will further increase sustainable production of this important cool-season food legume. See also: Chickpea: Overview.

## **Further Reading**

- Brennan RF, Bolland MDA, and Siddique KHM (2001) Responses of cool-season grain legumes and wheat to soil-applied zinc. *Journal of Plant Nutrition* 24: 727–741.
- Gan YT, Miller PR, Liu PH, Stevenson FC, and McDonald CL (2002) Seedling emergence, pod development, and seed yields of chickpea and dry pea in a semiarid environment. *Canadian Journal of Plant Science* 82: 531–537.
- Jettner RJ, Siddique KHM, Loss SP, and French RJ (1999) Optimum plant density of desi chickpea (*Cicer arietinum* L.) increases with increasing yield potential in south-western Australia. *Australian Journal of Agricultural Research* 50: 1017–1025.
- Knight R (ed.) (2000) Linking Research and Marketing Opportunities for Pulses in the 21st Century. Dordrecht, The Netherlands: Kluwer Academic.
- Loss S, Brandon N, and Siddique KHM (eds.) (1998) *The Chickpea Book: A Technical Guide to Chickpea Production*. Bulletin 1326, Department of Agriculture Western Australia, South Perth, Australia.
- Saxena NP (ed.) (2003) Management of Agricultural Drought: Agronomic and Genetic Options, 209pp. Enfield, NH: Science Publishers.
- Saxena MC and Singh KB (eds.) (1987) *The Chickpea*. Wallingford, UK: CAB International.
- Saxena NP, Saxena MC, Johansen C, Virmani SM, and Harris H (eds.) (1996) Adaptation of Chickpea in the West Asia and North Africa Region. Andhra Pradesh, India: ICRISAT Aleppo, Syria: ICARDA.
- Siddique KHM and Loss SP (1999) Studies on sowing depth for chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.) and lentil (*Lens culinaris* Medik) in a Mediterranean-type environment of south-western Australia. *Journal of Agronomy and Crop Science* 182: 105–112.
- Siddique KHM and Sedgley RH (1986) Chickpea (Cicer arietinum L.), a potential grain legume for south-western Australia: seasonal growth and yield. Australian Journal of Agricultural Research 37: 245–261.
- Siddique KHM, Regan KL, Tennant D, and Thomson BD (2001) Water use and water use efficiency of cool season grain legumes in low rainfall Mediterranean-type environments. *European Journal of Agronomy* 15: 267–280.
- Singh KB and Saxena MC (eds.) (1996) Winter Chickpea in Mediterranean-Type Environments. A Technical Bulletin. Aleppo, Syria: ICARDA.
- Singh KB and Saxena MC (1999) *The Tropical Agriculturalist: Chickpeas*. London, UK: MacMillan Education Ltd.
- Singh KB and Singh O (1997) Prospects of creating higher yield potential in chickpea. In: Asthana AN and Masood Ali (eds.) Recent Advances in

Pulses Research, pp. 65-88. Kanpur, India: Indian Society of Pulses Research and Development, IIPR. Summerfield RJ (ed.) (1988) World Crops: Cool Season Food Legumes. Dordrecht, The Netherlands: Kluwer Academic.

#### **Relevant Websites**

http://www.icrisat.org. http://www.icarda.cgiar.org. http://www.clima.uwa.edu.au.

# COOKIES, BISCUITS, AND CRACKERS

Contents

Methods of Manufacture **Wafers Chemistry of Manufacture** The Diversity of Products

## **Methods of Manufacture**

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#### Introduction

A few generations ago, biscuits and cookies were most familiar as items that came from the home oven. More recently, they are commonly the product of factory manufacture, reaching the home in a packet. Nevertheless, the methodology of production (in home or factory) is similar, involving the essential steps of mixing, shaping and forming, and baking. Obviously, there are major differences in detail involved in the mass-production process, compared to making a small batch of cookies at home. This article describes the processes used to produce the diversity of cookies/ biscuits and crackers that reach us in packets, following these three major steps-mixing, forming, and baking.

#### **Mixing**

The first step in manufacture involves the blending of the ingredients into a uniform, homogeneous mixture. In the context of cookie and cracker doughs, the term

"mixing" takes on a broader meaning in that it also applies to the development of gluten from hydrated flour proteins, the aeration of a mass to give a lower density, and the dispersion of solids in liquids. One or more of the functions are required for the formation of cookie and cracker doughs. These processes are accomplished with three principal types of mixers: vertical spindle mixers, horizontal drum mixers, and continuous mixers.

#### **Vertical Spindle Mixers**

Vertical mixers were used extensively before the development of the high-speed mixer. Although usage has decreased overall, this type of mixer is still used for crackers and semisweet doughs, for which the vertical spindle design is particularly well suited. The feature that all vertical mixers have in common is the portable dough trough, which serves as the mixing vessel. The trough is usually a wheelmounted, heavy tub with vertical sides, round ends, and a flat bottom. The trough is designed so that it may be wheeled into position below the mixer head and locked into place. Mounted on an overhead frame are the drive mechanism and spindles with horizontal paddles or arms. Most mixers of this type are equipped with two or three spindles. The spindles are lowered into the trough and move in either a planetary or a stationary, circular motion when activated. The shape of the arms forces the dough upwards and downwards in the trough, generating

a mixing action. The mixer blades are designed to provide a cutting action rather than kneading or stretching the dough.

The advantage of vertical spindle mixers is that they can be used for almost any product. They are very well suited for products such as soda cracker doughs. These doughs require a two-stage mixing sequence separated by a fermentation period. The sponges and doughs do not have to be transferred in and out of the trough at each mixing stage. Instead, they remain in the same trough for the entire mixing/fermentation sequence. Because there is almost no heat generated during the mixing process, this mixer is ideal for doughs that must remain cool. The mixing action is very gentle. Doughs containing ingredients or particulates that are easily damaged may be safely mixed with this mixer type.

The primary disadvantage of vertical mixers is their slow operating speed. The spindles rotate over a limited range at two or three speeds, up to a maximum of  $\sim 20$  rpm. For doughs requiring the development of a gluten network, the mixing time required may be as long as 90 min. Because this is slow relative to the speed of the remainder of the processing line, several mixers may be needed to maintain an uninterrupted dough supply. Other disadvantages include lack of uniformity of the mix, and the labor-intensive nature of the system's design. The latter disadvantage relates to the fact that the dough troughs are very heavy, requiring special equipment to move, lift, and tilt the tubs at each stage of the process.

#### **Horizontal Mixers**

The feature that all mixers of this type have in common is a horizontal bowl mounted on a rigid frame that encloses the drive motor. The bowl may be stationary with a vertical front wall that slides down so that the dough may be ejected. More commonly, the mixer bowl is designed to tilt, and the dough is ejected from the tilted position. In both designs, the top cover is fixed so that mixing takes place in an enclosed space.

The blades of horizontal mixers are mounted on a horizontal shaft and may have any of several different shapes, depending on whether a cutting, scraping, or kneading action is needed. Mixing times for cookie and cracker doughs range from 5 min for soft doughs to 30 min for the hard doughs, which require gluten development. Most mixers of this type are fitted with a jacket surrounding the mixing chamber. This allows a coolant to be circulated, so that dough temperatures can be controlled. Without such control, horizontal mixers can increase the dough temperatures to the point where dough

handling and finished product properties are adversely affected.

The advantages of horizontal mixers are their high speed, ability to supply dough to a processing line continuously, uniformity of the mixes they produce, and their potential for complete automation. Horizontal mixers may be operated at speeds of 15–80 rpm. Commonly, their power train is equipped for two-speed rotation. Unlike most vertical spindle mixers, ingredients may be added with the blades in motion, and the discharge of the dough from the mixer is simple. Accurate control of dough temperature is possible, because there is a continuously circulating refrigerant.

Horizontal mixers require that all ingredientcharging spouts be located overhead or that ingredients be added manually. Charging the mixer with ingredients is typically a significant portion of the total mixing cycle time. During mixing, the blades may throw material to the top of the chamber, so that it is never fully incorporated. With the shaft and mixing blades located in the center of the bowl, dough discharge is not always rapid and may occur in several large fragments. Only one operation (charging, mixing, or discharging) may be performed at any one time, so that fast cycling of the dough batches may not be possible.

Horizontal mixers usually have capacities of up to 550 kg. The weight and the vibrations generated by their relatively high operating speeds place special design demands on the production facility. In addition to a reinforced floor, special mounts are necessary to secure the mixer in place.

#### **Continuous Mixers**

Continuous mixers are best described as a rotor- or screw-operating within a barrel jacketed for temperature control. The ingredients are fed continuously, either from one end of the barrel or in successive ports at intervals along its length. The mixing action may be altered from gentle blending and dispersing to vigorous or high-intensity kneading by varying the arrangement of different mixing arms along the length of the barrel. The amount of work put into the dough during its transit may be controlled additionally by restricting the dimension of the outlet orifice, creating a back pressure inside the barrel.

Continuous mixers are favored in some plants because they are capable of providing a constant supply of dough to the production line. Continuous mixers are small in size relative to horizontal mixers and are suitable for complete automation. In spite of this advantage, continuous mixers are not common in cookie or cracker plants, where a single line may be required to produce a variety of products. They are usually

used only on high-output lines having a single purpose or similar product types.

The primary disadvantages of this mixer type are its high cost and the additional cost of the associated automated, continuous ingredient-feeding systems. Beyond this, continuous mix systems are not particularly flexible; different products usually require completely different types of machines. In addition, the initial process set-up can be difficult, requiring considerable experimentation to determine optimum mixing conditions and sequences. Finally, starting and stopping the process are difficult in the event of any problem along the rest of the production line.

## The Forming Process

This step is specific to each product type, whereas the mixing and baking stages may be similar for all types of products. Three processes are used to form cookie and cracker doughs: (1) cutting or stamping from a continuous sheet of dough, (2) rotary molding by shaping dough in die cavities cut into the surface of a metal cylinder, and (3) extruding dough through a shaped die.

For each of these methods, the rheology of the dough is different and is designed to be compatible with the process. In general, doughs that are to be sheeted possess a significant gluten network as a result of mixing, and are both elastic and extensible. Those destined for rotary molding lack gluten development and are best described as cohesive. Doughs intended for extrusion are soft, frequently high in shortening, and spread while baking.

#### **Sheeting and Cutting**

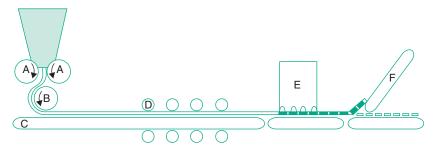
The most common and versatile method to form cookie and cracker doughs is sheeting and cutting. This method involves the production of a thick sheet of dough, evenly reducing the thickness of the sheet, cutting out the desired shapes, and returning the scrap dough to be reincorporated either in the mixer or early in the sheeting process. This method is used for the production of crackers, semisweet biscuits, and selected soft doughs.

After mixing, the dough is fed into a hopper, below which lie the sheeting rollers. There are typically three rollers below the hopper arranged in a triangular fashion (Figure 1). At least one of the top two rollers, known as forcing rollers (labeled A in the figure), is grooved so that a positive feed is provided to the gauge or gauging roller. The gauging roller (B), which is always smooth, serves to deliver the dough to the conveyor belt (C). The purpose of the sheeting unit is to compact the mass from dough hopper uniformly and provide a sheet of even thickness having the width of the processing line.

The relatively thick dough slab from the sheeter then passes through a series of reduction or gauge rollers (D). These are smooth steel rollers used to reduce the dough sheet to the thickness that is desired before cutting of the finished dough piece. The gauge rollers occur in pairs mounted vertically. For products having sticky or adherent doughs, it may be necessary to mount a scraper blade against one or both of the rollers to release the sheet of dough. On most process lines, there are two or three pairs of rollers. This ensures that the thickness is reduced no more than 50% at any one rolling operation.

Some doughs, such as those of saltines and cream crackers, are laminated before cutting. The lamination occurs by lapping the dough back upon itself in the process direction. At the lapper, the take-away conveyor lies at a 90° angle relative to the line delivering the dough. The number of layers is controlled by the relative rate of the lapper and take-away conveyor. The lapped dough then passes through several more sets of gauging rollers to bring the dough sheet to the desired thickness prior to cutting.

The repeated working of the dough in one direction results in the accumulation of stress. If the dough were cut at this point, the resulting pieces would shrink to



**Figure 1** Sheeting and cutting process. See text for explanation. (Reproduced from Macrae R, Robinson RK, and Sadler MJ (eds.) (1993) Biscuits, Cookies and Crackers: Methods of Manufacture. In: *Encyclopaedia of Food Science, Food Technology and Nutrition*. Academic Press.)

relieve the stress, and misshapen or distorted products would result. Therefore, it is normal to relax the dough after reduction and before cutting. The relaxation is accomplished by transferring the dough to a conveyor, still moving in the same direction, but at a slower speed.

Once the dough has been relaxed, it passes on to the cutting operation. Two different types of cutting methods exist: reciprocating cutters and rotary cutters. The reciprocating cutters are heavy block cutters that stamp out one or more pieces at a time. The cutter head (E) may have a dual action, whereby the cutter drops first, followed by a docking head or an embossing plate. The equipment operates via a swinging mechanism so that the dough sheet moves at a constant speed, the cutter drops and moves with the dough, and then rises and swings back to the original position. The second type of cutter, the rotary cutter, consists of a rotating metal cylinder. On the face of the roll are formed the desired shapes with a sharp metal edge. As the cutter rotates with the dough conveyor, the metal edges cut into the dough sheet to form the product. The product pieces are then conveyed into the oven.

As a result of either cutting process, 20–60% of the dough sheet remains as scrap. The scrap dough (F) is lifted away from the cut dough pieces and returned either to the mixer or to the sheeter. Return to the mixer permits uniform incorporation of the scrap into the dough mass. However, most systems route the scrap back into the sheeter either along the full length of the hopper or at the back side of the hopper. If dough is incorporated behind the new dough, imperfections will be on the bottom side of the dough sheet and will not be visible on the finished product.

#### **Rotary Molding**

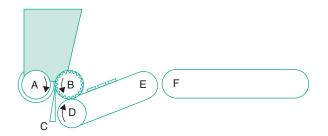
The principle of rotary molding is illustrated in Figure 2. Three rollers are placed in a triangular

arrangement below a dough hopper. A roller, called the forcing or feed roller (A), has deep grooves designed to pull dough down from the hopper. The dough is forced into the cavities of the engraved roller (B) by the forcing roller. A scraper blade (C) is mounted against the engraved roller to remove any excess dough and return it to the hopper via the forcing roller. Beneath the engraved roller is a rubber-covered extraction roller (D) that serves to drive the take-away belt (E). The extraction roller applies pressure to the engraved roller via the belt, causing the dough to adhere preferentially to the conveyor belt. Dough pieces are dropped from the take-away belt into pans or directly on to the baking belt (F).

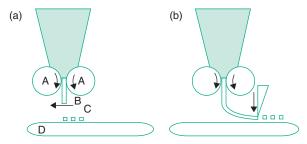
The rotary molding process is suitable only for dry, crumbly doughs. This process offers advantages over sheeting and cutting in that there is no scrap to recycle, and there are very low labor requirements to run the process.

#### **Extrusion**

There are two types of devices used in the production of extruded cookies: wire-cut machines and bar/ rout-presses. Both systems are very similar in design (Figure 3). A hopper is placed over a system of two or three rollers (A) that force dough into a pressure chamber (B). The rollers may run continuously or intermittently to force dough out of the pressure chamber at the die. For wire-cut cookies, the dough is extruded through a row of dies, and a wire or blade (C) mounted on a frame moves through the dough just below the die nozzle outlet. The cut dough pieces then drop into a conveyor band (D) for transport to the oven. The wire usually moves only in one direction through the dough, opposite that of the conveyor. The wire-cut machines operate at rates of up to 100 strokes per minute. Difficulties encountered with this type of production are distortion of the extruded



**Figure 2** Rotary molding process. See text for explanation. (Reproduced from Macrae R, Robinson RK, and Sadler MJ (eds.) (1993) Biscuits, Cookies and Crackers: Methods of Manufacture. In: *Encyclopaedia of Food Science, Food Technology and Nutrition*. Academic Press.)



**Figure 3** Extrusion process: (a) wire-cut machine; (b) bar/rout-press. See text for explanation. (Reproduced from Macrae R, Robinson RK, and Sadler MJ (eds.) (1993) Biscuits, Cookies and Crackers: Methods of Manufacture. In: *Encyclopaedia of Food Science, Food Technology and Nutrition*. Academic Press.)

dough piece during cutting, and inconsistent placement or drop of the cut piece on to the conveyor.

The design of the bar- or rout-press is very similar to that of the wire-cut machine. The hopper rollers and pressure chamber are essentially identical to their wire-cut counterparts. Unlike the wire-cut machine, the base of the pressure chamber has a die plate that is inclined in the direction of the extrusion. A continuous ribbon of dough is extruded from a nozzle, which is shaped to impart the desired finished product design. The dough ribbon can be cut into individual pieces by a vertically operating guillotine before the oven or after baking. If the product can be baked as a continuous ribbon the dough is extruded directly on to the oven band; otherwise, it is extruded and cut on to a conveyor belt.

## **Baking**

The baking of cookies, biscuits, and crackers is performed almost exclusively in band or traveling ovens. The band oven is essentially an insulated, heated tunnel equipped with a continuous conveyor. The ovens vary both in length (from 30 to 150 m) and in band width (from 1.0 to 1.5 m). More modern ovens frequently consist of a series of modular units or zones. Each of the zones is equipped with its own set of controls so that the temperature and air flow may be controlled within that zone. The oven band is typically continuous, passing on to a drive drum at the end of the oven and returning underneath the baking chamber to a tension drum at the feed or input end of the oven. The chamber through which the oven belt returns may or may not be enclosed. Frequently, the oven band serves as the baking surface for the product. Depending on the product type, the oven band may be solid or any of a variety of open wire-mesh types. The choice of mesh is a critical factor in the process, as it affects the heat transfer at the bottom of the product. This, in turn, can have a marked effect on the quality of the finished product.

There are three basic types of ovens: direct fired, indirect fired, and fully indirect fired. Ovens are usually heated by the combustion of gas, although there are a few manufacturers who use oil or electricity for economic reasons. The most common type is the direct-fired oven, in which gas is burned inside the baking chamber itself. In these ovens, the burners are placed across the width of the oven at regular intervals, both above and below the oven band. In other oven types, termed "indirect ovens," the gas or oil is burned outside the baking chamber, and the heated combustion gases are circulated into and throughout the baking chamber. Indirect-fired ovens typically have a single burner for each section. The hot

gases from the burner pass along pipes parallel to the length of the oven, both above and below the oven band. The products of combustion are circulated throughout the baking chamber by large fans. Fully indirect ovens are those in which the heat source is independent from the baking chamber and heat transfer occurs via a heat exchanger. None of the products of combustion circulate inside the baking chamber. This type of oven is not common, except when oil is used as a combustible material. If circulated, the products of this type of combustion would impart an unacceptable flavor to the products.

#### Cooling

Products hot from the oven must be cooled prior to packaging for several reasons: the products may not be firm enough to withstand the packaging process while warm, the packaging material may shrink around a warm product, or the quality of the products would deteriorate if palletized while warm because the cooling rate across the pallet would be quite slow.

The normal method of cooling products is to place them on an open conveyor and transfer them along a distance 1.5–2 times the length of the oven. The products cool naturally in the ambient factory atmosphere. In a few cases, it is necessary to provide forced air to aid the cooling process.

See also: Bakeries. Barley: Malting. Cakes, Pastries, Muffins, and Bagels. Cookies, Biscuits, and Crackers: Methods of Manufacture; Chemistry of Manufacture; The Diversity of Products. Consumer Trends in Consumption. Cultural Differences in Processing and Consumption. Extrusion Technologies. Food Safety through the Production Chain. Snack Foods, Processing. Starch: Analysis of Quality. Wheat: Dough Rheology. Appendix: Test Methods for Grain and Grain-Based Products.

## **Further Reading**

Almond N (1989) The Biscuit Making Process. Biscuits, Cookies and Crackers, vol. 2. London: Elsevier.

Faridi H and Faubion J (1995) Wheat End Uses Around the World. St. Paul, MN: American Association of Cereal Chemists.

Macrae R, Robinson RK, and Sadler MJ (1993) Biscuits, Cookies and Crackers: Methods of Manufacture. In: Encyclopaedia of Food Science, Food Technology and Nutrition. London: Academic Press.

Manley D (1991) Technology of Biscuits, Crackers and Cookies, 2nd edn. Chichester: Ellis Horwood.

Matz SA (1978) Cookie and Cracker Technology, 2nd edn. Westport, CI: AVI.

Matz SA (1988) Equipment for Bakers. McAllen, TX: PanTech International.

Pyler EJ (1988) Baking Science and Technology, 3rd edn., vol. II. Merriam, KS: Sosland.

Smith WJ (1972) Technology, Production and Management. Biscuits, Crackers and Cookies, vol. I. New York: Magazines for Industry.

Wade P (1988) The Principles of the Craft. Biscuits, Cookies and Crackers, vol. I. London: Elsevier.

## **Wafers**

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#### Introduction

The ice cream cone is the most familiar member of the wafer product group. Like many uses of wafers, the ice cream cone is the ultimate illustration of the concept of providing food in an edible container. Indeed, wafers usually serve the function of being edible carriers of an added food. However, the crispness of the wafer enhances the appeal of the added food, making the wafer much more than an inert carrier.

Wafers are a special member of the biscuit/cookie/cracker family of cereal products. The diversity of wafer shapes (illustrated in Figure 1) includes flat wafers, hollow wafers, molded cones, rolled wafer cones, and wafer sticks. Wafers are thin, crisp, and precisely shaped products, generally made from cereals. They may be made from quite different recipes, using a diversity of manufacturing equipment, and destined for a variety of uses. However, they share the same fundamental sequence of manufacturing steps detailed in this article. To understand the manufacturing process, the concepts of water activity, and glass transition are of critical importance.

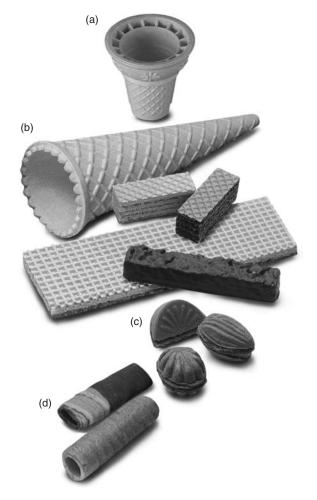
#### The Main Features of Wafers

Wafers are baked as sheets, cones, and sticks or with different fancy shapes. The characteristic features with respect to other bakery products are:

1. Wafers are very thin biscuits; the overall thickness is usually between less than 1 and 5 mm. They

- often have a typical "wafer pattern" on one surface or on both. The surfaces are smooth and precisely formed, with the dimensions and all the details – engravings, logos, etc. – of the baking molds. See Figure 1 for some examples.
- 2. Wafers are cereal-based low-fat products made of wheat flour, sometimes with addition of other flours or starches. The product density is in the range of 0.10–0.25 g cm<sup>-3</sup>. In cross-section, the wafer matrix is highly aerated and primarily of gelatinized starch.
- 3. Wafers, by their typical delicate and crisp texture, combine well with different fillings (cream, ice cream, foam) and coatings.

There is sometimes confusion in terminology between the crisp "wafers" as described here and "waffles,"



**Figure 1** Wafer products. *From top to bottom*: (a) molded cones; (b) flat wafers, creamed/enrobed; (c) hollow wafer pieces; and (d) wafer sticks. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 539, Elsevier Ltd.)

which are of a soft, cake-like texture but show some kind of wafer pattern, too.

## The Main Types of Wafers

There are two basic types of wafers:

- No- or low-sugar wafers. After baking, these contain from zero to a very low percentage of sucrose or other sugars. Typical products are flat and hollow wafer sheets, molded cones, cups, and fancy shapes.
- 2. Higher-sugar wafers. Well over 10% of sucrose or other sugars are responsible for the plasticity of the hot, freshly baked sheets. These are formed into different shapes before sugar recrystallization occurs. Typical products are rolled sugar cones, rolled wafer sticks or tubes, and deep-formed fancy shapes.

As in both wafer types, the main ingredient is flour, wafers fit very well into current dietary recommendations to consume more cereals. They are high-carbohydrate, low-fat products.

The baking of "wafers" between hot metal plates has been known since medieval times, but these first wafers were more similar to our waffles or pancakes in their high fat and egg contents and their texture.

Modern wafers are low-fat cereal products, very similar to the altar breads for Christian churches, and are basically made out of flour and water. The first wafer ovens were used after First World War, but more automatic manufacturing lines have been available since the mid-1950s.

#### **The Main Recipes for Wafers**

There are two questions to be answered before deciding upon a wafer recipe:

- What is the end use of the wafer? If it is part of a cream-filled, chocolate-covered biscuit, where contributing a crisp texture element is far more important than the taste of the wafer itself, recipes with few components are recommended. If the wafers are consumed directly as wafer bread or wafer sticks, more sophisticated recipes are chosen.
- 2. What kind and quality of raw materials are available? Low- to medium-protein soft wheat flours with a low water absorption work best, especially for no-sugar wafers. Problems with suboptimal flours must be balanced by variations in minor ingredients in the recipe. The use of wholemeal flour is possible, and in some regions, other cereals such as rice or corn are used for wafer production.

Table 1 lists the common ingredient ranges for both types of wafer.

## In-line Manufacturing of Wafer Biscuits

Wafer biscuits are the most important products by volume. The different steps of manufacture are illustrated in Figure 2.

#### **Batter Preparation**

Wafers are made from a fluid batter with a typical viscosity in the range of 300–2000 mPs. First, the water-soluble components are dissolved. By adding the farinaceous ingredients, a homogenous suspension, the "wafer batter," is obtained within a few minutes of mixing (Figure 2, process 1).

#### **Batter Transport and Depositing**

From an intermediate tank via a ring main, the batter is pumped to the oven and spread on to the baking molds by a depositor head (Figure 2, process 2).

#### **Wafer Sheet Baking**

The baking of wafer sheets is performed in "tongs," i.e., pairs of cast-iron metal plates with a hinge and latch on opposite sides. Baking plate sizes up to  $350 \times 730$  mm are available. The precisely machined baking plates carry reedings or other engravings. We call the resulting wafers "flat" wafer sheets, with an overall thickness of no more than 2-5 mm (Figure 2, process 3).

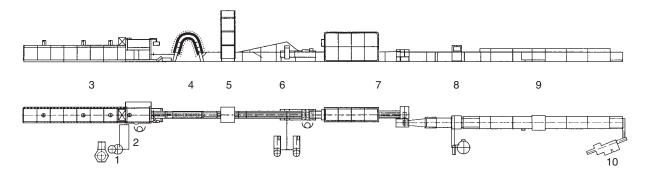
But such baking plates can also carry special figures (nuts, sticks, hemispheres, fancy shapes) up to a depth

**Table 1** Wafer batter ingredient ranges (weight parts, flour = 100)

	No (low)-sugar wafer	Higher-sugar wafer
Wheat flour	100	100
Water	120-160	100-140
Starch	0-12	0-5
Sucrose	0-4	25-75
Oil/fat	0.5-2	1-6
Milk powder	0-2	0-2
Soy lecithin	0.2-1	0.2-1.5
Salt	0-0.6	0-0.6
Sodium bicarbonate	0.1-0.5	0-0.3

Optional minor ingredients: other cereal flours, soy flour, other sugars and syrups, egg-based ingredients; whey powder, yeast, caramel color, cocoa powder, colors, ammonium bicarbonate, enzymes.

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**Figure 2** Manufacturing line for creamed and enrobed wafer biscuits from flat wafers. (1) Batter preparation; (2) batter transport and depositing; (3) wafer sheet baking; (4) wafer sheet cooling; (5) conditioning of sheets; (6) creaming and wafer book building; (7) cooling and cutting of books; (8) enrobing; (9) cooling of enrobed pieces; and (10) packaging. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 540, Elsevier Ltd.)

of  $\sim$ 20 mm, thus yielding the so-called "hollow" wafer sheets.

Modern wafer-baking plates often are surfaceplated, e.g., with chromium for easier release and reduction of cleaning stops. The plates are edged with metal strips to give a closed baking mold, except for small venting channels for steam release. Waferbaking ovens frequently have 32–104 pairs of plates, continuously circulating on a chain. They are mostly gas, sometimes electrically heated and operate at mold temperatures between 160°C and 190°C.

The baking process Within a few seconds after batter deposition, the baking molds close and are locked. At first, the batter is distributed mechanically, but then the mold is filled completely by the steam that evolves. A small quantity of batter is extruded as baking waste "bobbles" through the venting channels. As gelatinization of starch starts immediately, the pressurization of the mold by steam occurs at the right moment, resulting in a well-aerated starch foam.

When most of the water has been driven off, the glass temperature of the wafer matrix rises, and the stable structure is formed. The temperature of the wafer increases to 160–190°C, i.e., to the temperature of the baking mold. Then, from Maillard reactions, the typical wafer color and flavor develop.

The overall baking times are between 1.5 and 2.5 min, depending on the wafer thickness and baking temperature.

During the wafer manufacturing process, there is no substantial degradation of starch molecules compared with other bakery products such as extruded cereals. Therefore, wafers have two unique textural properties:

- 1. Extreme crispness on biting and initial chewing.
- Good mouth feel during prolonged chewing and swallowing owing to the absence of sticky, glutinous stimuli.

#### **Wafer Release and Cooldown**

At one end of the oven, the plates open to release the baked sheets and to spread fresh batter, and then reclose very quickly. The sheets are cooled to room temperature while passing over an arch-type sheet cooler (Figure 2, process 4).

#### **Wafer Conditioning**

Next, the wafers optionally pass a conditioning unit, where the moisture content of the sheets is carefully increased so as to achieve some stability in both the texture and size of the wafer (Figure 2, process 5).

Moisture sorption and wafer texture After baking, the residual moisture is 0.8-1.5%. Looking to the sorption isotherm, this corresponds to a water activity of  $\sim 0.1$  or even lower. As both the water activity of the air in the production area and the water activity of filling creams or coatings are well above that, wafers pick up moisture very easily. In line with this sorption, the dimensions of the sheet increase by 0.2-0.3% for every 1% of additional moisture. This can result in cracking of the coating in enrobed wafer biscuits.

To compensate for the low water activity, humidity conditioning up to ~4.5% wafer moisture is possible. This is recommended, especially if enrobed or chocolate molded wafer products are made, in order to anticipate this first dimension increase and to avoid any cracking of the coating during its shelf life.

Moreover, with increasing water activity, the wafer texture changes from a soft to a harder crispness, accompanied by a higher mechanical stability, which is good both for handling and for the final product texture. Up to 5-6% moisture content, the wafer sheets keep their typical crisp texture, but higher moisture levels will result in most cases in inadequate, tough, or even soft and soggy textures (see Table 2 for details).

Table 2 Water activity, water content, and texture of wafers

Wafer condition	Wafer texture	Water activity, approx.	Moisture (%), approx.
Freshly baked	Very tender, crisp	< 0.1	<2
After conditioning	Crisp, harder	0.3	4.5
Limit of crispness	Crisp to tough	0.5/0.55	>6
Wafers, foam-filled	Soft to flexible	0.7	_ > 12
Collapse of structure	Very soft, shrinks	> 0.85	>20

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#### **Creaming and Book Building**

The sheets then pass the creaming station, where a layer of a sugar- and fat-based cream is applied to one side. The type of cream flavor liked most varies regionally, but chocolate, vanilla, hazelnut, milk, strawberry, and lemon are the most common. Creaming is done at temperatures of 30–40°C either by contact spreading or by depositing a preformed cream film (Figure 2, process 6). For flat wafers, several creamed sheets together with a noncreamed top sheet form a so-called "wafer book."

For hollow wafers, the cream is added to the hollow parts either by spreading or by controlled single depositing. Again, either two hollow wafer sheets or a hollow and a flat sheet are combined to form a "book."

#### **Cooling and Cutting**

The wafer books pass a cooling tunnel to set the cream, after which they are wire or saw-cut into small biscuits (Figure 2, process 7).

#### **Enrobing or Molding in Chocolate, Cooling**

The cut biscuits may be enrobed with chocolate-type coatings, sometimes after the application of chopped nuts or crispies to the top wafer. Molding in chocolate is another possibility. After a final cooling step, the biscuits are ready for packaging (Figure 2, processes 8 and 9).

#### **Packaging**

The biscuits have to be packed tightly to protect against humidity, but also against oxygen and light to prevent oxidative deterioration and to insure a shelf life of 6–9 months. Inadequate packaging-film moisture barriers and bad sealing are the most frequent reasons for later complaints by customers. Laminated or specially coated films are used for the typical wafer product packaging in flow packs, boxes, or bags (Figure 2, process 10).

## **Manufacturing of Molded Wafer Cones**

Another type of "hollow" wafers, cones, cups, and fancy shapes with up to 185 mm in length are produced in cast-iron molds. Holes for four to six



**Figure 3** Molded wafer cone: example for new, nonflat top design. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 542, Elsevier Ltd.)

items are provided in each of these molds, and 12–72 molds are circulated in one oven. The lower part of the mold is made of two symmetric halves that open to release the baked pieces. After their reclosure, they take up a fresh batter deposit, and finally the "core," the upper part of the mold, closes the mold for a new baking cycle. The baked cones are cooled down and stacked for packaging.

Recipewise, there are two groups of molded cones:

- 1. No- or low-sugar cones, generally known as "cake cones," whose recipes are similar to those for sheets (see Table 1).
- 2. The so-called "molded sugar cones" have an intermediate sugar content, usually below 20 parts of sucrose for 100 parts of flour.

## **Recent Developments in Molded Wafer Cones**

Whereas traditionally molded cones have flat tops and regular, symmetric reedings now for a few years, molds for more sophisticated products can be manufactured. We now see cones and cups in the market showing curvilinear tops and artfully designed outsides. Figure 3 shows the first of these, which comes from a Japanese cone manufacturer.

## **Manufacturing of Rolled Wafer Cones**

"Rolled sugar cones" need a concentration of more than 20% of sucrose or other sugars in the finished product. The first three steps of rolled sugar cone manufacturing are rather similar to no- or low-sugar wafer sheet baking with the exception of reduced steam pressure as there are no baking ledges to build up pressure and to extrude "bobbles." The deposit is just to form an oval or circular sheet (see Figure 4):

- 1. Batter preparation (Figure 4, process 1).
- 2. Batter transport and depositing (Figure 4, process 2).
- 3. Wafer sheet baking (Figure 4, process 3). Nowadays, equipment is available to manufacture up to 13 000 cones per h.
- 4. Wafer take-off, rolling, and cone release (Figure 4, process 4). When, after baking, the mold reopens, the sheet is automatically stripped off the plate and rolled immediately on tapered mandrels to form the finished cone. A series of rolling devices mounted on a round table operates continuously: sheet removal, rolling, release, etc.

#### **Glass Transition and Formability**

During the rolling procedure, the wafer temperature is still above the glass transition point. The molten sugar acts as a plasticizer, and so any rolling or deep-forming operation can be performed. Besides the rolled cones, three more types of products are manufactured by the same principle:

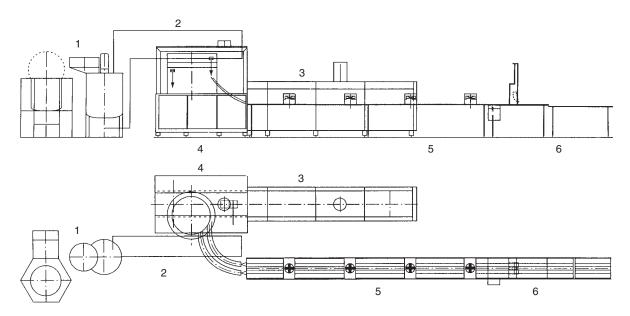
- 1. Wafer "rolls." Wafer sheets of rectangular shape are baked, stripped off the baking plate, and rolled into a sugar wafer "rod" without a center hole (different from wafer "sticks" with a center hole discussed later). The rod is later cut into smaller cylindrical pieces. Typically, these pieces are finally partially enrobed with chocolate-type coatings.
- 2. "Deep-formed" wafers (cups, shells), where the hot, flat wafer piece is introduced into a forming tool, sometimes with an additional embossing or stamping device, which defines the final shape.
- 3. Rolled wafer sticks. These are discussed in a separate section below.

## **Cone Cooling**

The fresh cones pass through a cooling section, where ambient or cooled air is applied. Here, sugar recrystallization finishes to give the final strong and brittle texture (Figure 4, process 5).

#### **Cone Sleeving and Stacking**

Now, paper sleeves are applied automatically, and these form the packaging material for the typical end product, industrially manufactured icecream cones. Next, is automatic cone stacking and packaging.



**Figure 4** Manufacturing line for rolled sugar cones. (1) Batter preparation; (2) batter transport and depositing; (3) wafer sheet baking; (4) take-off, rolling, and cone release; (5) cooling of cones; and (6) cones sleeving, stacking. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 543, Elsevier Ltd.)

Later in the ice-cream plant, the wafer cones are sprayed inside with chocolate and filled, and finally, the paper cone is closed (Figure 4, process 6).

## **Manufacturing of Rolled Wafer Sticks**

Rolled wafer sticks are hollow tubes with walls consisting of very thin multiple layers, similar in texture to crunchy cereals. These layers do not carry a wafer pattern and are  $\sim 0.5$  mm in thickness. But from the recipe, the sticks are typical higher-sugar wafers (see Table 1).

In manufacturing, a stripe of batter is applicated to a heated drum and baked into a continuous wafer band. The band is rolled immediately while hot into a continuous still formable tube with an internal diameter of  $\sim 6-36$  mm. From here, there are several options to create a large family of finished products:

- The tube is automatically cut into wafer stick pieces and cooled. These are consumed as sweet snacks or with ice cream, for example. Other end uses include intermediate products for confectioners to finish by additional filling or decorating operations.
- 2. During the rolling operation, the tube is coated inside with compound chocolate or filled with cream, either partially or fully. Then, cutting and cool-down, followed optionally by a coating and/or decorating process, give the finished product.
- 3. The filled tube is transformed into small pillow-like bits by a combined squeezing—cutting operation.
- 4. The wafer tube pieces, nonfilled, coated inside, or partially cream-filled immediately after cutting while still hot, pass a pressing station to form oval or even flat pieces. Such flat pieces may form the center of premium-type confectionery items with a chocolate coating, including chopped nuts or cereal crispies, for example.

5. Larger-diameter tubes are pressed flat, and by an embossing and stamping operation, so-called "fan wafers" or other elaborate shapes can be generated.

The diameter and length of the sticks as well as the number of very thin, "glassy" sheets forming the wall of the stick can be adjusted individually. Rolled wafer sticks have a unique brittle wall texture without being too hard in biting and chewing.

## **Savory Wafer Products**

Whereas many end uses of wafers in combination with sweet fillings, coatings, etc. are well known, savory or neutral-tasting wafer products are becoming increasingly popular in some areas. Some examples:

- 1. Wafers as crispbread, delivering a crunchy but softer texture than traditional hard crisp breads. Besides wheat flour there are several options for other cereal and noncereal flours as raw materials. The products are either neutral in taste to be eaten with sweet or nonsweet toppings or have spices, cheese, and other flavorsome ingredients.
- 2. Wafers flat or hollow wafers with savory fillings, e.g., peanut butter or cheese creams.
- 3. Wafer sticks, a recent development, made from a nonsweet but still rollable material. These are typically combined with savory cream fillings.

# Trends — From the Very Wafer to a More Sophisticated End Product

After discussing the different wafer types and their traditional end uses (for an overview, see Table 3), a sharply increasing number of new confectionery brand products during the last decade are worth mentioning. Here, wafers are only a smaller, but still important, part of the deal. These are small confectionery pieces, mostly in bite-size or sectioned

**Table 3** Which product from which wafer type?

End product	No (low)-sugar wafer	Higher-sugar wafer
Wafer crisp bread	Flat wafer sheet	
Sugar wafer biscuits, creamed	Flat wafer sheet	
Wafer biscuits, creamed and enrobed or molded in chocolate	Flat wafer sheet	
Containers, e.g., for ice cream	Molded cake cone	Molded sugar cone, rolled sugar cone
Confectionery, filled, enrobed, decorated	Hollow wafer or hollow + flat wafer	Wafer stick or sugar cone, small
Fan wafers		Wafer stick, pressed, embossed
Wafer rolls, partially enrobed		Rolled sheet
Wafer bowls		Deep-formed sheet



Figure 5 New, inductively heated, low-emission oven for rolled wafer stick manufacturing. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 545, Elsevier Ltd.)

bars, often chocolate-covered, where the wafer has three main functions:

- 1. To add its typical texture and crispness, e.g., to a soft filling cream with a piece of nut and a chocolate cover, thus imparting a multitextured impression.
- 2. To make the overall product "lighter" in calories and nutritionally, as the wafer part is of an extremely low density and cereal-based.
- 3. To define the precise structure of the piece, e.g., when holding a soft filling, or, for noncoated pieces, to keep the consumer's hands clean if the filling melts due to warmer conditions.

Looking to some of the current trends, we see increasing interest in combining lower fat fillings such as toffee (caramel). Another trend may be newer fatfree fruit-type fillings with water activities that are so low that the wafers stay crisp. Even some new examples of fillings with a higher moisture, resulting in a "soft" wafer part, can be rolled out.

## **Developments in Wafer-Manufacturing Equipment**

After a long period of just "routine" technical optimizations, recent years have seen a few new technical concepts in wafer-manufacturing equipment:

1. The "stack oven," with the baking plates vertically stacked. Here, the weight of the stack itself

- eliminates the need for hinges and for closing mechanisms as in the traditional chain oven. Moreover, the floor area needed is greatly reduced. The coming years will show whether that development will find its way into industrial practice.
- 2. A new "low-emission" heating concept together with a 60% reduction in energy consumption for rolled wafer stick ovens. The key is the replacement of the gas-heated drum by a ring, heated by induction. This results in a new, more consistent and more controllable product quality, and results in the elimination of flue gases and a huge reduction in energy consumption for baking, as the first industrial oven prototypes (Figure 5) showed.

See also: Bakeries. Cakes, Chemistry of Manufacture. Cakes, Pastries, Muffins, and Bagels. Cereals: Overview; Grain-Quality Attributes. Cookies, Biscuits, and Crackers: Chemistry of Manufacture; The Diversity of Products. Extrusion Technologies.

## **Further Reading**

Barron LF (1977) The expansion of wafer and its relation to the cracking of chocolate and "baker's chocolate" coatings. Journal of Food Technology 12: 73-84.

Blanshard JMV, Frazier PJ, and Galliard T (eds.) (1986) Chemistry and Physics of Baking. London: The Royal Society of Chemistry.

Haas F, Haas J, and Sachsenhofer J (1998) Bakers' oven for producing thin-walled shaped products with baking forms each consisting of two form halves which open and close. *PCT Patent Application* WO 98/51156.

Manley DJR (2000) *Technology of Biscuits, Crackers and Cookies*, 3rd edn., pp. 290–306. Cambridge, UK: Woodhead Publishing.

Negri G (1984) Waffeln und Verfahren zu deren Herstellung. European Patent 0,022.901.

Oliver G and Sahi SS (1995) Wafer batters: a rheological study. *Journal of the Science of Food and Agriculture* 67: 221–227.

Pritchard PE and Stevens DJ (1974) The influence of ingredients on the properties of wafer sheets. *Food Trade Review* 42(8): 9–15.

Roos YH (1995) Phase Transitions in Foods. London: Academic Press.

Schwartzberg HG and Hartel RW (eds.) (1992) *Physical Chemistry of Foods*. New York: Marcel Dekker.

Seibel W, Menger A, Ludewig H-G, Seiler K, and Bretschneider F (1978) Standardisierung eines Backversuches für Flachwaffeln. Getreide, Mehl und Brot 32: 188–193.

Stevens DJ (1976) The role of starch in baked goods. Part 1 The structure of wafer biscuit sheets and its relation to composition. *Starch* 28: 25–29.

Tiefenbacher K and Dobrovics M (1999) Mehle für die Waffelherstellung. *Getreide*, *Mehl und Brot* 53: 349–353.

Wade P (1988) Biscuits, Cookies and Crackers, vol. 1, pp. 139–150. Barking, UK: Elsevier.

## **Chemistry of Manufacture**

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#### Introduction

The term "cookie" obviously derives from the general verb "cook," but it has taken the specific meaning of a small flat baked product, generally sweet, made from cereal flour. The equivalent term "biscuit" means twice baked, referring to the low-moisture content of this family of baked products. Low-moisture content and brittleness is also inferred by the term "cracker." The term "biscuit" is more common in the UK, Australia, and New Zealand, whereas the words "cookie" and "cracker" are more common in the USA, where a biscuit is a chemically leavened bread-type item similar to a "scone" in the UK. In this article, the term "biscuit" is used to include the family of biscuits, cookies, and crackers. The biscuit group of products is distinguished from other baked

products by their moisture content of 1-5%, in contrast to finished breads and cakes with 35-40% and 15-30% moisture, respectively.

# **Characteristics of the Biscuit Family of Products**

Wheat flour, sugar, and fat are the basic ingredients, but thereafter the variety is almost endless. Biscuits can be grouped in many ways, based on their texture and hardness, their change in outline during shaping and baking, the extensibility or other characteristics of the dough, or the ways that the doughs are handled prior to biscuit formation. The groups often overlap, so it is important to see how several types of biscuits are related, based on their proportions of fat, sugar, and water, and then to compare their typical characteristics and processing means.

These differences in formulation, processing, and finished product attributes are all a function of the dough consistency or dough rheology. Certain key ingredients, such as flour, fat, and sugar largely determine the dough rheology and thus what type of *forming equipment* can be used to produce the biscuit. Based on these fundamental distinctions, five basic categories can be devised: (1) wire-cut; (2) rotary-molded; (3) bar-shaped; (4) deposited; and (5) cut or stamped. Regardless of the category, there are certain rheological requirements for all biscuits, namely the dough must be adequately cohesive for molding/forming, without excessive stickiness, and the dough must have a short, cuttable texture.

Probably the single greatest influence on (biscuit) dough rheology is the degree of gluten development within a dough. Biscuit doughs generally fall into two broad categories – hard and soft doughs. The viscoelastic properties of hard doughs represent the presence of a protein (gluten) matrix which is developed during mixing and sheeting. Hard dough are stiff. tight doughs that require extensive mixing (work) with a resulting increase in dough temperature. They are similar to bread doughs, except that the sugar and fat contents modify their viscoelastic properties. Hard doughs are usually laminated and sheeted before cutting or stamping. The formed pieces will generally shrink because of the elastic quality of the gluten. During baking, the biscuits may continue to shrink in outline, but become thicker. This type of dough formulation may also be suited for rotarymolded biscuits, due to its firm consistency.

Soft doughs do not have a formed gluten structure, because of their high levels of shortening and sugar, and are generally mealy or sandy in texture. They are usually formed by compressing into dies (rotary-molded) or by extruding and cutting, but some

types can be sheeted, then cut. Dough pieces formed from soft doughs tend to retain their shape until baking, but then they spread or flow, becoming thinner.

Deposit biscuits are the machine-made counterpart of the hand-bagged or "drop" version. Such formulae have been successfully adapted to automated production. Deposit biscuits contain about 25-40% sugar, 65-75% shortening, and 15-25% eggs and possess a spread factor of 79-80 (percentages based on 100 parts flour). (Spread factor is a measure of how much a cookie dough will spread out when it is baked. It is equal to the average width divided by the average height, and then multiplied by 10.) Formulations should be such that excessive spread does not occur and the top design of the biscuit is preserved during baking. Adequate adhesive characteristics of the dough are also needed, so that it will adhere to the band and separate from the main tube of dough when deposited (Figure 1). This type of formula may also apply to wire-cut biscuits, where the dough is forced through a die and cut into disks by a reciprocating wire.

Although there are many similarities between biscuit and cracker formulations, a few key differences should be pointed out. One is the obvious difference in sugar content. Unlike biscuits, crackers are usually made from laminated dough, where thin sheets of dough are alternately layered with fat. Whereas biscuits are chemically leavened, some crackers, i.e., saltines, are yeast-fermented over long time periods to develop the characteristic flavors. The flour used in

90 80 70 Wire-cut biscuit 60 Shortening (%) 50 40 30 Sheeted/molded biscuit 20 10 0 0 10 20 30 40 50 60 70 80 90 Sugar (%)

**Figure 1** Biscuit composition in relation to sugar and shortening, based on 100 parts flour. (Each dough is processed according to its consistency or water content.) (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 534, Elsevier Ltd.)

crackers is commonly stronger than biscuit flour and often dough relaxers, e.g., proteases, are required to increase the extensibility of the cracker dough. Salt further strengthens dough, increasing its resistance to extension. A series of biscuit and cracker formulae is shown in Tables 1 and 2.

Table 1 Representative formulae for various sweet biscuits

Ingredient	Animal cracker (molded) %	Sugar cookie (cut/stamped) %	Chocolate chip (wire-cut) %
Soft flour	55.72	45.00	22.00
Shortening	11.36	11.20	19.40
Granular sugar	11.00	27.10	29.70
Water	7.70	9.80	NA
Sucrose syrup	7.33	NA	NA
Honey	2.35	NA	NA
Arrowroot flour	1.83	NA	NA
Molasses	0.88	2.70	NA
Salt	0.88	0.40	0.70
Monopotassium tartarate	0.37	NA	NA
Sodium bicarbonate	0.37	0.40	0.10
Lecithin	0.22	NA	NA
NFDM	NA	1.30	NA
MCP	NA	0.30	NA
Liquid whole eggs	NA	1.80	9.10
Vanilla flavor	NA	NA	0.20
Chocolate chips	NA	NA	18.70

NFDM, non-fat dry milk; MCP, monocalcium phosphate; NA, not applicable. Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 534, Elsevier Ltd.

Table 2 Representative formulae for various cracker types

Ingredient	Snack cracker %	Soda (saltine) %	Graham %
Strong flour	66.37	69.60	25.40
Soft flour	NA	NA	25.40
Graham flour	NA	NA	12.60
Water	18.10	21.58	16.40
Granular sugar	3.85	NA	7.62
Shortening	3.85	6.61	5.08
Ammonia bicarbonate	2.05	NA	0.38
Corn syrup	1.90	NA	NA
Malt syrup	1.28	0.64	0.44
Brown sugar or honey	NA	NA	4.64
Meal	0.96	NA	NA
Sodium bicarbonate	0.71	0.44	0.63
Salt	0.60	0.97	0.89
Monocalcium phosphate	0.30	NA	0.25
Protease	0.03	NA	NA
Lecithin	NA	NA	0.25
Optional seasonings	As desired	NA	0.02
Yeast	NA	0.16	NA

NA, not applicable.

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#### **Ingredients on the Biscuit Dough**

#### **Wheat Flour**

Wheat flour is unique among the cereal grain flours in that, when mixed with water, its protein components form an elastic network capable of holding gas and developing a firm spongy structure during baking. The protein substances contributing these properties (gliadin and glutenin), when combined with water and mixed, are known collectively as gluten. The suitability of a flour for biscuit making is generally determined by its gluten. Gluten characteristics are determined by genetics, the wheat's growing conditions, and the milling process.

Wheats are described by millers as hard, medium, or soft, based on the grain's physical characteristics. Hard types tend to have higher protein quantity and quality, possessing a vitreous endosperm, with starch granules tightly packed in a protein matrix. During milling, some starch grains are damaged, resulting in an increased surface area that leads to higher water absorption. The softer wheats have a less compact starch—protein complex which results in less starch damage and lower water absorption. The protein level of soft wheats is usually lower, producing a less-resistant, more extensible dough. These "weaker" flours are traditionally deemed more suitable for biscuit making than the harder flours used for breads.

Flour protein levels needed for biscuit making typically range from 7% to 10% and are often selected by functionality for end use, price, and availability. Flour should contain no more than  $\sim 14\%$  moisture. Biscuit flour is typically left unbleached and unchlorinated.

#### **Sweeteners**

All biscuit formulas contain sweeteners, which constitute the bulk of dissolved materials in most doughs. Sugars impart sweetness, act as vehicles for other flavors, and create an attractive finish. Sweeteners also increase tenderness, crust color, volume, and moisture retention, while maintaining the proper balance between liquids and solids responsible for product contour/shape. Machining properties and baking characteristics are also closely related to sugar. Sweeteners tenderize the finished product by interfering with gluten hydration and starch gelatinization.

High-fructose corn (maize) syrups are becoming increasingly important, but sucrose from sugar cane or beet is still the major sweetener. Commercial sugars are often categorized as granulated or powdered. Granulated sugars range from coating sugar (extremely fine grain) to coarse. Powdered sugars are made by grinding granulated sugars and screening

through fine bolting cloths. Generally, as the size of the sugar crystal increases, the size and symmetry of the biscuit decrease, while the thickness and color increase. Often anticaking agents, e.g., corn starch, are added to insure proper flow of the sugar during raw material handling. Where pumpability may offer a processing benefit, granular sucrose can be combined with water to form "liquid sugar," usually at 67% sugar solids or 67°Brix.

Sucrose may be hydrolyzed (inverted) to glucose (dextrose) and fructose (laevulose) by heating it in the presence of a dilute weak acid or mixing it with invertase enzyme. Sucrose is the standard for sweetness, with an arbitrary rating of 100. Fructose and glucose are rated at 170 and 74, respectively. Their combined sweetness in a completely inverted sugar is 127, sweeter than the starting sucrose. Inverted sugars are used primarily for their hygroscopicity and browning reactions, which contribute moisture retention and color development in the biscuits.

#### **Shortenings and Emulsifiers**

Fats are the third major component used in biscuit making, but are considerably more expensive than flour or sugar. Besides being used in the doughs, fats or oils are used as surface sprays, in cream fillings and coatings (such as chocolate), and as release agents. In dough, they tenderize (impart shortness to) the crumb by being dispersed in films and globules during mixing, which interferes with gluten development. Shortening also aids dough aeration during the creaming step. The overall effect improves palatability, extends shelf life, improves flavor and, of course, adds caloric energy.

Animal fats, primarily lard, were originally used by bakers. Compound (part animal and part vegetable source) shortenings and all-vegetable shortenings were then developed. Soybean, cottonseed, palm, coconut, and peanut oils are the primary vegetable sources used in shortening production. Continued advancements in purification and hydrogenation developed vegetable oils that could replace animal fats with equal or better flavors, melting points, consistency, and availability. Because of current health concerns, most bakeries have switched to fats of plant origin. The hydrogenation process used to convert liquid vegetable oils into plastic shortenings suitable for entrapping the air and controlling spread is known to generate some transfatty acids which are sometimes believed to be harmful. This is not normally a major concern for cookie producers or consumers however, with the possible exception of sandwich cookies containing a large amount of a shortening-based filling.

Surfactants (surface-active agents) are given many names by bakers: crumb softeners, emulsifiers, antistaling agents, or dough conditioners. Examples include lecithin, mono- and diglycerides, diacetyl tartaric acid esters of fatty acids, polysorbate 60 and sodium stearloyl 2-lactylate. Surfactants at low concentrations act to modify the surface behaviors of liquids. They are believed to complex with the protein-starch structure, thereby strengthening the film, and to delay dough setting during baking. The behavior of surfactants is due to their amphoteric (possessing both hydrophilic and hydrophobic molecular regions) properties. Their behavior varies according to the charges on the molecules, their solubility, the hydrophilic-lipophilic balance, and the type of functional groups involved.

Surfactants modify dough consistency and reduce stickiness by reacting with the gluten. The greasiness of biscuits with high fat content is also reduced by surfactants. Crumb softeners also complex with the starch molecules to delay retrogradation and texture staling. The grain pattern and volume of the finished product are often improved, as surfactants increase dough gas-retaining properties.

Antioxidants retard the development of oxidative rancidity during product storage. All fats are subject to oxidative or hydrolytic rancidity, which causes objectionable odors and flavors, but antioxidants delay these reactions from occurring within the biscuits' shelf life. They are usually added to bulk shortenings and are important for preserving low-moisture products, which are expected to remain edible for several months.

#### Is Water an Ingredient?

Water is often thought of as a processing aid or catalyst, rather than as an ingredient. It is incorporated at the dough stage but driven off during baking. Water functions in several ways, including hydrating flour proteins and starch, dissolving sugars, salts, and various leavening chemicals, aiding in ingredient distribution and helping control dough temperature.

A dough's consistency is directly related to its water content, or absorption. Many factors affect dough absorption. Approximately 46% of flour's total absorption is associated with the starch, 31% with protein, and 23% with the pentosans. Acceptable consistency can be obtained only after sufficient water is present to hydrate the flour. This is regarded as bound water and controls the dough's consistency. As bound water layers are "stacked up," some of the water is held less and less strongly, resulting in water that can escape (evaporation) and/or migrate as free water. Water activity  $(a_{w})$  is an important way

to measure and monitor water's mobility in baked products.

#### **Making Biscuits Lighter**

Leavening agents aerate the dough or batter to make it light and porous. The leavening action is responsible for good volume, improved eating quality, and a uniform cell structure. Leavening can be achieved by various methods, including yeast fermentation, the mechanical incorporation of air by mixing and creaming, formation of water vapor during baking, and the creation of carbon dioxide and/or ammonia by chemical leaveners. However, creation of the initial air bubbles during the mixing phase is critical before any of the other leavening agents can take effect.

Small products like biscuits that bake quickly need a fast-acting leavener that will release the gas before the structure sets. The most widely used source of carbon dioxide in chemically leavened systems is the reaction of sodium bicarbonate or baking soda (NaHCO<sub>3</sub>) with an acid, usually the acidic salt of a weak mineral acid. The leavening acid promotes a controlled and nearly complete evolution of carbon dioxide from sodium bicarbonate in an aqueous solution. Some examples include monocalcium phosphate monohydrate (CaH<sub>4</sub>PO<sub>4</sub>)<sub>2</sub> · H<sub>2</sub>O, sodium acid pyrophosphate (Na<sub>2</sub>H<sub>2</sub>P<sub>2</sub>O<sub>7</sub>), and potassium acid tartrate (KHC<sub>4</sub>H<sub>4</sub>O<sub>6</sub>). When these agents combine with water, they react to form controlled amounts of carbon dioxide. Sodium bicarbonate also raises dough pH.

Ammonium bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>) generates carbon dioxide, ammonia, and steam when heated. It increases spread and gives a larger, more desirable surface "crack" in some types of hard, high-sugar biscuits. However, it can be used only with low-moisture biscuits that are baked sufficiently to drive off all residual ammonia.

#### Other Ingredients

Milk products, eggs, and salt are added for variety. Milk and eggs are viewed as wholesome ingredients by consumers; however, they are among the most expensive.

Milk and whey are good sources of protein and lactose, which aid in shape retention and browning reactions. They add flavor and nutrients, improve texture, crust color, moisture retention, and control spread. They are usually added in dried form.

Eggs contribute color, structure, nutritional value, and some flavor. They affect texture as a result of their emulsifying, tenderizing, leavening, and binding actions. The form of eggs used can be fortified or

whole components (yolks and whites) in the liquid, frozen, or dried state, or combined with sugar.

Salt performs two principal functions in biscuit doughs. The first is flavor. It accentuates or potentiates the flavor of other ingredients (e.g., the sweetness of sugar is emphasized), and it removes the flatness or lack of flavor in other foods. Moreover, salt has a slight effect on the consistency of hard doughs, because it has a strengthening effect on gluten. Salt also controls fermentation and aids in suppressing undesirable bacteria.

Minor ingredients include malt, proteases, mold inhibitors, spices, and flavorings. Though used in relatively small amounts, these ingredients have quite important effects on the sensory and physical qualities of biscuits.

Malt is prepared from barley by sprouting it, then drying it at controlled temperatures. Diastatic malt provides enzymes that break starch into simple sugars and add flavor and color. If the malt is heated sufficiently to inactivate the (amylase) enzymes, for use as a flavorant, it is called "nondiastatic."

Proteases are important in crackers (low sugar). They are often added to modify the gluten framework. The effect of a protease is to make the dough less elastic, so that shrinkage does not occur during sheeting and cutting. Proteases may occasionally be used in cookie production. A dough containing gluten that is too strong will decrease biscuit spread, so proteases can improve the spread ratio.

Mold inhibitors are not generally used in low-moisture cookies and crackers, but some types which are higher in moisture may benefit from their inclusion. Other food products used in biscuits (i.e., fillings, toppings, and creams) may require an inhibitor. Some examples include sodium diacetate  $(Na_2C_2H_3O_2 \cdot \frac{1}{2}H_2O)$ , calcium propionate  $(Ca(CH_3CH_2COO)_2)$ , and sodium propionate  $(CH_3CH_2COON_a)$ .

#### **Added Flavors**

Our choice of food is largely influenced by taste and flavor. Smell, taste, touch, and sight are all influenced by the chemical and physical properties of the food. Sweet bakery foods are often selected for this reason. Therefore, selecting the correct flavor is extremely critical. Selection of the best flavor is most often achieved through experience and trial and error.

Biscuits may be flavored in one or more of three ways: adding the flavoring to the dough before baking; dusting or spraying the flavor on after baking; or by flavoring a nonbaked portion such as cream filling, icing, or jam that is applied after baking.

Spices are aromatic vegetable products (tree bark, seeds, fruits, and roots), and are usually finely ground.

They improve quality through smells and tastes. The most commonly used spices are cinnamon, mace, nutmeg, caraway, anise, allspice, poppy seed, coriander, ginger, cloves, and fennel.

Flavorings are alcohol extracts from fruits or beans. Vanilla is the most common because it blends with and enhances other flavors. Flavorings, such as vanilla or almond, can be either natural or synthetic. Because flavorings are volatile, much of them may be lost during baking.

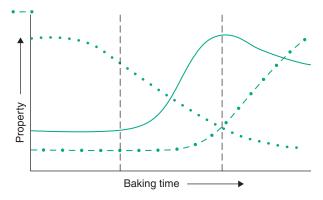
## **Added Colors**

Color additives are used in biscuits and in fillings, icings, and coatings to create a perception of quality and richness.

#### **Changes during Baking**

Chemical changes Biscuits are usually baked in a tunnel oven. The dough pieces are placed on either a flexible metal or wire mesh band that travels continuously through the oven's length, which may reach 100 m. Baking is controlled by varying the temperatures in the individual zones within the oven. It is not uncommon for the drier to consist of 5–7 zones. Baking time is regulated by the band speed. Crackers and some biscuits are docked by pressing blunt pins into the dough sheet. Docking dough pieces before baking creates air passages through the crust and seals the top to the bottom, reducing big blisters.

Dough undergoes several changes during baking (Figure 2). Changes in dimension and texture, loss of moisture, and color and flavor development are the most important. Baking is divided into three phases. The first involves dough expansion and the start of moisture loss. Dough expansion and water loss reach maximum rates and colour development starts during the second phase. The third phase



**Figure 2** Physical changes in biscuits during baking. Key: • - •, color; — , thickness; • • • , weight. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 537, Elsevier Ltd.)

concludes baking with a lower rate of moisture loss, thinning of the biscuit, and increasing surface color.

Changes in dimension and texture As dough pieces enter the oven, the biscuit's internal temperature rises, and the sugars and fats melt. The complex matrix contains liquified fat and dissolved sugars. Water evaporation causes the solution to become more concentrated, and the dough temperature continues to increase. Leaveners and steam cause the dough volume to expand. With sufficient heat, limited protein denaturation and starch swelling occur, helping to create some structure. However, the high sugar and fat content inhibit full starch gelatinization.

Doughs rich in fat and sugar, but containing little water, often have unhydrated proteins and ungelatinized starch when baked. With this formulation, a rigid structure cannot be achieved, and is therefore replaced by a soft, sugary matrix. During baking, the dough expands greatly via steam and leavening, but does not set properly, resulting in collapse of the biscuit upon removal from the oven. This expansion—collapse effect is responsible for the characteristic cracked surface of some biscuits.

Changes in moisture Dough moistures average 11-30% before baking and 1-5% after. Moisture can be lost only from the biscuit surface. It migrates from the center to the surface of the biscuit by capillary action and diffusion. The free water is evaporated very readily, but part of the bound water will also be liberated, in association with color development. Moisture content and water activity are critical parameters, as both directly influence storage life.

Color changes One of the most important color reactions is the Maillard reaction. It involves the interaction of reducing sugars with amino groups in the proteins, mainly from lysine, and produces an attractive reddish-brown hue. It is also associated with the dextrinization of starch and the caramelization of sugars. These reactions require very high temperatures, which are reached only at the biscuit surface.

#### Finishing, Storage, and Staling

In general, freshly baked biscuits are cooled before packaging or secondary processing, e.g., icing or sandwiching with a cream filling. Snack crackers are sprayed with oil and salted/seasoned prior to cooling. The cooling period is usually 1.5–2 times longer than the baking period. During cooling and storage, the biscuit continues to undergo texture changes. Hard dough biscuits are rigid and crisp immediately after baking. Soft dough products are still flexible

at the end of baking, but become firm and crisp after cooling, as a result of sugar recrystallization and glass transition temperature.

#### **Changes in Moisture Distribution**

The residual moisture is not uniformly distributed as a biscuit leaves the oven. Most of the moisture lies in a lamella near the center, leaving the surface and the outer periphery almost dry.

"Checking" is a change associated with uneven moisture distribution. Dimensional changes within the biscuit after baking cause it to crack. The center shrinks as it loses moisture, but the rim expands as it absorbs moisture. Checking can happen in almost any type of biscuit or cracker, but is most commonly found in semisweet products.

See also: Barley: Malting. Breads. Cakes, Pastries, Muffins, and Bagels. Canola: Processing. Cereals: Overview. Cookies, Biscuits, and Crackers: Methods of Manufacture; Wafers; The Diversity of Products. Consumer Trends in Consumption. Cultural Differences in Processing and Consumption. Enzyme Activities. Extrusion Technologies. Food Safety through the Production Chain. Lipid Chemistry. Nutrition: Effects of Food Processing. Snack Foods, Processing. Starch: Chemistry. Wheat: Grain Proteins and Flour Quality. Appendix: Test Methods for Grain and Grain-Based Products.

#### **Further Reading**

Blanshard JMV, Frazier PF, and Gailliard T (1986) Chemistry and Physics of Baking. London: Royal Society of Chemistry.

Fennema OR (1976) *Principles of Food Science*. New York: Marcel Dekker.

Manley DJR (1983) *Technology of Biscuits*, *Crackers and Cookies*. Chichester: Ellis Horwood.

Matz SA (1991) The Chemistry and Technology of Cereals as Food and Feed, 2nd edn. New York: Van Nostrand Reinhold/AVI.

Matz SA (1992) Cookie and Cracker Technology, 3rd edn. McAllen, TX: Pan-Tech International.

Pomeranz Y (1978) Wheat Chemistry and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Pyler EJ (1988) Baking Science and Technology, 3rd edn. Kansas City, MO: Sosland.

Smith WH (1972) Biscuits, Crackers, and Cookies: Technology, Production, and Management. Barking: Applied Science.

Stauffer CE (1990) Functional Additives for Bakery Foods. New York: Van Nostrand Reinhold/AVI.

Sultan WJ (1986) *Practical Baking*, 4th edn. Westport: AVI. Wade P (1988) *Biscuits*, *Cookies and Crackers*. New York: Elsevier.

## The Diversity of Products

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#### Introduction

The diversity of cookies, biscuits, and crackers is evident when one glances along the shelves of a western-style supermarket. This family of products, distinguished by having a low moisture content (below 5%), has a cereal-flour base of at least 60%. The flour is usually from wheat, but it may also be from oats, barley, rye, maize, or rice. The other major ingredients are shortening and sugar. Water is also added, but it is not so significant in quantity as it is for breads and cakes. This article describes the history and usage of cookies, biscuits, and crackers, and the classification of the various types. A range of types is illustrated in Figure 1.

## **Historical Origins**

The name "biscuit" comes from Latin, where "bis coctus" means twice-baked. There is also an Old French word, "bescoil," that has a similar meaning. It is thought that these products have been baked for thousands of years. The original process consisted of baking the biscuits in a hot oven and subsequently drying them in a cool oven. It is very rare to find this double baking technique in modern biscuit production. However, a special type of microwave oven is often connected after a gas-fired oven in factories. Cookie is derived from a Dutch word, "koekje," which means little cake, while the sound of a cracker being eaten probably led to use of that name.

Biscuit, as defined above, is a term used in the UK (and in New Zealand, Australia, and South Africa). The word biscuit is also used for a baked product in the USA. This is a leavened bread-like product that is similar to the UK scone and is not discussed in this article. In the USA, the word cookie describes the same type of products that are called biscuits in the UK. Cracker is a generic term used throughout the world and refers to products with very low sugar and fat contents.

The low moisture content of biscuits, cookies, and crackers means that these products have a long shelf life and a relatively low risk of spoilage by microorganisms. This has led to a history of usage in epic journeys, such as the flight of the Israelites from Egyptian slavery and the sea voyages of the fifteenth-century explorers. British and European traditions usually served biscuits in a semiformal situation with tea or coffee between main meals, especially in the afternoon. Small biscuits were usually made so that a range of appearances and flavors could be eaten without a large intake of food. Modern biscuits, cookies, and crackers are often used as a casual snack food, especially in the USA. Many of these are much larger than traditional biscuits, as they are sometimes eaten to replace meals by consumers who have busy lifestyles.



Figure 1 Biscuits come in a wide range of shapes, sizes, textures, flavors, and colors. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 526, Elsevier Ltd.)

#### Classification

Geographic location plays an important role in the classification of biscuit and cookie names. In particular, distinct names are used in the UK and USA, while in other parts of the world, the distinction between biscuits or cookies and cakes is not as clear; these products are often classified as small confectionery products. For example, biscuits in France are often classified under the title "petits fours sec." These words literally translate as small oven dry, giving the implication that these are small, dry baked goods. However, petits fours secs may also encompass other products that are outside the biscuit definition given above, such as cake-, meringue-, and pastry-based products. Biscuits that are too large to be put in the mouth at one time, would be called "les petits gâteaux secs," meaning small dry cakes. A similar distinction exists in Germany where biscuits are called "kekse" or "konfekt," but only if they are small.

In the UK, biscuits are classified on their formulation, the two major categories being hard and short doughs. Hard doughs are similar to bread doughs but have a much stiffer consistency. With hard doughs, the three-dimensional gluten network is well developed during the mixing process, so the dough is both elastic and extensible. The formula for hard doughs is very lean, as the content of fat and sugar relative to flour is very low. Crackers are made from hard doughs, as are semisweet, unsweetened, and savory biscuits. Short doughs are more closely related to cake doughs than bread doughs, although they have less water than a standard cake formula. Short doughs are named for the higher content of shortening or fat than hard doughs. They also have a higher sugar content. The increased levels of fat reduce dough extensibility, causing this type of biscuit or cookie to break easily. The flour in short doughs is given very little mixing, so the development of a gluten network is minimized. The consistency of short doughs has been compared with wet sand; they hold together under pressure but crumble easily. Short doughs have a subgroup called soft doughs that have higher levels of fat and sugar, resulting in a dough with an even softer consistency.

Biscuit classification in the USA is based on the method of processing and, in particular, the way the biscuits are shaped. The four main categories are sheeting or cutting (sometimes called cutting machine doughs), rotary molding, wire cutting, and depositing. Sheeting is used for doughs that would be classified as hard doughs in the UK and is always used for making crackers. The dough is passed through a series of rollers until it reaches the required thickness. The biscuit or cracker shapes are then cut out of the dough sheets using a plastic or metal die. It is

important that the dough is strong and elastic so that the biscuits and cracker shapes do not deform when excess scrap dough is removed from around the shapes. Rotary molding is used for short doughs, and requires a dough with a relatively stiff consistency that is not too sticky. The dough is compressed into dies mounted on the surface of a roller, and any excess dough is scraped off. The molded dough piece retains its shape as it is pushed out of the die on to the baking sheet or band. Short doughs may also be shaped by wire cutting, which involves extruding the dough through a die and slicing it with a tight wire at appropriate intervals. The tension placed on the dough during extrusion and the thickness of the wire will vary for different types of doughs. Because soft doughs are similar to a batter, having a semifluid consistency and lacking cohesiveness, they are shaped by depositing. The dough is extruded through a nozzle and dropped on to the baking sheet or band. To achieve uniformity in size and shape of the biscuits or cookies, the flow of dough is cut off at regular intervals.

## **Types of Crackers**

Attributes that distinguish crackers are very low levels of fat and sugar. They are often used as a basis for a savory topping, such as cheese or tomato, but the modern snack cracker, with its tasty coatings and flavors, may be eaten without additions. Crackers may be further subdivided into those that are fermented, including soda crackers, saltines, and cream crackers, and those that are chemically leavened like the popular snack cracker.

Soda crackers have been popular in the USA for over 150 years. They are typically 4 mm thick and  $50 \times 50$  mm square. The shortening content is 8-10%. Saltines are a smaller, more dainty type of soda cracker with an increased amount of shortening. Traditional soda cracker manufacture involves long fermentation using a sponge starter. Soda crackers have a significant amount of sodium bicarbonate added to the dough (1%), which increases the alkalinity and is the reason for the name of the cracker. Once the dough is mature, it is sheeted to about 4 mm and then laminated 6-8 times. The cracker is cut by making lines of perforations and baked as a whole sheet, a process that minimizes the amount of waste dough. A feature of soda crackers is the nine hole docking pattern on each cracker, set out in a  $3 \times 3$ grid pattern. After baking, the sheet of crackers is split along the perforation lines, usually to make a block of four crackers for packaging. The final product is quite flaky but crisp. The spring between the docking holes on the top of the cracker should be even, and the

bottom surface should be almost flat with numerous small blisters. Because soda crackers are quite dry and bland, they are not usually eaten alone and are often used as an accompaniment to soup.

In the UK, soda crackers are rare, and cream crackers fill a similar market niche. Despite their name, cream crackers contain no cream! They have a slightly higher fat content (12–18%) than soda crackers. Cream crackers are generally a 65 × 75 mm rectangular shape and are slightly thicker than soda crackers with  $\sim$ 6.5 mm between biscuits stacked in a column. Unlike soda crackers, they are usually produced as individual units. While, traditionally, the long sponge and dough fermentation process was used for manufacturing cream crackers, modern techniques involve a single-stage mixing and fermentation process, which takes from 4 to 16 h. As with soda crackers, cream crackers are sheeted and laminated. A feature of cream crackers is the laminating "dust," consisting of flour, shortening, and salt, that is applied between the layers of dough. This causes the laminations to lift apart during baking, giving an extra flaky structure. The movement of the laminations and subsequent surface blistering are quite irregular, giving the cream cracker its characteristic uneven surface. Blisters are present on both the top and bottom surfaces. Cream crackers have a final moisture content of 3-4%, which is quite high for a cracker, and along with the increased fat content, the cracker is relatively soft, will not crumble and should "melt in the mouth." Without chemical leavening, cream crackers are bland but have a slightly nutty flavor. They are mostly eaten with a savory topping and are often buttered.

Snack or savory crackers have a more recent history than soda and cream crackers. They have two distinguishing characteristics. The first is that the cracker is sprayed with hot oil as it leaves the oven, and the second is that a topping is applied to the crackers to add flavor. Snack crackers usually contain some sugar (4–10%), which also adds flavor and texture. They are usually chemically leavened, but some snack crackers are made from fermented doughs. Because snack crackers do not have the long fermentation to mature the dough, proteolytic enzymes or sulfites are used to relax the doughs so that the crackers do not deform during sheeting and cutting. Snack crackers come in a wide range of shapes and sizes, but are often round and have docking holes to allow an even lift during baking. Only snack crackers produced by fermentation are laminated. Toppings are generally applied before baking, and include herb, cheese, salt, chicken, and smoke flavors. Sometimes, the crackers are decorated with small seeds, such as poppy, sesame, or celery. Snack crackers have a dense texture and are quite soft. The hot oil spray improves the mouth feel and gives an attractive appearance to the finished product.

#### **Cookies and Biscuits Made from Hard Doughs**

Sweet and semisweet biscuits made from hard dough are generally more popular in the UK than the USA. While the gluten network is relatively well developed by mixing, the higher amounts of sugar ( $\sim$ 20% of flour) and fat (16-20%) than in cracker doughs make the gluten less elastic and more extensible. Chemicals, such as sodium metabisulfite or other chemical derivatives of sulfur dioxide, can also be used to condition and relax the dough to facilitate processing. Unlike cracker doughs, most hard sweet and semisweet biscuit doughs are chemically leavened. After mixing, the dough is sheeted and formed into shapes. The individual biscuits are cut out of the dough sheet, leaving a web of scrap dough to be removed and incorporated back into the main batch of dough. These biscuits are generally docked and marked with a name or pattern before baking. Sometimes, a milk or egg/milk wash is applied to enhance their appearance after baking, and occasionally, a garnish of sugar or other granular material is applied.

Baked biscuits should have a smooth, even surface with a pale color. The texture of the biscuit is open and even, giving a delicate bite, although this is somewhat dependent on the formula - lower levels of sugar result in a harder bite. The ingredients in sweet and semisweet biscuits are quite plain, so the flavor is usually a mild vanilla, caramel, or buttery flavor. The biscuits are generally served without accompanying food and are eaten with tea or coffee. There are some interesting variations of sweet and semisweet biscuits, such as those that are processed after baking to incorporate a cream sandwich or chocolate coating. The garibaldi fruit sandwich biscuits have a layer of currant or small sultana filling between two layers of hard dough.

#### **Cookies and Biscuits Made from Short Doughs**

Most of the biscuits and cookies consumed worldwide are made from short doughs, and consequently the range of shapes, sizes, flavors, and ingredients is huge. Formulae are correspondingly variable, but there are some consistent requirements. Flour is usually weak, with less than 9.5% protein. While there are no rules for the proportions of fat and sugar, which can range up to 100% and 200% of flour weight, respectively, the quality of these ingredients is important since they make up such a large proportion of the dough. Short doughs are usually mixed in a two-stage process with

an initial creaming of the fat and sugar, although modern techniques tend to use the "all-in" mixing method. The doughs are cohesive and plastic but lack extensibility and elasticity. The consistency of the dough will vary according to the requirements of the machinery used to form and shape the biscuits.

As described above, the two main processes for forming short dough biscuits and cookies are rotary molding and wire-cutting. Rotary molding originated from the simple wooden molds often used in monastery kitchens to produce biscuits and cookies containing inscriptions. With a rotary mold, dough is continually fed from a hopper and forced into a metal die on a rotating roller. The formed dough shape is pressed out of the mold on to the baking band as the roller rotates. Unlike hard doughs, which tend to shrink during baking, short and soft doughs generally spread because of the high sugar and fat content. This is a particular disadvantage of rotary molding as the inscription can become blurred. Tight control of the formula is necessary to reduce spread. Rotary cookies should be thin and smooth with no surface cracks or irregularities. Because of their regular size and shape, rotary cookies may be used to make cream sandwiches in the same way as hard sweet biscuits.

Wire-cutting is a form of extrusion; the dough passes through a die and is sliced at intervals by a tight wire so that the formed dough shape drops on to the baking band. Doughs intended for wirecutting are usually softer than rotary molding doughs, and more chunky ingredients, such as chocolate chips, nuts, or raisins, may be incorporated. A variation on the wire-cutting process is a rout press that extrudes dough continuously. The dies on a rout press are designed to produce strips, which are cut into short lengths before baking. Both of these techniques can be adapted to coextrude by having two different doughs or a dough and a filling coming from separate hoppers into a single die that forms a dual layer cookie (e.g., chocolate/vanilla) or a dough tube often filled with a fruit paste of similar consistency.

#### **Cookies and Biscuits Made from Soft Doughs**

Soft doughs have a pourable consistency, are typically rich in fat (65-76%) of flour weight), and may be based on whipped egg whites (15-25%). Sugar is  $\sim 35-40\%$  of the flour weight. Weak flour is used, and mixing is in a two-stage process. The flour and other dry ingredients are added last, and only minimal mixing takes place to prevent the dough from becoming tough. Often, rich, expensive ingredients, such as ground almonds, coconut flour, or cocoa, are used in soft doughs. However, coarse particles are avoided,

because they may block the nozzles during depositing. Dough temperatures are important to achieve the correct consistency for forming the specific type of biscuit or cookie required. Temperatures generally range between 10°C and 17°C. The dough flows from the hopper through a nozzle on to the baking band. The nozzles may be of different shapes and sizes to alter the appearance of the cookie, but because the dough flows a little after depositing, these designs may be rather irregular. Some depositor heads can be rotated to make swirls and circular shapes, while two or more depositors may be synchronized to combine doughs of different flavors or colors within the same biscuit or cookie. Soft dough biscuits and cookies have a soft, delicate texture and a "melt-in-the-mouth" feel. However, these properties make them fragile and subject to breakage, and packaging can also be difficult because of the irregular shapes.

## **Decorating Biscuits and Cookies**

The nature of the final product can be further altered by secondary processing after baking. A wide range of processes are used to finish and decorate biscuits, cookies, or crackers, some of which have already been referred to. Two of the most popular are cream sandwiches and chocolate coating or enrobing. In a cream sandwich, the cream usually makes up  $\sim$ 30% of the final biscuit weight and consists of sugar, fat, and flavorings, such as fruit acids, cocoa powder, and skimmed milk powder. The cream is either poured into a stencil positioned over the top of the biscuit base or deposited directly on to the base. Working with chocolate is technically difficult because of the necessity to control the temperature accurately. The properties of chocolate are quite variable and depend on the ingredients that were used to make the chocolate. Various methods of fully enrobing or partially dipping the biscuit or cookie into a chocolate bath are used, depending on product specifications. Icings, made from icing sugar, water, and sometimes fat or a gelling agent such as gelatine, are another popular finishing on biscuits. Jellies, jams, marshmallow, caramel, fruit, seeds, and nuts may also be used to enhance the taste and texture of biscuits, cookies, or crackers.

See also: Bakeries. Barley: Malting. Breads. Cakes, Pastries, Muffins, and Bagels. Cereals: Grain — Quality Attributes. Cookies, Biscuits, and Crackers: Methods of Manufacture; Chemistry of Manufacture. Consumer Trends in Consumption. Cultural Differences in Processing and Consumption. Extrusion Technologies. Food Safety through the Production Chain. Snack

Foods, Processing. Wheat: Dough Rheology. Appendix: Test Methods for Grain and Grain-Based Products.

## **Further Reading**

Faridi H and Faubion J (1995) Wheat End Uses around the World. St. Paul, MN: American Association of Cereal Chemists.

France WJ and Small M (1986) The New International Confectioner, 5th edn. London: Virtue and Co.

Kulp K (1994) Cookie Chemistry and Technology. Manhattan, KS: The American Institute of Baking.

Manley D (2000) Technology of Biscuits, Crackers and Cookies, 3rd edn. Cambridge, UK: Woodhead.

Matz SA (1992) Cookie and Cracker Technology, 3rd edn. McAllen TX: Pan-Tech International.

New Zealand Institute For Crop & Food Research (1999) Biscuit Production. Baking Distance Learning Course. Christchurch, New Zealand: New Zealand Institute for Crop & Food Research.

Smith WH (1972) *Biscuits*, *Crackers and Cookies*, vols. 1 and 2. London: Applied Science.

The American Institute of Baking (1999) The Science of Baking Correspondence Course. Saltines/Snack Crackers. Manhattan, KS: The American Institute of Baking.

The Biscuit and Cracker Manufactures Association (1979)

The Cookie and Cracker Manufacturing Correspondence Course. Unit IV Crackers. Washington, DC: The Biscuit and Cracker Manufacturers' Association.

Wade P (1988) The Principles of the Craft. Biscuits, Cookies and Crackers, vol. 1. London: Elsevier.

## COIX

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as an effective remedy for a number of maladies and particularly beneficial to the digestive system. Coix has been considered as a good potential germplasm resource for maize improvement because it is tolerant to lateritic soils, low pH, has a low variation in photoperiod, less prone to attacks by viruses, and less susceptible to waterlogging.

#### Introduction

Coix lacryma-jobi L., commonly called Adlay, Job's tears, adley, adley millet, or coix millet, is an annual crop. Botanically, coix belongs to the tribe Maydeae (Tripsaceae) of the family Poaceae, and it is classified as a close relative to maize. This annual grass is native to India, Burma, China, and Malaysia and has been grown extensively in Southeast Asia for several thousand years. The plant is typically  $\sim 1 \text{ m}$  high with knobbly bamboo-like stems, and the new "tillers" arise from the base of stems. The glossy deep green leaves are up to 5 cm wide with slightly wavy edges. The flowering and fruiting spikelets are insignificant, the grain is pear-shaped,  $\sim$ 5 mm in diameter, and has a hard, shiny dark brown to gray-black hull. The grain is used in soups and beverages, and is popular in Chinese traditional medicine. It is well known to have pharmacological properties. The ancient Chinese medical book "Pen-tsao kang mu" described it

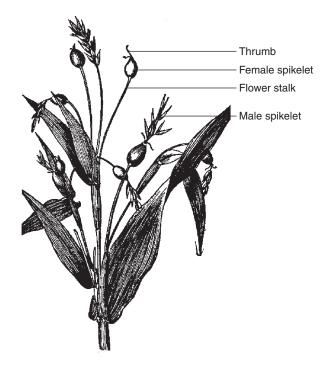
## **Botany**

#### Habit

Coix is a light-sensitive and short-day-length plant and prefers wet soils and cool weather. Although it is an annual crop, the plant is perennial in essentially frost-free areas. It is reported to tolerate annual precipitation of 6.1–42.9 dm, annual temperature of 9.6–27.8°C and soil of pH 4.5–8. It can be grown in a variety of environments – from cool-temperate moist to wet through tropical very dry to wet forest life zones, forest margins, and swamps. Although this annual grass is native to Southeast Asia, it is now distributed throughout the tropical, subtropical, and temperate zones Figure 1.

#### **Vegetative Organization**

The coix plant is 70–400 cm high. Its stems are erect or straggling with branches and prop-roots from the lower nodes. The internodes of the culms are solid.



**Figure 1** Inflorescence of *Coix*. (Adapted from the images from http://www.desert-tropicals.com/plants/Poaceae/coix\_lacryma-jobi. html.)

Leaves are not basally aggregated and nonauriculate, the leaf blades are lanceolate and broad,  $\sim 30-70$  mm wide, the shape is cordate or not cordate but not sagittate, they are flat and persistent without cross-venation, they are rolled in bud with a ligule present, and there is an unfringed to a fringed membrane.

# **Reproductive Organization**

The coix plant is monoecious with all the fertile spikelets unisexual but without hermaphrodite florets. The spikelets of sexually distinct forms (female-only or male-only) are on the same plant, the glumes of the fertile spikelet are several-nerved and all enclosed finally in a bony beadlike involucre, the involucre containing a minute fertile female flower and two sterile flowers. Pollen-bearing male flowers are produced on a slender stalk that extends out of the bead through a tiny pore. Two feathery stigmata from the fertile female flower also protrude from the pore — ready to receive pollen from the male flowers.

 Female-sterile spikelets: male spikelets grow in pairs or triads, several per disarticulating raceme. Rachilla of male spikelets is terminated by a male floret. The male spikelets have glumes and proximal incomplete florets or not (the lower floret sterile or male).

# H HW HINNYKKK

**Figure 2** The karyotype of *Coix*. (From Zhang Feixiong and Yi Zili (1994) *Natural Science Journal of Changsha Normal University of Water Resources and Electric Power* 9(2): 187–191.)

2. Female-fertile spikelets: Spikelets fall with the glumes. Rachilla terminated by a female-fertile floret. Usually, it is pollinated by the wind.

#### Seed

The shiny gray to black beads are dispersed and planted like seeds, but they are like little shells containing flowers and grains. The seeds are usually oval-shaped or egg-shaped, with a milky white outer surface and a slightly sweet taste. Fruit medium sized with globular form,  $6-12 \, \mathrm{mm}$  long. Close-up view of flowering coix shows the hollow, beadlike involucres, which have a natural hole in them.

# Cytology

The standard karvotype formula is 2n = 20 = 20m(Coix puellarum). But there are other karyotype formulas outside of the standard types, such as 2n = 20 = 18m + 2sm (C. stenocarpa); 2n = 20 =18m + 2sm and 2n = 20 = 16m + 4sm (Coix *lacryma-jobi*); lacryma-jobi var. 2n = 20 =12m + 2m (sat) + 6sm (Coix lacryma-jobi var. mon-2n = 20 = 14m + 6sm (+1-2B) lacryma-jobi var. formosana), 2n = 20 = 18m (2 sats) + 2 sm (Coix lacryma-jobi var. mayuen (Roman) Stapf). In another research, the chromosome complement of 2n = 10 and 2n = 20 was also found in different populations of coix of Indian origin Figure 2.

# **Biochemistry**

Like other members of the grass family, prolamin is the major storage protein in coix seeds. The coix prolamin is known as coixin and represents more than 70% of the endosperm protein. Based on differential solubility, coixins can be separated into two factions:  $\alpha$ - and  $\gamma$ -coixins.  $\alpha$ -Coixins are constituted by four size classes, while  $\gamma$ -coixins comprise only one molecular weight class.  $\alpha$ -Coixins are synthesized at earlier developmental stages than  $\gamma$ -coixins. The deduced amino acid sequence of a full-length cDNA clone encoding a sulfur-rich coix prolamin predicted a polypeptide of 194 residues, which shared 64%

homology with the 17 kDa  $\beta$ -zein. The mature protein contains the familiar composition of prolamins and an unusually high content of the sulfur-containing amino acids methionine (11.6%) and cysteine (5.2%). Hydropathy analysis showed that  $\alpha$ -coixin is slightly more hydrophobic than  $\beta$ -zein.

The anticomplementary polysaccharides CA-1 and CA-2 purified from the seed of *Coix lacryma-jobi* L. var. *ma-yuen* both consist of rhamnose, arabinose, xylose, galactose, galacturonic acid, and glucuronic acid but in different ratios. CA-2 contains more mannose and glucose. CA-2 showed more potent anticomplementary activity than CA-1 in low dose. The seeds of *C. lacryma-jobi* L. var. *ma-yuen* Stapf are used as a traditional Chinese medicine reputably possessing antitumor activity, attributed to an acidic fraction. Infrared spectroscopy and gas-liquid chromatography showed that this acidic fraction was composed of four free fatty acids: palmitic, stearic, oleic, and linoleic acids.

A protein inhibitor of locust gut  $\alpha$ -amylase which was purified from coix seeds consists of two major isomers, each a dimer of two closely similar or identical subunits of  $M_{\rm r} \sim 26\,400$  Da, and associated by interchain disulfide bonds. These isomers also have closely similar amino acid compositions. The major isomer shows no inhibitory activity against amylases from other sources (human saliva, porcine pancreas, Bacillus subtilis, Aspergillus oryzae, and barley malt). This highly specific function may be relevant to protection of the grain from insect feeding and fungal infection.

The gelatinization temperature of waxy coix starches varies between 63.4°C and 76.4°C and water-binding capacity ranges from 103% to 108% respectively. The starch yield from coix grains averages  $\sim$ 45%, the birefringence loss of starch granules begins at 65°C and finishes at 75°C, and breakdown during pasting in a Brabender viscoamylograph is substantial. The properties of waxy coix starch are similar to those of waxy maize starch. Amylose content is the main factor controlling differences in starch properties of coix starches. The amylose contents of normal coix ranged from 15.9% to 25.8%, and those of waxy coix were 0.7–1.1%. Swelling power of waxy coix starches varied between 28.6 and 41.0 g per g, generally higher than waxy maize. Normal coix starches had significantly higher gelatinization peak temperature (71.1–71.4°C) than normal maize (71.9–75.5°C), similar to waxy maize. Rapid visco analyzer (RVA) pasting profiles of normal coix shows some variation but closely matches the normal maize starch profile. Pasting profiles of waxy coix have more variation and lower peak viscosities than waxy maize starch. Waxy coix starches form very weak gels, while the gel hardness of normal coix starches was 11.4–31.1 g.

The complete amino acid sequence of the polypeptide of the major trypsin inhibitor from coix seeds showed 64 amino acids with a high content of cysteine. The sequence exhibits strong homology with a number of Bowman-Birk inhibitors from legume seeds and similar proteins recently isolated from wheat and rice.

The exochitinase praratins, enzymes that catalyze the hydrolysis of colloidal chitin and regenerated chitin to 2-acetylamine-2-deoxyglucose, have an average  $M_{\rm r}$  of 10 000 Da as determined by SDS-PAGE. The pH optima of the two enzymes are 5.0 and they are stable in the range of pH 5–8. The temperature optima are 45°C for (I) and 55°C for (II) (which is a glycoprotein) respectively. They are not stable at temperatures above 55°C.

Whole kernel oil yields and levels of four phytonutrients (free phytosterols, fatty acyl phytosterol esters, ferulate phytosterol esters, and  $\gamma$ -tocopherol) in oils were measured from three coix accessions (*C. lacryma-jobi* L.). Oil yields ranged from 3.03 to 4.83 wt.%, ferulate phytosterol esters in oils ranged from 0.109 to 0.119 wt.%, free phytosterol varied from 0.54 to 0.61 wt.%, phytosterol fatty acyl esters in the oil ranged from 0.79 to 0.98 wt.%, total phytosterols were from 1.43 to 1.69 wt.%, and  $\gamma$ -tocopherol varied between 0.030 and 0.045 wt.%. Whole kernel oil yields of coix are generally higher than *Zea mays* ssp. *mays* and teosinte accessions.

# Molecular Biology

The seed storage proteins of coix, sorghum, and maize are coded by homologous genes, which are coordinately expressed in the endosperm in a temporal-specific fashion. Opaque-2 (O-2), a bZIP protein originally isolated from maize, has been described as a transcription activator of  $\alpha$ - and  $\beta$ -prolamin genes. The coding region of the coix O-2 gene is interrupted by five introns and codes a polypeptide of 408 amino acids. Comparison of the deduced amino acid sequence with two different sequences of maize O-2 protein showed that the coix O-2 protein is similar to the maize O-2 isolated from a maize inbred line, W22. The coix O-2 protein has the same binding specificity and expression pattern of the maize O-2.

Dihydrodipicolinate synthase (DHPS) is the main enzyme of a specific branch of the aspartate pathway leading to lysine biosynthesis in higher plants. The open reading frame of the DHPS-encoding *DapA* gene from *C. lacryma-jobi* is interrupted by two introns and encodes the 326 amino acid-long coix DHPS protein, which is 95% identical to the maize

DHPS protein. DHPS transcripts are present in coleoptiles, embryos, endosperms, and roots but are almost undetectable in blades of young leaves of both coix and maize. The 5'-flanking region of *DapA* gene contains a TGACTC GCN4-like element located 372 bp upstream from the putative translation start codon. Steady-state levels of DHPS mRNA are slightly reduced in the endosperms and embryos of the maize lysine-rich O-2 mutants when compared with those in normal kernels. Also, the DHPS gene is not under the control of O-2.

Using random amplified polymorphic DNA (RAPD) markers, 21 coix accessions were characterized for genetic variations and relationships. The results indicated considerable variation at the DNA level among the coix germplasm, and the classification by RAPD data reflected the differences in geographic origins and evolution of coix. Recently, a coix genetic linkage map with RFLP and AFLP markers was constructed. The map consisted of 10 linkage groups, consistent with the chromosome numbers observed cytogenetically.

# **Utilization**

Coix may be a weed to some, a source of ornamental beads, a staple sustenance, and a productive fodder grass increasingly viewed as a potential energy source. It has long been consumed both as a nourishing food and as an herbal medicine.

#### Food

Before maize became popular in South Asia, coix was rather widely cultivated as a cereal in India and still occurs as a minor cereal. Coix was used as food, particularly by peasants in the Far East, very early in agricultural history. In wild varieties, the fruit has a hard, shiny coat. After domestication, this coat became less hard and easier to cook, for example, into porridge. Coix ranks along with wheat and barley in the Near East; beans, maize, squash, and pepper in the Americas; and rice in Asia as one of the earliest domesticated plants. As with other cereals, there are many coix cultivars, including soft-shelled, easily-threshed types with a sweet kernel.

Though the hard seedcoat makes extraction of the flour rather difficult, this is a potentially very useful grain because it has a higher protein to carbohydrate ratio than any other cereal (**Table 1**). Reported data differ somewhat. According to List and Horhammer, 100 g seed contains 380 calories, 11.2 g H<sub>2</sub>O, 15.4 g protein, 6.2 g fat, 65.3 g total carbohydrate, 0.8 g fiber, 1.9 g ash, 25 mg Ca, 435 mg P, 5.0  $\mu$ g Fe, 0  $\mu$ g  $\beta$ -carotene equivalent, 0.28 mg thiamine,

**Table 1** Composition of coix seed compared with other grains (on 100 g basis)

	Coix	Rice	Wheat	Maize	Millet
Energy (cal)	358	349	354	362	353
Protein (%)	14.2	7.8	9.9	8.4	9.7
Fat	3.6	1.3	1.8	4.3	3.5
Starch (%)	67.2	76.6	74.6	70.2	72.8
P (mg)	299	203	268		240
Mg (mg)	126				
Ca (mg)	49.0	9	38	34	29
Fe (mg)	2.90		4.2		4.7
Zn (mg)	1.89				
Mn (mg)	1.89				

From www.fao.org, www.chinafeedbank.com.cn and www.cmymy.51.net.

Table 2 Amino acid (aa) composition as % of crude protein

 aa
 Arg Cys Gly Hys IIe
 Leu
 Lys Met Phe Thr Try Tyr Val

 Ratio 4.4
 1.7
 2.8
 2.3
 4.0
 14.4
 1.9
 3.0
 4.8
 3.1
 4.2
 5.6

From www.fao.org.

0.19 mg riboflavin, 4.3 mg niacin, and 0 mg ascorbic acid. According to *Hager's Handbook*, there is 50–60% starch, 18.7% protein (mainly glutamic acid, leucine, tyrosine, arginine, histidine, and lysine) and 5–10% oil with glycerides of myristic and palmitic acids. The partial amino acid composition of crude protein in coix seeds is shown in Table 2.

Coix seeds can be used for baking, mixed with wheat flour, and for making porridge and beer. They are usually pounded, threshed, and winnowed. The pounded flour is sometimes mixed with water, as barley for barley water. The pounded kernel is also made into a sweet dish by frying and coating with sugar. The grains are also utilized in soups, broths, porridge, drinks, and pastries. In India, the Nagas use the grain for brewing a beer called "zhu" or "dzu." A Japanese variety called "Ma-Yuen" is brewed into a tea and an alcoholic beverage, and roasted seeds are made into a coffee-like drink. In some cases, the hulled grain is adapted for parching or boiling like rice, it is also husked and eaten like a peanut. Beers and wines are made from the fermented grain.

#### **Folk Medicine**

According to traditional Chinese medicine, coix seeds serve several functions. They stimulate function of the spleen and lung, remove heat, help in the drainage of pus and induce diuresis. Coix seeds have been widely employed as a diuretic, stomachic, analgesic, and antispasmodic agent from ancient times. They are

also used to treat the symptoms of diarrhea and arthritis. The fruits are anodyne, anti-inflammatory, antipyretic, antiseptic, antispasmodic, hypoglycaemic, hypotensive, sedative, and vermifugal. According to Hartwell, the fruits are used in folk remedies for abdominal tumors, esophageal, gastro-intestinal, and lung cancers, various tumors, as well as excrescences, warts, and whitlows. This folk reputation is all the more interesting when reading that coixenolide has antitumor activity. It is also a folk remedy for abscess, anodyne, anthelminthic, anthrax, appendicitis, arthritis, beriberi, bronchitis, cancer, catarrh, diabetes, dysentery, dysuria, edema, fever, headache, phthisis, pleurisy, pneumonia, puerperium, rheumatism, small-pox, splenitis, tonic, etc.

Coix seeds are reportedly effective in treating verrucas caused by the human papilloma virus. A tea from the boiled seeds is drunk as part of a treatment to cure warts. It is also used in the treatment of lung abscess, lobar pneumonia, appendicitis, rheumatoid arthritis, beriberi, diarrhea, edema and difficult urination. The action of coix seeds against many kinds of disease can be attributed to various components with pharmacologically different activities. Coix roots can be also used in the treatment of menstrual disorders.

# **Fodder**

The leaves and stems provide a useful fodder for cattle and buffalo. The grass is very suitable for growing in waterlogged areas. Its average yield is 12 t per acre. The grass can also be turned into silage.

# **Genetic Resources**

Coix is reported to tolerate lateritic soils, low pH, slope, viruses, and waterlogging, and to be photoperiod insensitive. It is a potential gene resource for improvement of other cereal crops. It is a perennial plant, which forms several stalks and its pollen can be collected throughout the year when the plant is maintained in a controlled environment. It can be used as the pollen parent for wheat crosses for haploid production without requiring synchronization of flowering dates.

#### Cultivation

Generally, coix is propagated by seeds. The plant prefers light (sandy), medium (loamy), and heavy (clay) soils. It prefers neutral and basic soils, but can grow in very acidic soil, and it requires moist soil. It is sown at rate of  $6-10 \text{ kg ha}^{-1}$ . Seed is planted 2.5 cm deep, at a spacing of  $60 \times 60 \text{ cm}$ . One intercultivation, before the plants tiller and cast shade on the ground may be necessary. The largest plants result when the plant is

given partial shade although it will tolerate full sun. It adapts to wet soils so may be used near water. Sufficient rains in early stage of growth and a dry period when grain is setting are necessary for good yields. Plants respond well to liberal applications of organic manure. The grain is usually harvested in the fall when the plant matures, and is dried in the sun. Plants are cut off at base and grain is separated by threshing. Coix yields depend on varieties cultivated in different countries. Usually, unhusked grain yields vary from 2.1 to 3.5 t ha<sup>-1</sup>.

Leaf blight, caused by *Bipolaris coicis*, is one of the most destructive and major yield-limiting diseases in coix. Plant response to leaf blight is quantitative rather than qualitative, varying greatly among cultivars or lines tested. Yield reductions are primarily associated with decrease in kernel numbers per plant, thousand kernel weight, and percent kernel ripeness. Other diseases common in coix are rusts and smuts. Rusts are caused by *Puccinia* while smuts are caused by *Tilletia* and *Ustilago*. Leaf-gall virus and the nematode *Meloidogyne incognita acrita* also attack this plant.

See also: Amaranth. Genomics. Maize: Breeding. Nutraceuticals from Grains. Protein Synthesis and Deposition. Pseudocereals, Overview. Quinoa.

# **Further Reading**

Duke JA and Ayensu ES (1985) Medicinal Plants of China. Algonac, MI: Reference Publications.

Hartwell JL (1967–1971) *Plants Used Against Cancer*, A survey Lloydia, pp. 30–34. Cincinnati, OH: American Chemical Society.

Jain SK and Banerjee DK (1974) Preliminary observations on the ethnobotany of the genus coix. *Economic Botany* 28: 38–42.

Li SC (1596) Pen-tsao kang mu. Systematic Pharmacopoeia, China.

Li XH, Huang YQ, Li JS, and Corke H (2001) Characterization of genetic variation and relationships among Coix germplasm accessions using RAPD markers. *Genetic Resources and Crop Evolution* 48: 189–194.

List PH and Horhammer L (1979) Hager's Handbuch der Pharmazeutischen Praxis, vols. 2-6. Berlin: Springer.

Schaaffhausen RV (1952) Adlay or Job's tears: a cereal of potentially greater economic importance. *Economic Botany* 6: 216–227.

Yeung HC (1985) Handbook of Chinese Herbs and Formulas. Los Angeles: Institute of Chinese Medicine.

Zhang Feixiong and Yi Zili (1994) Cytological studies of the genus Coix. Natural Science Journal of Changsha Normal University of Water Resources and Electric Power 9(2): 187–191.

# **Relevant Websites**

http://seed.agron.ntu.edu.tw — The website was built by the Seed Laboratory of National Taiwan University and provides more information about crops. http://www.agron.missouri.edu — Maize DB is a comprehensive source of information on the genetics and molecular biology of maize and is a service of USDA/ARS, enhanced by NSF support to the maize mapping project. The website also provides some information about other closely related crops.

http://www.biodiversity.uno.edu — A website about grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution.

http://www.chinafeedbank.com.cn — A website about feed information. It also provides more information about feed including from cereal crops. http://www.cmymy.51.net — A website providing information about famous doctors and medicine of China and also provides many Chinese medicine prescriptions.

http://www.fao.org — The website of Food and Agriculture Organization of the United Nations. It also contains a very wide range of information about food and agriculture.

http://www.hort.purdue.edu — The website of the center for new crop and plant products at Purdue University. New CROP provides windows to new and specially crop profiles.

http://www.ibiblio.org – (US) A resource and information center for edible and other useful plants.

# **CONSUMER TRENDS IN CONSUMPTION**

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# Introduction

Effects of consumer trends on food consumption are a challenge to the entire food system because they are continually changing. A trend is a general change in direction of behavior and attitudes that persists and is strong enough to change consumption patterns. For example, consumer trends influence food behavior and consumer attitudes over time and thus cause changes in food consumption. It is important for all sectors of the food industry to pay attention to consumer trends in order to successfully market their products. This article first presents data that follow changes in the consumption of grain-based foods in the United States for nearly a century, and then describes consumer trends that help explain recent changes in the consumption data.

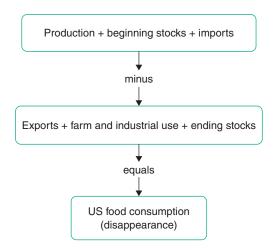
# Data for Consumption of Grain-Based Foods

Food consumption data give quantities for food either available for human consumption or actually eaten by individuals during a designated time period, such as per year, month, week, or day. In the US, the Economic Research Service (ERS) in the United States Department of Agriculture (USDA) compiles high-quality data for food available for consumption, also called food disappearance data. ERS is the only governmental source for this type of data in the US. ERS data are in the public domain and therefore available to everyone. In contrast, food consumption data compiled by private consumer information companies specializing in market research, marketing, and advertising are variable in quality and are proprietary data, i.e., access is controlled by the firms and the data are only available to those who pay for it.

ERS data for food available for consumption are called time series data. These data estimate consumption at the national level expressed as amount per capita (person) per year. The data are published annually for several hundred basic commodities and commodity ingredients. The time series food consumption data presented in this article are for the US. These data show consumption trends over time, which for some commodities date back to 1909. Time series food consumption data are available for most developed countries and for some developing countries.

Food consumption data compiled from information collected from individuals about food actually eaten in a day, week, or month are called cross-sectional data. Such data in the public domain are available from USDA. Proprietary cross-sectional data are available from private consumer information companies.

Time series data for food available for consumption (disappearance data) use supply and utilization balance sheets to estimate the kinds and amounts of commodities moving through the food distribution channels from production to consumption, Figure 1. The amount of food available for consumption



**Figure 1** Estimation of food available for consumption (disappearance data) in the US. (Data from USDA/Economic Research Service.)

(disappearance) is the difference between available supplies (production, beginning stocks, and imports) and utilization (nonfood and other uses such as exports, end-of-year inventories, seed, feed, and industrial uses). To express consumption on a per capita per year basis, amount of food available for consumption is divided by the resident population in the US plus the armed forces overseas on July 1 of the year studied. Amounts reported are weights of food as sold in retail stores.

Time series data have some limitations. They give overall increases or decreases in consumption based on the entire population, but do not give specific food items or amounts of each that are actually consumed by individuals. Changes in consumption in different regions or by different subgroups of the population cannot be determined from time series data. Also, these data overestimate actual consumption because they include waste and spoilage losses throughout the marketing process and in the home. This has been estimated at  $\sim\!25\%$  of food available for consumption.

The time series data presented in Figure 2 give the total flour and cereal products available for consumption along with data for several specific products – wheat flour, rice, corn products, and oat products – available for consumption in the US from 1909 to 2000. Additional consumption data for specific products show trends in more detail for products with low consumption, rye flour, rice, oat products, and barley products in Figure 3 and corn products in Figures 4 and 5.

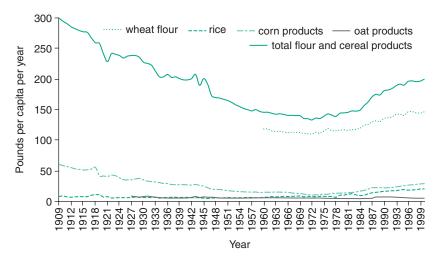
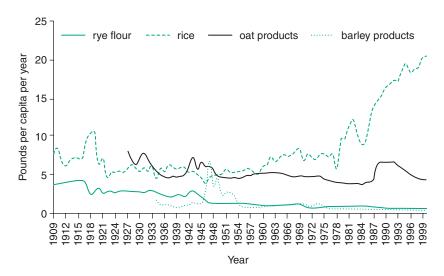
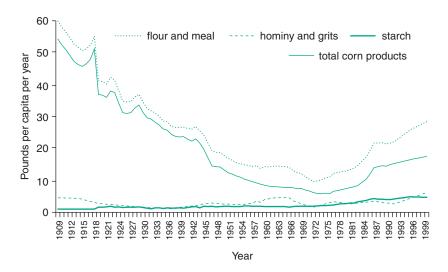


Figure 2 Time series data for flour and cereal products available for consumption in the US. Includes total flour and cereal products (consumption of most items at the processing level, computed from unrounded data, excludes wheat not ground into flour and quantities used in alcoholic beverages and fuel); wheat flour; rice (milled basis); corn products (includes flour and meal, hominy and grits, and cornstarch, excludes corn sweetners); and oat products (includes rolled oats, ready-to-eat cereals, flour, and bran). (Data from USDA/ Economic Research Service.)



**Figure 3** Time series data for different types of flour and cereal products available for consumption in the US showing details of trends at the processing level, excluding quantities used in alcoholic beverages and fuel, for rye flour, rice (milled basis), oat products (includes rolled oats, ready-to-eat oat cereals, flour, and bran), and barley products (includes flour, pearl barley, and malt and malt extract used in food processing). (Data from USDA/Economic Research Service.)



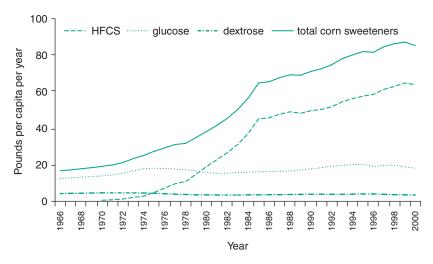
**Figure 4** Time series data for corn products available for consumption in the US including total corn products (computed from unrounded data, excluding sweeteners) and for flour and meal, hominy and grits, and starch. (Data from USDA/Economic Research Service.)

The overall trend for total flour and cereal products, Figure 2, shows a continual decrease in per capita consumption from 300 pounds in 1909 to a low of 133 pounds in 1972, followed by increases to 145 pounds in 1980, 181 pounds in 1990, and 200 pounds in 2000. Products included in that total are wheat flour, rice, corn products, and oat products, Figure 2, and rye flour and barley products Figure 3.

Per capita consumption data for the specific products listed above as shown in Figures 2 and 3 indicates an overall trend of decreasing consumption reaching a low for rice in 1946, wheat flour and

corn products in 1972, and oat products in 1984, followed by increases since then till 2000 except for rye, and barley products with lowest consumption in 2000. Consumption in 2000 for all products except wheat and rice was lower than in 1909 or in the first year data were available for the products. In 2000, total wheat flour consumption (including 91% wheat flour and 9% durum flour) was higher than that of any other grains at nearly three-fourths of total flour and cereal products consumption, Figure 2.

Per capita consumption of total corn products (except corn sweeteners) was  $\sim$ 14% of total flour and cereal products consumption, Figure 2. Detailed



**Figure 5** Time series data for corn sweeteners available for consumption in the US, including total corn sweeteners (computed from unrounded data), HCFS (high fructose corn syrup), glucose, and dextrose. (Data from USDA/Economic Research Service.)

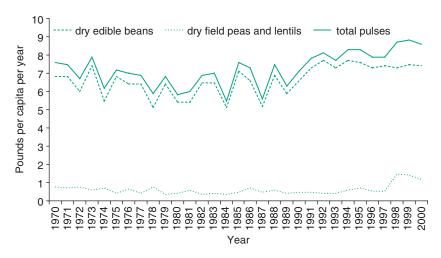


Figure 6 Time series data for pulses available for consumption in the US, including total pulses (computed from unrounded data), dry edible beans (cleaned basis), and dry field peas, and lentils. (Data from USDA/Economic Research Service.)

data for consumption of corn products, Figure 4, show total corn products consumption decreased until 1972, and then increased since then till 2000. Consumption of flour and meal was highest for the corn products and lowest for starch and the hominy and grits. But these consumption data for corn products did not include corn sweetener data, which was first reported in 1966, Figure 5. By 2000, the total for these sweeteners (high fructose corn syrup (HFCS), dextrose, and glucose) was 85.3 pounds per capita, dry weight. Three-fourths of this total was HFCS.

Per capita consumption data for pulses (grain legumes) started in 1970. Total consumption for pulses and dry edible beans followed the same

pattern and was higher for pulses than for dry peas and lentils (Figure 6). Dry bean consumption fluctuated at about the same level from 1970 until 1990 when it increased, stabilized, and then slowly decreased until 2000. Dry pea and lentil consumption was stable at a low level until 1998, when it more than doubled, and then decreased slightly until 2000.

Total per capita peanut consumption was relatively stable from 1967, when data started until it decreased to the lowest point in 1980, steadily increased to a high in 1989, and then gradually decreased until 2000, Figure 7. Consumption of peanut butter was the highest of all peanut products and followed the same consumption pattern as total peanuts. Consumption patterns for snack peanuts and candy



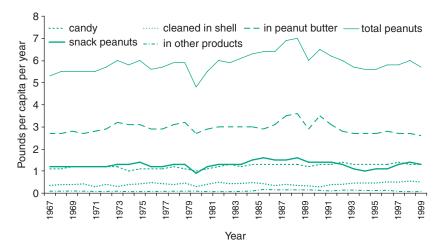


Figure 7 Time series data for peanut products available for consumption in the US including total peanuts (computed from unrounded data), snack peanuts, cleaned in shell (roasting stock, shelled equivalent), in peanut butter (peanut butter made by manufacturers for use in cookies and sandwiches but excludes peanut butter used in candy), in candy, and in other products (includes grated and granulated peanuts and peanut flour). (Data from USDA/Economic Research Service.)

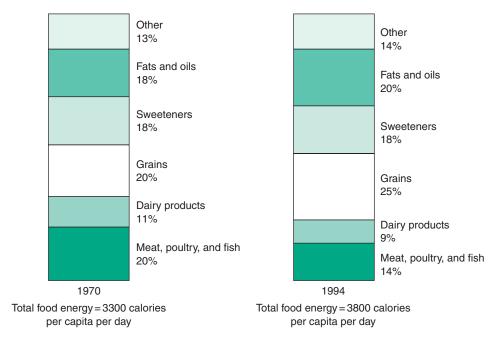


Figure 8 Contribution of grains and meat, poultry and fish groups to calories available for consumption per day in the US food supply in 1970 and 1994. (Data from USDA/Center for Nutrition Policy and Promotion.)

were similar – i.e., lower than for peanut butter. Peanut consumption was lowest for peanuts cleaned in shell and for other products.

Time series consumption data can also be used to calculate the nutrient content in food available for consumption. Nutrient data show that most of the protein intakes in food, in 1909, was from grain-based foods. By 1970, calculations showed that the same number of the calories available for consumption per capita per day were available from the grains group, 20%, and from the meat, poultry, and fish group, 20%, Figure 8. By 1994, the contribution of the grains group to calories available per capita per day had increased to 25% and that for the meat, poultry, and fish group had decreased to 14%, indicating that consumer consumption patterns had changed between 1970 and 1994.

The increases in per capita consumption for most grain-based foods in the US since the 1970s shown by the data described above indicate a continuing

consumer demand for them. Cross-sectional and sales data also show the same trends. This is not surprising considering the wide variety of grain-based foods and ingredients for these foods that is available to consumers and food processors/manufacturers. The food composition table for grain-based foods in the Appendix: Grain Composition Tables gives an edited version of these foods and ingredients derived from a large detailed food and nutrient database for grain-based foods and ingredients.

Examples from sales data show the highest sales for the wide variety of fresh breads and other baked products from in-store bakeries, bakery chain restaurants, and for pizza. Complete meal sandwiches, breakfast sandwiches, foreign sandwiches, and mini sandwiches are increasing in popularity. Specialty breads such as artisan breads, stuffed peasant breads, and other ethnic breads, flat breads, and breadsticks are in high demand. Breads with highest sales in restaurants are dinner rolls, bagels, and French/Italian breads. Bread in different forms also is used to package popular portable foods such as wraps, hot pockets, tortillas, pitas, focaccia, and others. Bread also is the main ingredient in the over 45 billion sandwiches consumed each year.

Industry response to consumer demand for quick breakfasts is a wide variety of ready-to-eat and quick-cooking breakfast cereals available. These include whole grain and enriched/fortified rice-based cereals, oat-based items such as rolled oats, oat bran, and quick-cooking flavored oat cereals, and corn products such as cornmeal and hominy grits. Refrigerated and frozen grain-based breakfast items are other convenience products in the marketplace.

Additional examples of popular grain-based foods marketed include many snack foods — crackers, popcorn, pretzels, corn chips, and doughnuts. Fast-food items in greatest demand are various types of buns used in burgers made from beef, poultry (chicken and turkey), fish (tuna, salmon, cod), and other meats. Specialty food service and frozen meal items include bowl meals (noodle bowls), noodle soups, rice salads, sticky rice cakes, lasagna, spaghetti, and other Italian dishes, and many desserts.

# **Consumer Trends**

Consumer characteristics that influence trends in eating behavior and food consumption include demographics, lifestyles, and attitudes toward food. All these characteristics affect the marketing, processing/manufacturing, and agricultural production of grain-based foods. The US consumer trends described here are similar to those in other developed countries.

# **Demographics**

Demographic characteristics that affect consumer trends include age, household size, household income, women in the labor force, education, geographic location, and ethnic background.

Age The increasing age of the population is one of the major consumer trends in the US and in developed and developing countries. By 2050, the median age in the US is projected to be 42, up from 23 in 1900, 33 in 1991, and 37.4 predicted in 2010. The elderly population over age 65 is expected to increase from 35 million in 2000 to 54 million in 2020. By 2030,  $\sim$ 21% of the population will be over 64 years, 12% will be over 74 years, and the ratio of old to young will increase for all races. By 2050,  $\sim$ 50% of the population is projected to be over 50 years.

Age groups, expected to have the largest population increase by 2005, are children and young adults who are the children of the 78 million baby boomers born between 1946 and 1964. After 2005, the number of children is expected to stabilize and then decrease.

Household size Household size in the US decreased from an average of 2.8 persons in 1980 to 2.5 persons in 2000 and is expected to be 2.4 persons in 2020. Smaller households, many of them elderly people in one or two person households, are changing consumer food demands. By 2010, the highest proportion of all households, about one-third, will be "empty nesters" without children under the age of 18. Single person households will increase by 12% and by 2010 and will surpass the number of married couple families with children. No change is expected in number of family households with children by 2010.

Household incomes An overall decrease in household incomes has occurred in the US. The rich are getting richer, the poor are getting poorer, the middle class is decreasing, and a class of unemployable people has developed. Low incomes from the downturn in the US economy and higher than usual unemployment plus an aging retired population with limited incomes will tend to increase economic stress on those segments of the population, while high-income households will be unaffected.

Women in the labor force The number of women in the labor force has more than doubled since the middle of the twentieth century creating two-income families with increased incomes but less time to spend on cooking and housework, more single-parent families headed by working women and more working women in single person households. Their

demands for convenience foods have given product developers opportunities to create products that will increase consumption of grain-based foods.

Amount of education The average educational level of US consumers is increasing overall, but differs within some population groups and in some regions of the country. As education increases, incomes increase. In general, educated affluent consumers have the most information about what to eat to improve their diets.

Geographic location The US population is increasing most rapidly in the south and west with accompanying small increases and some decreases in northern states. People also are moving from farms and small towns to large cities. Only  $\sim 2\%$  of the population live on farms and about three-fourths live in metropolitan areas.

Ethnic background Ethnic groups in the US population are increasing. By 2020 the Hispanic, segment is expected to have the greatest increase, and non-Hispanic Whites, Blacks, and Asians are expected to increase at slower rates. Demands for a wide variety of ethnic foods will increase as immigrants continue to come to the US from many different countries. The most popular ethnic cuisines are Italian, Mexican, and Chinese.

# Lifestyles

Lifestyle trends of US consumers reveal their diversity. Many lifestyle classification systems have been developed by consumer information firms to describe consumer lifestyles and to help marketers understand how lifestyles affect buyer behavior and food consumption. These systems use a variety of interesting descriptive terms or phrases to identify consumer characteristics for marketers, i.e., who and where customers are, what they want, what they need, what they buy, and how to contact them. Small groups of consumers with similar lifestyles and specific characteristics are called nichés. Using many different marketing techniques to reach nichés of specific consumers identified by lifestyle classification systems is replacing former marketing strategies directed to the mass market of homogeneous consumers that no longer exists. Marketing to nichés helps food retailers choose products to offer in their stores to attract consumers and thus increase profits. Niché marketing of grain-based foods has the potential to increase consumption of these foods.

# **Attitudes toward Food**

Trends in consumer attitudes toward food (shopping, meals, time, cost to prepare) cover many aspects of how consumers feel about food. These include their food habits, food preferences, practices related to food shopping, purchasing and preparation, and overall philosophy about how to provide food for themselves and/or others.

**Food habits** Food habits indicate how consumers use food. They are based on culture and are passed on from one generation to the next. Food habits specify how and where food is obtained, stored, prepared, served, and consumed.

Food preferences Food preferences express food likes and dislikes. They determine which foods consumers purchase and eat. Food likes and dislikes that influence consumption include familiarity with the quality characteristics of food and acceptance of the aroma (smell), appearance, taste/flavor, and texture.

Food shopping, purchasing, and preparation Although enjoyed by some consumers, many dislike these tasks because they are time-consuming and inconvenient.

# Impact of Consumer Trends on Food System Trends

The retail food marketplace, food processing/manufacturing/distribution, and agricultural production sectors are all affected by consumer trends. Grain-based foods are important consumer products that move through each sector of the food system.

Food marketplace trends The retail food marketplace includes food stores and food service establishments. These firms provide food for consumers to buy at reasonable prices. Food stores differ in size, variety of foods offered for sale, number of departments, methods of check out, and customer services. Because of significant decreases in home cooking using individual ingredients ("scratch cooking") food stores popular with consumers are those that offer a wide variety of convenience foods. All consumers benefit from high-quality convenience foods that are nutritious and require minimal or no preparation and clean up. Those who need these foods the most are the elderly for whom food preparation may be difficult, two-income families with working women, young adults who do not know how to cook, and families in which children have major responsibility for food choices and meal preparation.

Shopping convenience offered by food stores is a response to consumer needs. For example, one-stop shopping in large stores provides busy consumers with everything needed in one shopping trip. And services provided for all, but particularly needed by older shoppers are convenient parking, efficient checkout, grocery packing, and assistance in moving groceries from stores to their transportation home. Large stores are a problem for aging shoppers because it is difficult for them to walk the long distances of the aisles when shopping. To accommodate older consumers, some smaller new stores are now being built.

Food service offers wide choices for food away from home to consumers who now eat almost half of their food away from home. Food service establishments vary from economical fast-food outlets to expensive formal dining, and everything in between. Demand for food away from home increases for small households except those that have low incomes. Most establishments sell takeout food that is usually eaten at home. Trends toward very large servings in commercial food service outlets make consumers feel as if good value is received for the money spent, but another trend has emerged — the tendency to overeat and gain weight.

Food processing/manufacturing trends Food processors add value to basic food commodities by processing them into ingredients used by food manufacturers in the wide variety of grain-based foods in the marketplace. Wheat, for example, is milled into flours used in many grain-based foods.

Food product development adds value to basic ingredients in the form of convenience foods. This gives consumers the option of "not to have to cook," and makes portable foods available that are easy to eat on-the-run. Low calorie, nutrient dense, inexpensive, convenient grain-based foods in small packages that are easy to open are being developed for the aging US population with decreased food and calorie needs and increased nutrient needs. These changes in food and calorie needs of the elderly could decrease consumption of grain-based foods in the future. Special dietary foods and nutraceuticals (foods containing certain beneficial ingredients) are being developed for the increasing number of people of all ages with chronic diseases. However, development of unique and expensive grain-based foods, particularly ethnic foods, which are being developed for affluent consumers can be expected to increase in the future. These foods contain a variety of grains as ingredients and are from the most popular ethnic cuisines, Italian, Mexican, and Chinese, as well as from cuisines increasing in popularity such as non-Chinese Asian, Caribbean, Middle Eastern, and Mediterranean.

Increased consumption of creative, convenient, and nutritious grain-based foods, including popular ethnic foods, can be expected by the increasing number of young adults in the population who are noted for their inability to cook and their need for quick solutions to their food needs. The problem of overweight and obesity in adults and children in the US has increased demand for development of low calorie grain-based foods.

Agricultural trends Agricultural trends are changing as consumers are more demanding, particularly about the quality of their food. Consumers have an impact on what farmers produce and sell. Fewer farms are small independent units with the farmer deciding what to produce, harvest, and sell each year. Specialized contract farming is more prevalent in which the farmer contracts with a large, integrated corporation to produce one product. In the case of grains, farmers contract with a corporation to grow specified grain(s) according to the directions provided. The farmer knows ahead of time what product quality is expected and both the farmer and the corporation will know what will be paid for it. This arrangement may or may not be advantageous to both parties depending on the contract. Only a small proportion of the retail price of manufactured grain-based foods goes to farmers for the grains used in them.

Organically produced food is increasingly being demanded by consumers. This has lead to expanded marketing opportunities for organic farmers and the establishment of governmental standards for the production and labeling of organically produced foods. Organic production standards include methods for handling crops, livestock, and processed agricultural products. In 2002, the USDA approved national labeling standards for organically produced agricultural products that give consumers assurance of consistent organic product labeling throughout the country. In recent years, organic farmers have increased their use of direct-to-consumer marketing, mostly in farmers markets. Supermarkets also have increased offerings of clearly labeled organic foods. Organic grain and oilseed farmers produce wheat, corn, soybeans, oats, barley, sorghum, rice, spelt, millet, buckwheat, rye, dry peas, lentils dry beans, flax, and sunflowers mostly for food processors.

### **Globalization Trends**

Grain-based foods are staples of the food supply in every country. Grain imports and exports are very important not only to help distribute grain supplies globally, but also to contribute to the agricultural economy. Seasonal effects on the market occur

because crops are harvested at opposite parts of the year in the two hemispheres. Time series data provide the information from which appropriate quantities of staple grains can be either exported or imported by different countries to balance supplies and also to meet the nutritional needs of the population.

# **Summary**

Consumption data for grains and grain-based foods change over years because changes occur in consumer trends. Time series data from ERS for grain-based foods available for consumption from 1909 to 2000 in the US show that per capita consumption for some grain products increased and some decreased over that time period. A gradual increase in rice consumption occurred since 1909 and in wheat flour consumption since 1960 when data were first available. Wheat flour had the highest consumption of all grain products. Data for the other grains since ~1970 show a continuing increase in consumption until 2000 for all products except rye flour and barley products. Sales data for grain-based foods in recent years show similar trends.

Consumer trends help explain consumption trends for grain-based foods. Major consumer trends include demographics (age, household size, household income, women in the labor force, amount of education, geographic location, ethnic background), diverse lifestyles, and consumer attitudes toward (food habits and food preferences). Consumer trends that affect movement of grain-based foods through the food system include food marketplace trends, food processing/manufacturing trends, and agricultural trends. Major trends in the food system include niche-marketing, development of grocery stores to improve food shopping convenience, increased demand for and development of convenience foods, and the growing demand for food away from home. Globally, grain-based foods are staple foods in every country. Consumption trends provide information on grain supplies available to export or import among countries to balance supplies and provide for nutritional needs worldwide.

# **Acknowledgments**

The author thanks Dr. B Senauer for helpful suggestions on this manuscript and H Van Schooten for making the line graphs. Both are in the Food Industry Center, Department of Applied Economics, University of Minnesota.

See also: Grain Production and Consumption: Europe; Cereal Grains in North America; Oilseeds in North America; Oceania; South America. Nutrition: Soy-Based Foods. Rice: Chinese Food Uses. Snack Foods, Processing. Soybean: Soy-Based Fermented Foods.

# **Further Reading**

Kittler PG and Sucher KP (2001) Food and Culture, 3rd edn. US: Wadsworth.

Nestle M (2002) *Food Politics*. Berkeley and Los Angeles: University of California Press.

Nestle M (2003) Increasing portion sizes in American diets: more calories, more obesity. *Journal of the American Dietetic Association* 103(1): 39–40.

Senauer B, Asp E, and Kinsey J (1991) Food Trends and the Changing Consumer. St. Paul, MN: Eagan Press.

Sloan AE (1999) The upper crust. Food Technology 53(10): 26.

Sloan AE (2001a) Demo memos. Food Technology 55(6): 18.

Sloan AE (2001b) Ethnic foods in the decade ahead. *Food Technology* 55(10): 18.

Sloan AE (2002a) The natural and organic foods marketplace. *Food Technology* 56(1): 27–37.

Sloan AE (2002b) Fast and casual: today's foodservice trends. *Food Technology* 56(9): 34–53.

# **Relevant Website**

http://www.ers.usda.gov – An excellent website for data, briefings, and publications.

# **CONTAMINANTS OF GRAIN**

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# Introduction

Ideally, grain should be harvested in sound and clean condition, without any form of defect or contamination. Realistically, however, there is always the likelihood that the grain will be harvested together with nongrain material and with weed or crop seeds, all of which detract from the value of the harvested crop. The loss in value relates partly to the fact that the resulting consignment of grain does not contain 100% of the type of grain ordered, but worse than that, it is likely that the nongrain materials will cause problems with the processing of the grain, or that they may even render the grain unfit for the designated purpose or for any use.

# **Historic Perspective**

Primitive man, as the hunter-gatherer, had to accept whatever material grew with the seeds that were collected laboriously from wherever they could be found. As a result, the presence of contaminants must have been considerable. Presumably, this unsatisfactory situation was one of various stimuli for early man to progress to cultivation and seed sowing, thereby to improve the purity and quality of grain foods. Nevertheless, throughout the Middle Ages, the presence of contaminating material was generally extensive, because of poor agricultural practice and also due to the lack of purity of the seed for sowing.

Significant improvements in grain quality have been forced as a result of the progressive change from subsistence agriculture (the farmer's family consuming what they produce) to trading in grain (provision of the grain produced for sale, in competition with others). The past century has seen the extension of trading from the local situation to major international marketing and transport on a large scale (*see* Cereals: Overview). As a result, standard specifications have been established for the various grades of grain, based on quality, offered for trade within a country and internationally. Examples of these specifications are provided in Tables 1–3.

An important part of these specifications is the levels of contaminating materials, the higher-value grades being those with the least foreign material. Examples of these specifications can be examined on the websites of the export corporations of major grain-trading countries. The first two examples in Table 1 contrast a premium Australian wheat grade (prime hard) with a lower grade (general purpose). The former has tighter specifications for all aspects of physical quality, compared to the lower-value grade, including the requirement for less material in the category of contaminants.

# **Types of Contaminants**

Nongrain material goes under various names, depending on local terminology and on the species of grain involved. Common terms include "extraneous matter," "unmillable material," "dockage," "besatz," and "screenings." This last term alludes to the general practice of screening the grain through sieves of suitable size to separate material that is larger or smaller than the normal size range of the grain involved. The act of sieving to determine screenings is illustrated in Figure 1. The amount of these screenings is a significant measure of grain quality, as it indicates the proportion of the grain consignment that is not the grain purchased. In addition, the nature of the nongrain material is very important, depending on which of the following categories are involved.

# **Plant Material from the Grain Crop**

Most obviously, nongrain contaminants are derived from the plants that produced the harvested grain. This includes parts of the husks, leaves, and stalks. For the cereal grains, it is not uncommon for the tips of the heads to be included with the harvested grain. This occurs for varieties that may not fragment as readily as others during harvesting. This is due to the dilemma facing the breeder in selecting the best genotypes for the harvest operation. On the one hand, it is desirable to have a variety that "shatters" readily, so that the grain is released from the head on harvesting. However, this characteristic is likely to carry the significant disadvantage that some of the grain will be prematurely shed onto the ground before or during harvest, thus reducing the yield of available grain.

Table 1 Some of the specifications for physical aspects of grain quality for contrasting grades of wheat involved in international trade

Quality attribute	Australian prime hard	Australian general purpose	Canadian No 1. CWRS	Canadian CW feed
Test weight, kg hl <sup>-1</sup>	74	68	75	65
Varietal mix	Specified	Specified	Specified	Specified
Falling number	350	200	•	·
Sprouted grains	Nil	Nil	0.5	No limit
Unmillable material:				
above 2 mm screen	0.6% max.	1.2% max.		
below 2 mm screen	Active scale	Active scale		
Small foreign seeds	0.6% max.	1.2% max.	0.05%	0.05%
Contaminants in 1/2 I measure				
Seeds, see Table 4	From 1 to 50, acc. to species	From 2 to 150, acc. to species	Not stated	Not stated
Tainting material	Nil	Nil		
Chemicals, dyes, etc.	Nil	Nil		
Ergot pieces	1	1	0.01%	0.10%
Loose smut pieces	3	3		
Grain insects	Nil	Nil		
Field insects	0-10	0-10	1.0	No limit
Earth pieces	1	3		
Sand grains	20	50		
Earcockle	10	15		
Head scab	1	2		
Heat damaged or moldy	Nil	Nil		
White-grain disorder	2	5		
Dry green, sappy or	1	10	7.0%	No limit
frosted/distorted grains				
Smudge, black point			10.0%	No limit
Shrunken, broken grains			7.0%	No limit
Sclerotinia			0.01%	0.10%
Objectionable matter			0.03%	0.10%
(stone, sticks, glass, concrete)				
Foreign material			0.2%	1.0%
Excreta			0.01%	0.03%
Vitreous kernels			65.0%	No limit
Grass green			0.75%	No limit
Fusarium damage			0.25%	5.0%
Fireburnt			Nil	2.0%
Degermed			4.0%	No limit
Dark, immature			1.0%	No limit
Artificial stain, no residue	1	1	Nil	2.0%
Pink grain	2	5	1.5%	No limit
Broken grain, seive #5, buckwheat			0.30%	0.50%

Sources: (1) AWB Ltd. Wheat Receival Standards (2002-03); (2) http://www.grainscanada.gc.ca - Canadian Grains Commission Official Grain Grading Guide, dated 1 August, 2002.

In addition, if the settings of the grain-harvesting equipment are not correct, there will be excessive inclusion of plant parts with the grain or damage to the grain. The resulting presence of shattered or half grains is undesirable, because they are more susceptible to insect attack and to further damage during grain handling and transport. The risks of such undesirable consequences during harvest and transport are greater in very dry, hot conditions.

Hot, dry conditions are also conducive to the production of grain dust - fine particles, comminuted from the plant material and even from the grain itself. Ongoing handling and transport of dry grain is likely to produce more grain dust, as grains rub against each other when it is "turned" or moved from storage facilities to transport containers (trucks or rail cars). Figure 2 shows that grain dust is largely composed of fragments broken from the surfaces of grains. In the wheat dust (Figure 2a), there are short hairs from the brush end of the grain, and also fragments of the bran layers, as well as starch from the endosperm of grains that have been completely broken. The dust from barley (Figure 2b) shows long spear-like structures (broken pieces of rachilla hairs) as well as fragments of awns, lemmas, and paleas (see Grain and Plants, Morphology). It is a controversial

Table 2 Specifications for physical aspects of grain quality for grades of barley involved in international trade

Quality attribute	Australian malt 2 six-row malting barley	Australian malt 3 six-row malting barley	Australian food 1 six-row malting barley	US no. 1 six-row blue malting barley	US no. 4 six-row blue malting barley
Test weight	65 kg hl <sup>-1</sup>	65 kg hl <sup>-1</sup>	68 kg hl <sup>-1</sup>	47.0 lb bu <sup>-1</sup>	43.0 lb bu <sup>-1</sup>
Varietal mix	Specified	Specified	Specified	Specified	Specified
Falling number	300	300	300	Not stated	Not stated
Sprouted grains	Nil	Nil	Nil	3.0%	13.0%
Unmillable material	38.0 below 2.5 mm screen	42.0 below 2.5 mm screen	30.0 below 2.5 mm screen	4.0%	10.0%
Small foreign seeds	0.6%	0.6%	0.6%	0.5%	3.0%
Contaminants in 1/2 I measure					
Seeds, specified species	From Nil to 85, acc. to species	From Nil to 85, acc. to species	From Nil to 85, acc. to species	2.0%	5.0%
Tainting material	Nil .	Nil .	Nil	Nil	Nil
Chemicals, dyes, etc.	Nil	Nil	Nil	Not stated	Not stated
Ergot pieces	0.5 cm	0.5 cm	0.5 cm	Nil	Nil
Loose smut pieces	Nil	Nil	Nil	Nil	Nil
Grain insects	Nil live, 10 dead	Nil live, 10 dead	Nil live, 10 dead	Nil	Nil
Field insects	3	3	3	Nil	Nil
Earth pieces	3	3	3	0.5%	3.0%
Sand grains	50	50	50	0.5%	3.0%
Objectionable matter (stone, sticks, glass, concrete)	Nil	Nil	Nil	Not stated	Not stated
Frosted	5	5	5	0.4%	0.4%
Dark tipped (per 100 g)	10	10	10	Not stated	Not stated
Odors, moldy	Nil	Nil	Nil	Nil	Nil
Chemical residues (chemicals not approved for use on grain)	Nil	Nil	Nil		
Heat damage				0.1%	0.1%

Sources: (1) Australian GrainCorp Receival Standards 2002/2003; (2) USDA (1995) USDA Grain Inspection Handbook.

Table 3 Specifications for physical aspects of grain quality for grades of oilseeds involved in international trade

Quality attribute	Australian canola CSO-1	Australian sunflower CSO-4	Australian soybean CSO-9	US no. 1 grade canola	US no. 3 grade canola	US no. 1 grade soybeans	US no. 4 grade soybeans	US no. 1 grade sunflowers	US no. 2 grade sunflowers
Test weight	6.2 kg hl <sup>-1</sup>		Not stated			56 lb bu <sup>-1</sup>	49 lb bu <sup>-1</sup>	25 lb bu <sup>-1</sup>	25 lb bu <sup>-1</sup>
Sprouted grains Unmillable material	5%	5%	5%	5.0%	5.0%	1.0%	5.0%		
Contaminants in 1/21 mea	asure								
Seeds, specified species	From nil to 200, acc. to species	From nil to 200, acc. to species	From nil to 200, acc. to species	Not stated	Not stated				
Ergot pieces				0.05%	0.05%				
Oil content	42%	40%							
Green seeds Chlorophyll	5% 12 mg per kg	Nil	Nil	2.0%	20.0%				
Impurities	3%	4%	4%						
Broken seed	7%	7%	20%						
Damaged seed Heat damaged Sclerotinia Objectionable matter (stone, sticks, glass, concrete)	3%	3%	3%	0.1% 0.05% 0.05%	2.0% 0.15% 0.05%	2.0% 0.2%	8.0% 3.0%	5.0% 0.5%	10.0% 1.0%
Splits Grains of other colors De-hulled seed						10.0% 1.0%	40.0% 10.0%	5.0%	5.0%

Sources: (1) AOF Incorporated Technical and Quality Standards, December, 2001; (2) USDA (1995) USDA Grain Inspection Handbook.

matter in international trade as to whether the accumulated dust should be removed before shipping grain, or whether it is a legitimate part of a grain cargo. In any case, the dust generated during grain handling is a significant health problem, causing irritation to the bronchial tract as well as to the



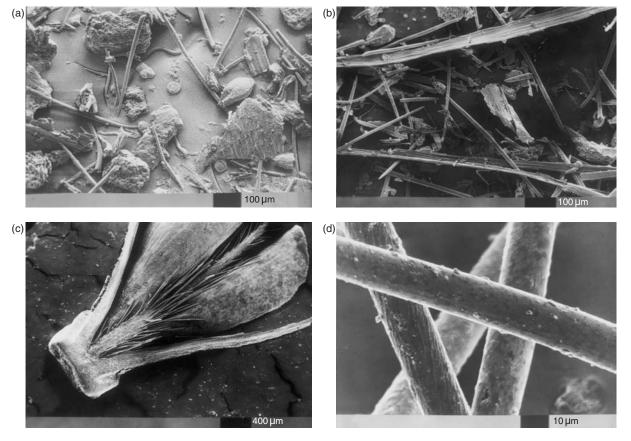
**Figure 1** Sieving of a grain sample to remove screenings (nongrain material).

skin. Dust also creates safety concerns due to its explosive potential; therefore dust reduction is a high priority at grain storage centers.

#### **Defective Grains**

Defective grains may be regarded as undesirable contaminants, even though of the same grain species. Such defects include:

- Immature cereal grains these may have come from side tillers that formed at a late stage compared to the main tillers of the plant. Alternatively, it may indicate that varieties of different maturity have been sown as a mixture. These grains are likely to be green in color and "sappy" in texture.
- Sprouted grains (Figure 3) these are grains that have become wet, and have thus started the germination process (*see* Cereals: Grain Defects).
- Sun-cracked rice grains these are grains that will probably fall to pieces during rice milling (the removal of the outer bran layers), thereby reducing the yield of whole rice grains.



**Figure 2** Scanning electron micrographs of grain dust from (a) wheat and (b) barley. The photo of half a barley grain (c) shows where the rachilla hairs have been broken from the base of the rachilla. The rachilla hairs are also shown at greater magnification (d). In each photo, the black bar at the bottom, near the right side is: (a) 100 μm, (b) 100 μm, (c) 400 μm, and (d) 10 μm.

 Vitreous content, pinched grains, black point, frosted/distorted are very important but controversial defects. Ear cockle (seed-gall nematode), a disease caused by *Anguina tritici*, must be detected as it is an object of exclusion due to quarantine in certain countries, especially in Iran.

# **Infected Grains**

Various forms of infection with microorganisms cause grains to become dangerous contaminants (see Cereals: Grain Diseases). For example, grain that is harvested with a high moisture content (>16% moisture) may be infected with molds, commonly called field fungi, such as Fusarium and Alternaria causing, in turn, the production of mycotoxins. Because these toxins are active at very low concentrations, e.g., a few ppb, their detection is difficult. They are not necessarily present in visually spoiled grain (e.g., moldy), so there is the need for ready means of testing. Immuno-assay kits are a means of doing so quickly and in "field" situations. If not detected, mycotoxin-affected grain may cause severe illness or death to humans or animals. Grain affected by field fungi may be dark brown, gray, or various shades of black discoloration or pink, especially when affected by Fusarium spp.

Ergot infection is another serious contaminant, mainly present in rye, rye grass, canola, and to



**Figure 3** Sprouted heads of wheat. The heads have become wet when harvest ripe. The one at left is a sprout-susceptible variety and the grains have started to sprout inside the head.

a lesser extent, wheat. In this case, the ergot fungus (Claviceps purpurea) infects the flowers of the cereal grain, producing an ergot body in place of the grain (Figure 4). The ergot bodies often thresh intact, easily recognizable in grain samples as black bodies (sclerotia) larger and longer than the normal grain length. The extent of ergot contamination is generally specified as a percentage by weight, but the length of ergot bodies placed end-to-end is a simple measure used in practical situations. Harvested grain of any species may contain ergots from other species, especially from rye grass (Lolium perenne). Any of these sources of ergot produce toxic alkaloids, which may cause injury in cattle when present at a level as low as 0.05%. On the other hand, some poultry species appear to be much more tolerant to ergot poisoning (see Cereals: Grain Diseases).

Another serious infection of cereal grains is bunt, also known as ball smut or stinking smut, caused by *Tilletia caries* or *T. foetida*. This defect also involves the replacement of the endosperm of the grain by bunt spores, which have a pungent unpleasant odor. As a result of this form of contamination, sound grain may be tainted by admixture with bunt-affected grain (Figure 5). If these grains are broken, black bunt spores spread through the grain as a further source of contamination. In the case of wheat, the bunt spores catch in the brush hairs of sound grains (Figure 6).

In grain storage, excessive moisture can lead to moisture migration and "bin burn" of the grain (causing heat damage and moldy appearance) resulting



**Figure 4** Ergot-infected heads, in which ergot bodies have replaced grains in the head. (Reproduced with permission from Ferns GK, Fitzsimmons RW, Martin RH, and Wrigley CW (1978) *Australian Wheat Varieties: Supplement No 1.* Melbourne, Australia: CSIRO.)



Figure 5 Bunt of wheat. (Reproduced with permission from Ferns GK, Fitzsimmons RW, Martin RH, and Wrigley CW (1978) Australian Wheat Varieties: Supplement No 1. Melbourne, Australia: CSIRO.)



Figure 6 Bunt spores caught in the brush hairs of sound grains. (Reproduced with permission from Ferns GK, Fitzsimmons RW, Martin RH, and Wrigley CW (1978) Australian Wheat Varieties: Supplement No 1. Melbourne, Australia: CSIRO.)

from the development of field fungi, which may produce dangerous toxins and offensive odors.

# **Other Crop Seeds**

Commonly found contaminants are the seeds of other crop plants. These are likely to be species whose plants are of similar size and maturity to the target seed. It is thus difficult to avoid harvesting them together with the intended crop. Such foreign seeds are likely to be other agricultural species (e.g., barley or rye in a wheat crop) that has been present in the seed sown, or that have been carried over from a previous crop at this growth site. Such contaminants may not be critical unless they interfere with the subsequent processing of the grain, such as milling into flour. A further consideration is how readily they can be removed, if their removal is essential, because this exercise will add to processing costs. Oilseeds, for example, pose the threat of disrupting wheat-milling equipment with the build-up of oil residues, although contaminants such as canola are relatively easy to separate from wheat before milling.

For a wheat consignment, there may be no need to remove minor contamination with other cereal grains, such as barley and oats (up to  $\sim$ 5%). Canadian research has shown that flour yield decreased by  $\sim 0.4\%$ for each addition of 1% of barley added to a wheat sample. Milling stocks fed well during flour milling, despite the barley contamination. Likewise, the effects of contamination of wheat with cultivated oats (or even wild oats) were similar to that of barley, except that at 5% addition, oats caused difficulty with the milling process.

#### Weed Seeds

Weed seeds present problems of greater severity than crop seeds (Table 4). Some weed seeds are the same size as grain kernels, which make it virtually impossible for them to be sieved out of a parcel of grain (e.g., Phalaris). For a start, agricultural authorities must restrict the seeds of noxious weeds, due to the threat that they pose to cropping and grazing. However, in small amounts, their presence may not be serious to agriculture because they are likely to be destroyed by processing, such as flour milling. If wheat contains seeds with a dark seedcoat, they are likely to cause dark specks in flour, which can decrease the visual appeal of the end product for the market. On the other hand, some weed seeds cause serious problems because some are toxic to humans and animals. In this category are species such as castor oil seed (Ricinus communis), Mexican poppy (Argemone species), and thornapple (*Datura* species). Ricin, the toxic principle in the castor oil seed, is reported to be one of the most toxic plant substances known.

Tainting seeds, whose scent may taint a whole consignment of grain, must be avoided, and they are generally specified as "nil tolerance," i.e., none of this type is allowed. In Australia, for example, seeds called "Hexham scent" (Melilotus indicus) impart their scent to the grain with which they may be mixed, with the risk of a large consignment of grain being tainted by the presence of a relatively small

Table 4 Groupings of contaminating seeds (a few examples only) used by the Australian wheat industry to indicate their severity as contaminants

Seed type	Botanical name	Local common name	Maximum number allowable in 1/2 l			
			Most grades	GP1 <sup>a</sup>	Feed	
1	Ricinus communis	Castor oil plant	Nil	Nil	Nil	
	Coriandrum sativum	Coriander				
	Allium vineale	Crow garlic				
2	Datura spp.	Thornapple	1	1	1	
	Gossypium spp.	Cotton seed				
3	Zea mays	Maize	1	50	100	
	Lupinus spp.	Lupin				
	Helianthus annuus	Sunflower				
4	Melilotus indicus	Hexham scent	5	50	50	
	Argemone spp.	Mexican poppy				
5	Sorghum halepense	Johnson grass	20	80	200	
	Lolium temulentum	Drake, darnel				
6	Secale cereale	Cereal rye	50	200	500	
	Sorghum bicolor	Sorghum				
	Avena sativa	Oats				

<sup>&</sup>lt;sup>a</sup>General Purpose 1.

Source: AWB Ltd., Melbourne, Australia.

contribution of grain with this contamination; similar problems may be produced by the presence of eucalyptus leaves. Animals will probably reject feed grain if it is tainted. Alternatively, if they do consume it, the taint may be passed through to the animal by-products, such as milk.

There is naturally great variation in the range of weed-seed species that are relevant to specific regions and grain species. Regional and national booklets are published for many grain-growing regions, illustrating a relevant range of weed and crops seeds. Examples of weed seeds that must be identified and restricted are shown in Figure 7.

#### Insects

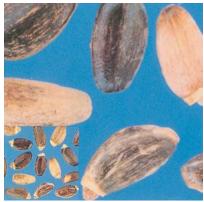
Insects that attack grains are a serious case of contamination (Figure 8). Accordingly, there is a "nil tolerance" for live insects in many regions (Tables 1–3). This specification may be relaxed to a small extent in the cases of dead insects and for species of insects that do not attack grains, (on a case-by-case basis only) such as field insects. Infestation with grain-attacking insects (previously or currently present) may be indicated by the presence of grains that have been partly eaten by insects (Figure 9).

There are two main types of grain insects. Primary insects are more destructive as they can attack sound grain, while secondary insects can only attack grain that has been damaged. Insects can deplete the energy value of the grain, greatly reducing its value even for stock feed. Insect infestations are often associated with bad odors. Due to the serious threat that some

insects can impose, there is a strict nil tolerance on specific species. For example, the Khapra beetle, *Trogoderma granarium*, is the world's most serious insect pest. This insect does not occur in some countries (e.g., Australia). However, the warehouse beetle, *T. variable*, is often mistaken for the Khapra beetle and this can jeopardize not only the acceptance of a parcel of grain but the whole country's reputation.

#### **Animals and Animal Products**

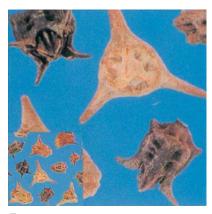
Further contaminants include snails, rodents, urine, feces, rodent hairs, birds, bird feathers, and snakes. Snails rarely live more than 2 weeks in storage, so that they are unlikely to reproduce during storage. Most animals contaminate grain through the grain-storage system (with the exception of snails) due to their reproduction and spread throughout the grain store. Rodents and birds are attracted to the grain as a food source and they will often nest there. As a result, there can be the added contamination from the accumulation of their urine, feces, rodent hairs, feathers, eggs, and decaying carcasses. For example, a single rat can produce 12 000 droppings, 2.91 of urine, and 0.9 million shed-hairs in 6 months. Rodents thus become an important issue, especially when there is a plague. Animals and birds are also potential vectors for diseases. Snakes and some bird species will be present around storage sites, preying on rodents and other birds. All of these animals can become included in a consignment of grain accidentally if present on one of the transport belts or elsewhere on the grain-transport path when it is turned on.



Silybum marianum variegated thistle



Carthamus lanatus saffron thistle



Emex spp. double gee threecornered Jack spiny Emex cats' heads



Galium tricornutum (Galium tricorne) threehorn bedstraw

Figure 7 Weed seeds that are likely to cause problems if identified in grain consignments. (Reproduced with permission from Ferns GK, Fitzsimmons RW, Martin RH, and Wrigley CW (1978) *Australian Wheat Varieties: Supplement No 1.* Melbourne, Australia: CSIRO.)

# **Agricultural Chemicals**

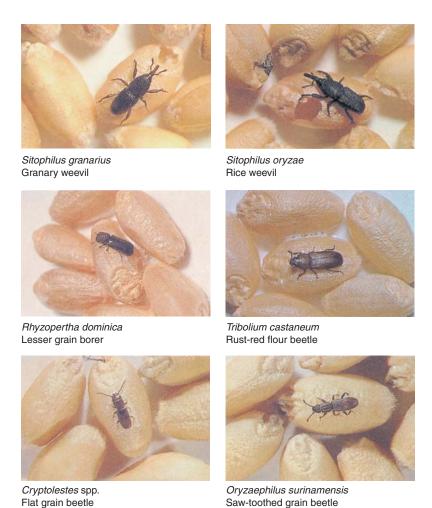
Pickling compounds (fungicides) applied to seed grain render the grain unfit for consumption (human or animal). Pickling colors range from bright pink or green through to a slight pink or green coloration. There is a strict nil tolerance for pickled grain as a small amount can render a whole stack unusable. Parcels are regularly checked for pesticide residue levels (obtained through the application of herbicides and/or insecticides). There are tolerances by national regulatory bodies, known as maximum residue limits (MRL). Grain with levels above these limits cannot be used or sold legally.

Grain that is fed to cattle and chickens can transfer the chemicals, thus posing a threat to their markets, with the likelihood that the resulting meat will be prohibited from human consumption. Examples include endosulfan in cottonseed hulls and wheat resulting from spray drift. It is necessary to observe and follow chemical instructions for correct application rates and withholding periods to ensure that MRL levels are not exceeded and to reduce the chance of insect resistance building up.

Heavy metals (mercury, cadmium, and lead) are contaminants that must be tested for routinely as a precautionary measure, especially in industrialized countries.

#### **Inanimate Materials**

Sticks, stones, and other inanimate materials cause problems in both analysis and processing, as they can damage grinders and technical equipment. The severity of the material depends on their size. If the material is a similar size to the grain, it cannot be sieved out easily and this can become a serious issue. Such materials can be picked up during the



**Figure 8** Insect species that attack grains. (Reproduced with permission from Ferns GK, Fitzsimmons RW, Martin RH, and Wrigley CW (1978) *Australian Wheat Varieties: Supplement No 1.* Melbourne, Australia: CSIRO.)



**Figure 9** Wheat grains that have been partly eaten by insects, indicating previous (or current) infestation. (Reproduced with permission from Ferns GK, Fitzsimmons RW, Martin RH, and Wrigley CW (1978) *Australian Wheat Varieties: Supplement No 1.* Melbourne, Australia: CSIRO.)

harvesting process or introduced through the storage and handling processes if equipment is not carefully calibrated and cleaned prior to use.

# **Tainting Contaminants**

In addition to the contamination of grain with tainting materials as a result of harvest, tainting of a grain consignment can occur during storage and transport if a taint or odor is acquired from materials with the grain. One such source can be the packaging material used, in the case of grain that is stored and transported in bags, for example, hessian bags.

# **GM Grain**

With the introduction of genetically modified (GM) grain (initially involving maize, canola, and cotton), and in response to various international market demands, the presence of GM grain within conventional grain delivered can lead to rejection of that grain. Methods are being put into place that will permit



Figure 10 Sampling grain from a truck using a spear sampler.

the detection of such contamination thereby guaranteeing the required grain quality required by specific markets.

# **Analysis of Contaminating Materials**

Given the wide range of contaminants that may be harvested with grain, plus the variations in allowable levels, it is important that there should be rapid means of identifying them. This requirement applies especially to identifying the species of contaminating seeds, since the range of species varies considerably from one region to another. Accordingly, manuals illustrating the range of contaminants are provided for staff training, to inform growers and for use when grain is delivered.

# Sampling

However, an essential prerequisite of valid analysis is the initial step of taking a representative subsample of the load being analyzed. Sampling procedures are generally specified together with the manuals illustrating the contaminants. Procedures are also published by grain-trading authorities. The website of the US Department of Agriculture (www.usda.gov/gipsa/pubs/farm-proc/practical\_proc.htm) specifies standard sampling procedures for various grain-production situations. Standard procedures for sampling are provided in the Methods of the American Association of Cereal Chemists (AACC) and the International Association for Cereal Science and Technology

(ICC) (see Appendix: Test Methods for Grain and Grain-Based Products).

A critical stage of grain sampling is taking a representative set of samples from a truck when grain is first delivered from harvest to the elevator (Figure 10). In this case, sampling is done so that a whole truck's contents can be observed from above. Routinely, the grain in the truck is sampled by pushing a sampling spear into the grain at several points. This form of sampler is designed to take grain from several vertically separated points throughout the depth of grain. Alternatively, vacuum probes are used to spear the truck in a straight vertical line right down through to the bottom of the load. If the grain is delivered by road, train, or other vehicle with multiple compartments, each bin is sampled three times.

# **Sieving**

An example of the use of mechanical sieving is provided by the routine practice of GrainCorp in eastern Australia using the Agtator (Figure 11). Forty shakes are automatically performed to and fro, as specified by Australian receival standards. The use of a mechanical sieve such as this ensures repeatable movement, speed, and duration. The following screen sizes are appropriate: wheat, 2.0 mm slotted; for barley, 2.5 mm and 2.2 mm slotted; for sorghum, 2.0 mm slotted (wheat screen); for sunflowers, 2 mm round; for chickpeas, top 3.97 mm slotted over bottom 2 mm slotted; and for canola, top 2.58 mm round over bottom 1.00 mm round.



Figure 11 Agtator equipment for sieving grain.

# Visual Examination and On-the-Spot Instrumental Analysis

Traditional methods of analyzing contaminants involve visual inspection, and this approach is still the best with respect to providing an immediate outcome and requiring no expensive equipment. However, this approach is subjective and highly dependent on the experience of the inspector. This expertise is acquired and maintained by ongoing training, the use of illustrated manuals and actual samples, requiring ongoing monitoring to ensure the standards are applied consistently. By this approach, grain samples are first evaluated visually at the site of grain receival, and then a representative portion may be sent back to a regional laboratory for a second cross-reference assessment. In addition, a further portion may be presented to buyers of the grain for their additional evaluation. The identification of the species of insects is also an important part of this process, because the various insect species differ in the severity and consequences of infestation.

The immediate detection of serious contaminants is critical at the time of delivery, so that defective grain loads are not combined with sound grain, as this might downgrade the grain to which it is added. Subsequent analysis in the laboratory provides results too late to prevent damage of a large grain consignment by, for example, the incorporation of a relatively small load of grain that is tainted or insect infested. On-the-spot analysis is thus critical.

It has become usual for visual inspection to be complemented by on-the-spot testing to provide objective results for specific critical aspects of contamination. Image analysis has great potential for replacing the subjectivity of human involvement by analysis of the image of grains and contaminants provided by a television camera. However, the introduction of image analysis is a slow process due to the expense and technical input required to develop methods and monitoring procedures.

NIR analysis has been used to detect the presence of insect infestation, probably due to the ability of this spectroscopic method to detect the distinctive presence of insect protein and/or chitin. The use of NIR methodology has the potential advantage that NIR equipment is already in routine use to determine moisture, protein content, and oil content, but the NIR units in routine use may not be adequate for more complex analyses. Ultraviolet light inspection provides an immediate means of checking for rodent urine, which fluoresces with a blue-green color (see Appendix: Test Methods for Grain and Grain-Based Products; AACC Method 28-85). Rapid immunoassay kits are now available to detect and quantify a range of chemical contaminants, aflatoxins, and damage from sprouting.

# **Analysis in the Laboratory**

Contaminant identification may continue by visual examination at a regional or central laboratory, where a higher level of expertise may be expected of the inspectors. There is also a role for image analysis and sophisticated NIR equipment at a central site, where the volume of samples would warrant the expense and need for operator expertise. A wide range of laboratory methods is available for the detection of specific contaminants. For example, the AACC Methods provide many standard procedures in section 28 "AACC Method Group" (see Appendix: Test Methods for Grain and Grain-Based Products). Laboratory methods include gas or liquid chromatography to analyze for agricultural chemicals and ELISA immuno-assays for many contaminants, especially aflatoxins.

# **Avoidance of Contaminants**

# **Purity of Seed Sown**

An obvious source of contaminants is the seed originally sown. If it contains foreign seeds, these are likely to multiply and downgrade the harvested grain. Additional risks include the presence of diseased seed, which will lead to the spread of diseases during growth. It is thus important to obtain seed of guaranteed purity at the time of purchase.

# **Farm Management**

Use of "best farm-management practices" includes cleaning and calibrating of harvester and sowing equipment before use, proper preparation of fields before sowing, ensuring that the crop is mature before harvesting, and storage of the grain so that it does not become contaminated. During growth of the crop, weeds must be controlled. In addition, it is valuable to use crop rotations as "bio-fumigants" to avoid the buildup of microbial spores in soils.

### **Quality Assurance Methods**

All these methods of contaminant avoidance should be combined for implementation via a system of quality assurance, to provide systematic recognition of potential hazards, intentional approaches to avoid the hazards, and records to indicate how and when the preventive measures were taken. This approach extends beyond the farm, to involve, for example, the use of traceable and repeatable methods of appropriate farm practices, working only with companies that are themselves quality-assurance certified.

# International Regulations on Contaminants

International regulations on many food matters are administered by parts of the Codex Alimentarius. The Codex Committee on Food Additives and Contaminants (CCFAC) establishes or endorses maximum or guideline levels of contaminants in food and animal feed, as well as dealing with food additives and naturally occurring toxicants. CCFAC is developing risk-analysis approaches to be applied to all foods. The resulting document is the General Standard for Contaminants and Toxins in Foods. Further details of the latest developments are available at the USDA website: www.fsis.usda.gov/oa/codex/fac.htm.

# **Future Prospects**

Traditionally, the presence of contaminants of any type in a grain consignment has prompted a reduction in its market value, because any variation in appearance can provide grounds for bargaining and for price reduction. More recently, there has been a growing awareness that some defects and contaminants may have relatively small financial consequences. In some instances, the economic effects can readily be assessed. For example, the presence of 2% screenings (by weight) at a 5% reduction in price would be a worthwhile bargain, if the cost of cleaning is more than covered in the price difference. Similar considerations apply to other innocuous contaminants, if their dimensions make them easily removable. Another approach to overcoming such problems may involve the intelligent blending of diverse grain samples. Relatively simple research activities can establish the extent of economic penalties for the presence of one grain type as a contaminant in another, e.g., oilseed in cereal or the reverse.

There is a continuing need for more effective methods of analysis to characterize and quantify the presence of contaminants in grain deliveries at the point of receival and during grain handling. New screening methods should preferably be deployable on-the-spot and be nondestructive of the grain. Most of all, they need to be cost-effective, the cost of deployment warranting the benefits, and the risks thereby avoided. Detection of contaminants by image analysis is likely to increase in its application. We can expect that large grain sorters will be placed into grain-storage sites and cleaning facilities.

Increases in quality demands will mean the need to increase the efficiency of detection and measurement requirements at the receival point. In summary, these new methods must be rapid (taking less than 5 min), easy to operate with minimal operator skill required, nondestructive and cost-effective, being able to operate in a wide range of temperature variation, under dusty conditions, occupying minimal bench space... but the fulfillment of these requirements is a "tall order."

See also: Cereals: Grain Defects; Grain Diseases. Plants: Diseases and Pests.

# **Further Reading**

AOF Incorporated Technical and Quality Standards, December, 2001.

American Association of Cereal Chemists (1983) Approved Methods of the American Association of Cereal Chemists, 8th edn. St. Paul, MN: American Association of Cereal Chemists.

Australian GrainCorp Receival Standards 2002/2003.

AWB Ltd. (2001) Australian Graincrop Receival Standards 2002/03. Melbourne, Australia: AWB Ltd.

AWB Ltd. (2001) Wheat Receival Standards 2002/03 Season. Melbourne, Australia: AWB Ltd.

CCFRA (2002) Manual of Methods for Wheat and Flour Testing. Guideline No. 3, (3rd edn.). Chipping Campden, Gloucestershire, UK: CCFRA Technology.

Ferns GK, Fitzsimmons RW, Martin RH, and Wrigley CW (1978) Australian Wheat Varieties: Supplement No 1. Melbourne, Australia: CSIRO.

Fitzsimmons RW and Wrigley CW (1979) Australian Barleys: Identification of Varieties, Grain Defects and Foreign Seeds. Melbourne, Australia: CSIRO.

National Institute of Agricultural Botany (2000) Botanical Descriptions of Cereal Varieties. Cambridge, England: National Institute of Agricultural Botany.

USDA Grain Inspection, Packers and Stockyard Administration (1995) *Grain Inspection Handbook: Book 1*. Washington, DC: USDA (http://www.usda.gov/gipsa).

Wrigley CW (ed.) *Identification of Food-Grain Varieties*. St. Paul, MN: American Association of Cereal Chemist.

# **Relevant Websites**

http://www.grainscanada.gc.ca — Canadian Grains Commission Official Grain Grading Guide, dated 1 August 2002.

http://www.aaccnet.org - American Association of Cereal Chemists.

http://www.aocs.org – American Oil Chemists' Society; Standard Methods.

http://www.awb.com.au - AWB Ltd, Melbourne, Australia.

http://www.campden.co.uk - Campden and Chorleywood Food Research Association.

http://www.cgc.ca; www.grainscanada.gc.ca - Canadian Grains Commission, Winnipeg.

http://www.fsis.usda.gov — Codex Committee on Food Additives and Contaminants.

http://www.pi.csiro.au – CSIRO Plant Industry, Australia.

http://www.wheat.pw.usda.gov - Graingenes.

http://www.icc.or.at — International Association for Cereal Science and Technology.

http://www.seedtest.org — International Seed Testing Association.

http://www.crop.cri.nz - New Zealand Institute of Crop and Food Research.

http://www.sgrl.csiro.au — Stored Grain Research Laboratory, Canberra, Australia.

http://www.usda.gov — United States Department of Agriculture.

Corn see Maize: Genetics; Breeding; Quality Protein Maize; Dry Milling; Wet Milling; Foods from Maize.

# **COTTONSEED**

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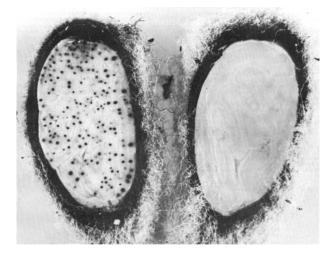
# Introduction

Most varieties of cotton (Gossypium hirsutum L., G. aboreum L., G. barbadense L., or G. herbaceum L.) are grown mainly in warm climates around the world. Over 98.5 million tons (Mt) of cotton were produced worldwide in 2001–02 and over 33.6 Mt of cotton-seed. Cotton is grown for its fiber (over 80% of its value) and the seed is used mostly for oil recovery and feed. Whole cottonseed can be fed to dairy cattle, and the meal resulting from oil extraction is fed primarily to ruminants and, in limited amounts, to poultry and swine.

Traditional varieties of cottonseed contain gossypol, a yellow-green polyphenolic compound considered toxic to man and monogastric animals (Figure 1), reportedly affecting the heart, liver, and reproductive organs. It has been used in China as a male contraceptive but the practice was abandoned because of permanent side effects. Gossypol is dispersed in the plant as deposited structures or "glands," which can be seen as black specks in the stems, leaves, and seed (Figure 2). The glands in the seed are ovoid structures containing 35–50% gossypol and are 0.025–0.178 mm in diameter. These gossypol glands are difficult to break by mechanical means, but heat generated in extraction of oil by pressing, binds gossypol to protein, turning it nontoxic. Switching to solvent extraction with hexane, where no appreciable heat is generated, increases the free gossypol content in the meal over ten times.

Raw cottonseed kernels may contain 0.6–2.0% free gossypol. The Food and Drug Administration in the US (FDA) limits free gossypol in human food products and ingredients at 450 ppm, and the Protein Advisory Group of the United Nations Food and Agriculture Organization (FAO) and World Health Organization (WHO) has set maximum guidelines of 600 ppm for free gossypol and 12 000 ppm total

Figure 1 Gossypol.



**Figure 2** Cross-section of glanded cottonseed (left) and glandless seed (right). (Data from Miravalle RJ (1972) *J. Am. Oil Chem. Soc.* 49: 24–26.)

gossypol. The feed industry in the US set limits for free gossypol levels in poultry diets at 100 ppm maximum for broilers and 40 ppm for laying hens.

Removal by mechanical separation of the gossypolcontaining glands has also been reported. Also addition of iron salts, such as ferrous sulfate, which bind the gossypol in feeds and render it biologically inactive, is practiced in some countries.

Solvent extraction with hexane of cottonseed oil, commonly used commercially, removes only small amounts of gossypol with the oil. However, extraction with more polar solvents is effective in removing gossypol. Examples of polar solvents used include aqueous acetone, a mixture of acetone and hexane, and sequential extraction with hexane, aqueous acetone, and anhydrous acetone. Blends of methylene chloride and hexane-acetic acid have also been used. These solvents are not widely used commercially.

Techniques have also been reported that mill cottonseed in the presence of hexane and then remove the intact, heavier gossypol glands by the liquid cyclone

process. Also, an air classification process has been developed to separate intact gossypol glands from solvent-extracted ground flour.

# **Glandless Cottonseed**

Some of the first studies on the development of glandless cottonseed in the US were done in the early 1950s. These studies described that selection of plants from the "Hopi Moencopi" variety could result in almost complete elimination of pigment glands from leaves and bolls. When crossing Moencopi with upland cotton varieties (*G. hirsutum*), it was found that glandless seed appeared in later segregating generations. Production of glandless cotton was first conducted in Iguala, Mexico in 1960.

Basically two genes, gl-2 and gl-3, in the plant are found to control the production of gossypol pigments in the seed. When present in the homozygous recessive condition (gl2gl2-gl3gl3), all parts of the plant aboveground, including the seed, have no pigment glands. The presence of pigmented glands can be easily seen as black specks by cross-sectioning a seed (Figure 2).

Recently, the value of the whole seed as cattle feed has increased making it less profitable to extract the oil and sell the meal. In some cases cottonseed is left at the gin in exchange for ginning and baling services. Sometimes glandless cottonseed is considered to produce fiber of lower yield and quality than traditional "glanded" varieties. Some studies have shown that the glandless factor in itself does not decrease fiber yield or quality as long as care is taken to prevent weed and insect infestation.

Gossypol is regarded also to be a natural insecticide. Reports show that glandless cotton attracts more insects than glanded varieties. Entomology studies have shown that cotton bollworm (*Heliothis zea* Boddie), tobacco budworm (*H. virescens* F.), pink bollworm (*Pectinophua gossyprella* Saunders), and lygus insects prefer glandless cotton. It is generally recognized that glandless varieties require closer supervision to intercept and control insect infestations.

Processing and utilization of glandless cottonseed was reported in the early 1960s in the US. Glandless cottonseed oil and meal were evaluated in poultry broiler and layer rations. Glandless cottonseed meal was found nearly as effective as soybean meal in achieving broiler gains and did not produce the green yolk discoloration in eggs commonly experienced with feeding glanded cottonseed meal.

The first commercial glandless cotton variety was a storm-proof, boll-type cotton. It was evaluated in farm-scale trials and made available for commercial planting in the late 1960s in the US. Commercial sale

of glandless cottonseed began in the early 1970s, with FDA approval. In 1976, under title 21 (Food and Drugs) of the Code of Federal Regulations, sale of glandless cottonseed kernels and cottonseed flour as food additives was allowed. It included restrictions on gossypol (450 ppm). In 1978 the National Cottonseed Products Association (NCPA) in the US established grades of glandless cottonseed products: class A, to contain not more than 400 ppm of total gossypol; class AA, to contain not more than 100 ppm total gossypol; and class AAA, to contain not more than 10 ppm total gossypol. Glandless cottonseed kernels started to be sold for use as snack foods, in baked goods and in soft candy.

Research and production of glandless cotton for food and feed is also reported in Europe, Africa, and Asia. Some US cotton varieties were adapted to African crops. Development and study of some Egyptian varieties of glandless cotton have also been reported. From studies done in the Ivory Coast, it has been suggested that glandless cottonseed cultivation and oil mill processing is more economically viable in that part of Africa. Conditions there may be better for glandless cotton considering that in larger industrialized countries other competitive oil seed proteins are more readily available. An additional advantage is that large, isolated land areas can be dedicated to this crop and less problems of cross-pollination would be encountered.

# **Processing and Products**

Other than gossypol content very few differences are found in glandless compared with glanded cotton-seeds. Evaluations of eight varieties each of glanded and glandless cottonseed were reported (Table 1). Amino acid profiles were similar for glanded and glandless seed. The storage and processing characteristics of glandless and glanded cottonseed are also reported to be essentially identical with regard to development of free fatty acids, moisture levels, and refining losses in the crude oils.

No major differences were encountered either in solvent extraction of glanded versus glandless cotton-seed. Extraction rates of oil from glandless cotton-seed flakes are about the same as from glanded flakes, using commercial hexane. It has also been suggested that glandless cottonseed can also be a source of a new type of lecithin. Cottonseed has the highest content of phospholipids after soybeans, present at  $\sim$ 2.2% in the oil, but due to gossypol presence it was not commercially viable. Currently, the major commercial source of lecithin is soybean oil. Composition of cottonseed phospholipids consists of phosphatidylcholine, phosphatidylethanolamine, and

**Table 1** Comparative analysis means of eight varieties each of glanded and glandless cottonseed and their products<sup>a</sup>

Product and assay	Glanded cottonseed	Glandless cottonseed
Whole cottonseed		
Oil (%)	21.0	21.1
lodine no.	108.9	109.9
Protein ( $N \times 6.25$ ; %)	23.1	22.5
Wt. 100 fumed kernels (g)	10.0	10.6
No. fumed seed/100 ml	542	510
% Kernels in lint-free seed	61.7	59.6
Cottonseed kernels		
Oil (%)	37.8	39.7
Protein ( <i>N</i> × 6.25; %)	39.3	38.9
Crude fiber (%)	1.6	1.7
Total phosphorus (%)	0.8	0.9
Total sugars (%)	7.4	6.8
Total gossypol (%)	1.2	0.02
Wt. 100 kernels (g)	6.5	7.0
No. kernels/100 ml	912	844
Hexane-extracted flour (meal)		
Oil (%)	0.8	0.8
Protein (N × 6.25; %)	63.2	62.6
Crude fiber (%)	2.7	2.8
Ash (%)	8.0	7.8
Total phosphorus (%)	1.3	1.4
Total sugars (%)	13.4	13.7
Total gossypol (%)	1.6	0.02
Color, Hunter "L" values		
Dry	84.3	89.8
Wet (5 water: 1 flour)	48.1	71.3
Crude oil		
Cyclopropenoid fatty acids (%)	0.23	0.23
Fatty acids (%)		
Myristic	0.9	0.7
Palmitic	23.0	22.6
Stearic	2.2	2.1
Oleic	17.7	17.7
Linoleic	55.8	56.5
Unknown	0.4	0.4
Refined oil		
Refined oil color, red	6.9	3.7
Bleached oil color, red	2.9	2.2

<sup>&</sup>lt;sup>a</sup> Dry weight basis. (Source: Lawhon JT, Cater CM, and Mattil KF (1977) Journal of the American Oil Chemists' Society 54: 75.)

phosphatidylinositiol (33%, 22%, and 37%, respectively) and is considered more oxidatively stable than soybean phospholipids due to lower content of unsaturated fatty acids.

One of the main advantages of milling glandless cottonseed is that it simplifies direct solvent extraction. Current commercial practice of refining glanded cottonseed oil requires that the oil be refined in its miscella state (oil—hexane solution) right after extraction. This is necessary because if the solvent is removed from the oil prior to refining, the color fixes into the oil and is very difficult to remove in subsequent bleaching operations. This means the equipment in refining of miscella has to be explosion-proof, which is more expensive and more difficult to maintain. This is no longer the case with glandless seed processing, where binding of gossypol (by cooking and hard press or prepress) or miscella refining become unnecessary. The oil from glandless seed is in lighter colored oil and the meal has greater amounts of soluble protein. Protein solubility of processed glandless cottonseed flakes or press cake has been reported to be higher than that of glanded seed (89.6%) compared with 84.8% for extracted flakes, and 59.3% for press cake.

Advantages of processing glandless cottonseed over glanded seed include:

- 1. reduction in electrical energy used for flaking;
- 2. elimination of prepress operations with larger solvent extractors with lower maintenance costs, and reduction in processing energy;
- 3. since there is no color setting problem, crude cottonseed oil can be held longer in storage;
- elimination of more dangerous miscella (oil-solvent solution) processing or on-site conventional refineries;
- 5. lower refining loss of glandless seed oil due to reduced use of alkali to eliminate color;
- 6. reduction of bleaching earth needs; and
- 7. marketing of a light-colored, gossypol-free oil.

Some emulsification problems have been reported in refining desolventized crude due to higher concentration of lecithin.

There are some drawbacks when switching to glandless crops; varying levels of gossypol can exist among individual seeds from the same plant. It is also difficult growing large acreages of glandless cottonseed, without cross-fertilization by windblown or insect-carried pollen of glanded varieties. In order to preserve the variety and seed lot purity in the production of glandless cottonseed products, it is important to monitor both, cross-fertilization from cotton plants in nearby fields and reversion to the glanded condition during successive plantings due to fertilization between heterozygous plants. Furthermore, segregated handling of glandless cottonseed in gins and oil mills can also be costlier. As a result large cottonseed processors may be hesitant to handle glandless cottonseed and dedicate separate processing facilities.

# **Foods Uses**

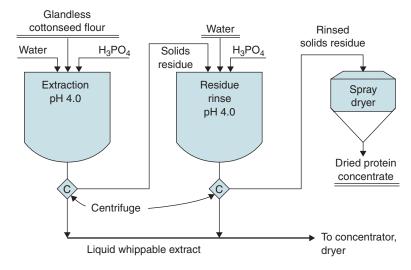
Several food uses of glandless cottonseed kernels have been reported as well as applications on the use of glandless cottonseed blended with other foods. Even though glanded cottonseed is not considered suitable for human consumption, there have been cases where cottonseed flour with bound gossypol has been used in nutrition intervention feeding products, such as Incaparina in South America.

Food products from glandless cottonseed include whole roasted seeds, flour, protein concentrates, and isolates. From these products many foods were reported to be prepared. The technologies for the production of high-protein products have been well established and apply similarly to all vegetable proteins. Specific technologies were developed for the production of cottonseed protein products that were later applied for processing of other vegetable proteins like soybean.

The preliminary steps in preparing glandless cottonseed products are similar to the process used with glanded cottonseed, i.e., ginned cottonseed is first cleaned to remove dirt and other impurities, conditioned, de-hulled, and separated to produce kernels. The kernels may be size-sorted and the larger particles roasted and color-sorted. The whole kernels are separated for roasting or conditioning. The smaller or broken kernels may then be further conditioned, flaked, and solvent-extracted for production of flours, concentrates, and isolates.

Whole glandless cottonseed kernels are prepared either for direct use as whole nut substitutes or for food ingredients. These are also used in the production of high-quality flours and food protein concentrates or isolates. The use of glandless cottonseed kernels for the production of nutlike products by means of various roasting methods has been reported, including dry roasting at several temperatures, roasting under vacuum followed by steam injection, pressure steaming followed by oven roasting, and deep fat frying in various oils (including corn, cottonseed, peanut, safflower, soybean, and sunflower oils). When the main product is to produce high-protein flours, less care needs to be taken to minimize breakage during de-hulling.

Glandless cottonseed flour can be prepared by extracting de-hulled glandless seed flakes with hexane. The extracted flakes are then desolventized and ground to a desired mesh size. In the production of cottonseed concentrates, the defatted flakes are reextracted with acidified water or ethanol to remove the soluble sugars and flavor compounds, and dried to produce protein concentrate. A spray dryer can also be used in preparing concentrates where the defatted flakes are ground prior to extraction. Concentrates have been prepared from flour by a dry air classification method and by acidic water extraction. Figure 3 shows a simplified diagram of the process to be used



**Figure 3** Protein concentrate production from glandless cottonseed. (Data from Lusas EW and Jividen GM (1987) *J. Am. Oil Chem. Soc.* 64(6): 839–854.)

to generate protein concentrate from glandless cottonseed.

Three major techniques have been reported for making glandless cottonseed isolates. These isolates can be conventional protein isolates, storage proteins (SP) (soluble at high and low pH), and nonstorage proteins (NSP) (soluble at near neutrality):

- 1. Ground flour is first extracted with dilute alkali (at pH 10) and the insoluble residue removed by continuous centrifuging or decanting. The clarified liquor is then precipitated at one pH (5.0). The resulting solids are concentrated by centrifugation and then dried to produce a mixture of storage protein and nonstorage protein.
- 2. In the "selective precipitation" procedure the protein is precipitated with alkali at a pH 10, the solids are then separated by centrifugation. The solids in the liquor are also precipitated at pH 7, and the storage protein curd removed by centrifugation and then dried. The liquor is further acidified to pH 4 to precipitate the NSP, also removed by centrifugation and then dried. The soluble matter remaining in the whey is then removed by either the selective extraction or selective precipitation method. A relatively pure storage protein fraction, containing over 90% protein (dry weight basis), can be prepared.
- 3. The "selective extraction" procedure consists basically of leaching the proteins soluble with water at neutral pH, where the solids are then centrifuged out. The liquor is then acidified to pH 4; this results in a precipitated protein curd. The curd is dried to produce an NSP isolate. The solids

from the original water leaching are then solubilized in alkali at pH 10 and centrifuged to remove insolubles. The liquor is then precipitated at pH 7 and the resulting curd is concentrated and dried to produce an SP isolate.

A patented process for the preparation of glandless cottonseed protein concentrate from solvent-extracted flour using industrial membranes has also been used. Defatted flour is sieved through an 80-mesh screen to break up any agglomerates and remove hull particles, and suspended in acidified water at pH 4.0–4.5. This solution is passed through an ultrafiltration membrane (100 000 MW cutoff) and the solids retained are either dried directly or neutralized before drying. The liquid fraction permeate may then be passed through a reverse osmosis membrane to concentrate the soluble solids and to recover water which may be reused in the process.

# **Feed Uses**

Glandless cottonseed has been reported to be suitable for monogastric animal feed not just for its lack of gossypol but also for higher available energy. The lower heat treatment required for glandless cotton-seed during milling is reported to degrade less protein than the prepress method normally used with glanded cottonseed. Commercial glanded cottonseed meal intended for poultry feeding usually receives considerable heat during processing to intentionally bind the gossypol. Also in order to increase the amount of glanded meal gossypol, addition of iron to the meal is used to inactivate gossypol. In the cases of glandless

cotton meal, it would be expected that lower heat treatment needed for glandless cotton would have beneficial effects in the protein availability and quality of the meal. Some heat pretreatment of the meal is still recommended, however, before hexane extraction, to improve protein efficiency. Heating glandless cotton-seed with 12% added water for 10 min at 82°C, followed by 105°C for 20 min before hexane extraction, was reported to appreciably increase broiler weight gains when compared with glanded cottonseed feed. It has also been shown that meal from glandless cotton-seed had an equal protein availability to soybean meal in supporting chick growth. Feed trials with glanded cottonseed meal with swine, catfish, fish, and shrimp have also been described.

See also: Animal Feed. Lipid Chemistry. Oilseeds, Overview. Grain Production and Consumption: Oilseeds in North America.

# **Further Reading**

- Berot S, Chenu M, Coutret J, and Gueguen J (1995) Cottonseed protein-rich products from glandless African varieties: 1. Pilot-plant scale production of protein concentrates. *Sciences-des-Aliments* 15(3): 203–215.
- Camire ME, King CC, and Bittner DR (1991) Characteristics of extruded mixtures of cornmeal and glandless cottonseed flour. *Cereal Chemistry* 68(4): 419–424.
- Cater CM (1968) Processing glandless cottonseed. Oil Mill Gazeteer 72(7): 8–15.
- Cherry JP, Gray MS, and Jones LA (1981) A review of lecithin chemistry and glandless cottonseed as a potential commercial source. Seed-oil content of glanded and glandless, cottons. *Journal of the American Oil Chemists' Society* 58(10): 903–913.
- Glandless cotton: its significance, status, and prospects:
  Proceedings of a conference, December 13–14, 1977,
  Dallas, Texas/sponsored by Agricultural Research
  Service, United States Department of Agriculture and
  National Cottonseed Products Association, Inc.,

- Agricultural Research Service Southern Region, US Dept. of Agriculture, 1978.
- Kohel RJ and Lee JA (1984) Genetic analysis of Egyptian glandless cotton. *Crop Science* 24(6): 1119–1121.
- Lawhon JT, Cater CM, and Mattil KF (1977) Journal of the American Oil Chemists' Society 54: 75.
- La-Rue DC, Knabe DA, Izard RS, and Tanksley TD (1986) Glandless cottonseed kernels for starter, grower and finisher swine. *Journal of Animal Science* 64(4): 1051–1059.
- Lusas EW and Jividen GM (1987) Glandless cottonseed: a review of the first 25 years of processing and utilization research. *Journal of the American Oil Chemists' Society* 64(6): 839–854.
- Miravalle RJ (1972) The plant geneticist's contribution toward changing the lipid and amino acid composition of cottonseed. *Journal of the American Oil Chemists' Society* 49: 24–26.
- Percival AE, Wendel JF, Stewart JM (1999) Taxonomy and Germ plasm resources. In: Smith CW and Cothren JT (eds.) Cotton. New York: Wiley.
- Robinson EH, Rawles SD, Oldenburg PW, and Stickney RR (1984) Effects of feeding glandless or glanded cottonseed products and gossypol to Tilapia aurea. *Aquaculture* 38(2): 145–154.
- Ryan JR, Kratzer FH, Grau CR, and Vohra P (1986) Glandless cottonseed meal for laying and breeding hens and broiler chicks. *Poultary Science* 65(5): 949–955.
- Simmons RG and Golightly NH (1981) Cottonseed Cookery. College Station, TX: Food Protein R&D Center, Texas A&M University.
- Silva-Ramos LC and Kohel RJ (1987) Seed-oil content of glanded and glandless cottons. *Journal of the American Oil Chemists' Society* 64(9): 1337–1340.

# **Relevant Websites**

http://www.cottonseed.com — National Cottonseed Products Association (NCPA) is a trade association for the cottonseed processing industry. Besides commercial information, it has further links to technical information on cottonseed products.

# CULTURAL DIFFERENCES IN PROCESSING AND CONSUMPTION

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Most cultures use a cereal grain as a staple. These staples have a myriad of uses throughout the day from appetizers to desserts. In some cases they are eaten alone and may be the main course of the meal. When eaten in combination with other foods, they can remain the main course or can act as carriers, as bread in sandwiches, noodles in pad thai or rice in pilafs, congees, or risottos. Some cereal-based foods are common to all cultures — a flat or griddle-cooked bread; pastas, noodles, or dumplings; gruels and porridges; snacks, biscuit (cookie), or crackers. While there is commonality, there can also be great differences imparted by the base grain and the added ingredients.

Even with the same base grain a number of factors affect the nutritional composition. Even within the same cultivar factors such as climate, soil, storage time and conditions, processing, and maturity at harvest affect the nutritional value. Grains may be minimally processed or highly pulverized and/or treated by intense heat. They may undergo germination or fermentation processes that either increase or decrease their nutritional content. Other ingredients may increase or decrease their nutritive contribution. The frequency with which the foods are eaten, the accompanying foods, and the occasion all have an impact on overall diet quality.

The actual nutritional impact depends on the nutritional status of the population, their reliance on a particular grain as a staple, and particular processing methods in that country. This article discusses the place of grains in the diet, the specific grain, and whether the cultural practice of consumption of grains, either eaten customarily as whole grain or refined, can have a significant impact on the diet.

# Cultural Differences in Processing and Consumption – Energy Requirements

Cereals can provide well over 50% of both energy and protein in parts of the developing world. In the developed world, particularly the USA, cereals provide a much smaller percentage of energy and are

not relied on for protein except in subgroups such as vegetarians.

Highly refined grain offers more available carbohydrate and slightly more calories than unrefined grains. Furthermore, antinutrients present in the outer layers of grain may inhibit amylases and proteases diminishing the total number of calories available. This can be a particular problem in areas where sorghum is a staple. In countries where calories are in short supply, refined grains may be beneficial. In developed countries where obesity threatens, the inclusion of whole grains with their slightly less available carbohydrate may be advantageous.

# Cultural Differences in Processing and Consumption — Protein

In terms of protein, each cereal offers distinct nutritional characteristics. These become important only if the staple is relied on as a major protein source. For example, oats offer a higher protein content than other grains and an amino acid pattern closer to the FAO standard than other grains. Historically, the high quality of protein from oats was once important in the UK and in parts of northern Europe. Similarly the high protein content of pseudocereal buckwheat provided important protein nutriture in parts of the former Soviet Union.

In most Western countries, protein quality and quantity is not an issue so cultural practices have little impact. However, for parts of the Indian subcontinent, Asia, and Africa, processing of cereals such as milling, fermentation, and cooking have important nutritional impacts. Milling may make protein more available and fermentation may improve biological availability of essential amino acids.

Fermentation and germination are important for improving the nutritional quality of grains. Fermentation, together with cooking and de-hulling, reduces the tannin of sorghum and millet. Since tannins complex with protein, they inactivate digestive enzymes and reduce protein digestibility. The presence of tannins in food can therefore depress growth and increase protein loss. Germination not only increases the synthesis of lysine and tryptophan, it also improves digestibility.

Cereals are incomplete proteins and have lysine as their most limiting amino acid. Corn protein is also limiting in the essential amino acid tryptophan, while other cereals are often limiting in threonine. Throughout the world, the practice of combining cereals with pulses makes a complete protein, so-called protein complementarity. In reciprocal fashion cereal grains contribute methionine, which is deficient in legumes. A survey of national dishes from around the world shows the many interesting and unique ways that different cultures have chosen to complement the protein. These time-honored national dishes are popular for vegetarians around the world. Some of them are listed below:

- India/Sri Lanka "rice pilaf," "dhal" (legume);
- India/Sri Lanka "chapati," "dhal";
- Middle East "pita" bread, "Hummus" ("garbonzo" bean/"tahini spread"); and
- China "congee" (rice gruel), small amounts of meat, tofu, or fish.

# Cultural Differences in Processing and Consumption – Carbohydrates Including Dietary Fiber

# Starch and Sugars

Many processing and cultural factors have nutritional impacts on sugars and starches. Carbohydrate energy provided in large part by the starch may be more available when the bran and germ portions are removed or if people from a particular culture regularly eat very finely pulverized grain or products such as broken rice. This is important in areas where calories are in short supply.

Germination and fermentation of grains have two impacts on carbohydrates: (1) improved starch digestibility as amylases partially degrade starch and (2) decreased flatus as some indigestible sugars degrade.

The glycemic response of grain-based foods is affected by processing because accessibility of the starch to digestive enzymes is changed. Inclusion of low glycemic index foods has been shown in some studies to positively influence blood sugar control, blood lipids, and other chronic diseases.

Cultural practices with regard to processing affect the glycemic index. For instance, wheat made into porous bread is easily penetrated by the digestive enzymes and has a high glycemic index. Bread made by the sourdough process reduces glycemic index because of the pH lowering. Wheat as an *el dente* pasta has a still lower glycemic index because the compact structure is less accessible to digestive enzymes.

For rice, varietal preference as well as processing affects the glycemic response. Medium-to high-amylose

rices, such as "basmati" preferred in India, have lower glycemic indexes. Glutinous rice used in Asia contains as much as 100% amylopectin and has a high glycemic index. Processing, such as parboiling to make converted rice and noodle making, tends to reduce the glycemic index.

Chemically modified starches which are used in many processed foods in developed countries have low glycemic responses. However, waxy cornstarch, which is 100% amylopectin, used in canned and frozen foods has a high glycemic index.

Processing can also impact whether or not a starch is digested and absorbed, e.g., forms of resistant starch. Grains which are minimally processed have slowly released carbohydrates and also show lower glycemic responses. High-temperature extrusion to make products such as corn flakes increases the amount of resistant starch. Resistant starch behaves nutritionally like dietary fiber, e.g., it is not absorbed in the small intestine and is fermented in the large intestine. More information is needed on the effects of various processes on the amount of resistant starch formed.

#### **Fiber**

Lack of adequate fiber has been associated with decreased gut motility and increased risks of heart disease, diabetes, and some types of cancer. For diets in developed countries, intakes of whole grains are low as are the extractions typically used for making flour-based products.

The degree of milling, or extraction rate, has a nutritional impact. Higher extraction rates are used in countries such as Norway, which regularly uses 80% extraction. The net result is a diet that has a higher dietary fiber and phytochemical intake from grains than when a low extraction rate is used, such as in the USA.

The cultural practice of including whole grains also impacts fiber nutriture. In Finland, regular use of breads and other products made from rye, oats, and barley and more frequent use of whole grains contributes to higher dietary fiber intakes than other European nations. In Mexico, traditional diets with high intake of whole meal corn tortillas and beans result in very high fiber intakes.

The grains which are frequently chosen in a culture also affects nutriture as different grains offer different fiber components, each with its own unique physiological characteristics. For instance, Germans and Scandinavians often choose rye. It contains a relatively high content of pentosan, a fiber with an arabinoxylan backbone containing small amounts of glucose and ferulic acid. Wheat, more frequently used in the

US and southern Europe, contains fewer pentosans than rye. Northern Europeans also use barley and oats. Their  $\beta$ -glucans are of interest because of their ability to lower serum cholesterol and attenuate blood glucose. Therefore, the consumption of these grains and brans from these grains is encouraged for heart patients and diabetics.

In countries of Africa exceedingly high levels of dietary fiber, especially fibers from sorghum with its high tannin content, have negatively impacted mineral nutriture. Inclusion of some types of hull material can irritate the bowel. Oat hulls, for example, have razor sharp pieces that can actually cut gut tissue.

# **Cultural Differences in Processing and Consumption** — **Micronutrients**

Most vitamins, minerals, and phytochemicals reside in the bran and germ. Thus, the cultural practice around the amount of the diet consumed as whole grain and the degree of milling affects the micronutrient contribution of grains. When the cultural practice is to consume more whole grains, the amount of micronutrient ingested goes up. However, ingestion of micronutrients does not always equal absorption. Tightly bound microcomponents may act locally on the gut but may not be absorbed. Milling reduces the level of all micronutrients but may release some and make them more absorbable. For strongly bitter grains such as sorghum, milling confers consumer acceptability. Over-milling or a high degree of extraction removes the aleurone layers and germ-rich vitamins and minerals and other phytochemicals.

Cultural processing practices can affect the availability of micronutrients. For example, without heat treatment of rice bran, lipases will destroy essential fatty acids, fat-soluble vitamins, and antioxidants in the bran. However, severe heat treatments may destroy vitamins, bind nutrients, or even initiate rancidity, which could decrease fat-soluble vitamins and produce free radicals that could have adverse effects on a number of vitamins. Liming of corn releases its bound niacin.

Several processes such as enrichment and fortification are used in many countries replacing vitamins and minerals lost during milling. Niacin enrichment of corn and flour helped eliminate pellagra in southeastern US. Folate enrichment of grain products has reduced birth defects such as spina bifida.

Enrichment of rice by adding a vitamin-rich talc has made an important nutrient contribution. However, enrichment's effect may be nullified due to certain cultural practices such as washing rice prior to cooking or cooking in large amounts of water, which is discarded. In areas where food availability is limited, the actual grain chosen and whether the grain is enriched can be critical. For example, in areas selecting unenriched milled rice as a staple, peripheral neuropathy and other symptoms indicative of early stages of beriberi may be present in the population. Such symptoms are seen in parts of Indonesia, where steamed white rice is used and few other thiamine-containing foods are eaten.

Fermentation and malting can change nutrient quality. Vitamin B often increases with fermentation, although the actual amount depends on the starting grain and the particular process. For example, in the preparation of "kaffir" beer (from maize), thiamine levels are virtually unchanged, but riboflavin and niacin contents almost double. The malting or germination of grains and pulses as practiced in certain parts of the world such as India increases vitamin C and phosphorus availability.

Cultural practices surrounding grain choice and processing can affect mineral status. Tannins, phytates, and other ligands in grain bind iron, zinc, and other metals thereby contributing to mineral deficiencies. High concentrations of tannins and cyanogenic compounds in grains such as sorghum can adversely affect the thyroid and contribute to the incidence of goiter. Milling removes much of the tannin and phytate or pulverizes the grain, making absorption of some minerals more likely.

Fermentation can affect phytic acid and hence mineral status. Thus, cultural practices around fermentation affect the amount of time the phytase enzyme is allowed to degrade phytate with longer fermentations improving mineral status. For example, in the early 1970s it was noted that many adolescent males in poor, rural parts of the Middle East failed to mature sexually (a syndrome called hypogonadal dwarfism). In this case the diet was comprised of nearly 85% whole-wheat pocket bread. The short fermentation coupled with high-temperature baking failed to allow enough phytase reaction; so the zinc remained tightly bound by the phytate and unavailable to the young boys.

# Cultural Differences in Processing and Consumption — Toxic Components

In addition to tannins and phytates, cereals and other plant foods may contain significant amounts of toxic or antinutritional substances. Milling removes a number of these:

 Protease and amylase inhibitors occur widely in cereal bran, which is lost during milling. They may be further reduced during fermentation but are not greatly affected by heat.

- Saponins, noted for their hemolytic activity, can cause growth inhibition. Interestingly, recent evidence indicates that these compounds may have some anticancer activity in the colon.
- Mold toxins found in all parts of the grain may be partially removed. Further reduction of mold toxins such as aflatoxin B1 occurs during fermentation because the lactone ring is opened.

# **Cultural Choice of Specific Grains and Their Effect on Nutriture**

The remainder of the article will focus on each grain, how it is processed and consumed and what nutritional implications result from the selection, processing, and preferred methods of consumption.

#### Rice

Rice is used throughout the world in diverse ways from Chinese stir-frys, Spanish "paellas," Japanese "sushi," Indian "biryanis," Thai "salads," Turkish "pilafs," Italian "risottos," Senegalese "yassas," Norwegian rice pudding to American "gumbos." Many of these dishes use white rice as the basis for important nutrients and energy, but rely on the other ingredients to contribute more nutrients and phytochemicals to the total diet.

Colored rice, such as black rice ("wehini"), used by Asian cultures have more antioxidants and nutrients than some other varieties. Some varieties are said to have medicinal properties such as a "body-strengthening" fraction or "nerve rejuvenating" function in paralytic conditions.

Wild rice, technically not a true rice, is comparable in nutritional quality to oats. It is almost always eaten as a whole grain so there is very little nutritional loss from processing wild rice.

Rice and wild rice are often the grain of choice for those allergic to wheat or those with gluten enteropathy (celiac sprue) where sufferers must avoid wheat and its relatives, rye, barley, and any grains that might have been contaminated by being processed on the same line as any of the aforementioned grains.

Broken rice During the milling of rice some grains are broken, or "damaged." These broken pieces are separated from the intact kernels. A grain of broken rice retains its high energy content but has a higher glycemic index than unbroken rice because the gut enzymes have ready access to the starch. In parts of Africa, broken rice is preferred because of its cheaper import price and fast uniform cooking. Broken rice is also used to make rice flour and rice noodles.

Broken rice can be processed to improve nutrient intakes. Vitamin A deficiency in children is common in many countries where the staple is rice. Broken rice grains can be made into a dough fortified with vitamin A and then reconstituted into rice-shaped kernels that have the same taste and texture as regular rice. Because the majority of the added nutrient is inside the shaped kernel, it is protected from oxidation, washing, and cooking. This technology also holds promise for other fortifiers such as iron, folic acid, and other micronutrients not present in normal rice.

The development of golden rice using biotechnology to add vitamin A is another process with potential. Addition of vitamin A is one tool to help prevent the WHO-estimated 250 000 to 500 000 cases of blindness due to vitamin A deficiency in children annually.

Rice is also made into processed food. It is puffed for use as breakfast cereal and rice cakes. They are popular with dieters in developed countries because of their low caloric value, but they tend to have a higher glycemic index than some other forms of rice. Those trying to avoid wheat also enjoy these. In Asian countries, rice-based snack foods are popular. In Japan, they account for  $\sim 10\%$  of total rice consumption. Other Asian countries eat processed rice products, primarily noodles. In terms of nutrition, the glycemic index may change and thiamine may be unaffected or completely destroyed depending on the temperature used in the process.

#### **Wheat and Its Parent Grains**

Wheat is important in most Western cultures and is basic to most breads, rolls, crackers, cookies, biscuits, cakes, doughnuts, muffins, pancakes, waffles, noodles, pie crusts, ice cream cones, macaroni, spaghetti, puddings, pizza, and hot, and cold breakfast foods. It is also used in baby foods, and is a common thickener in soups, gravies, and sauces. Germ, bran, bulgur, and malt are additional types of wheat products.

The largest nutritional impact is determined by the amount of whole wheat and refined wheat utilized and whether the refined grain is enriched or fortified. The glycemic index of wheat products can also be affected by the process and the end product. For example, bread from a sourdough method reduces the glycemic index over ordinary bread. Addition of grain particulates also slows starch digestion.

Processing and cooking methods affect the nutrition of products. Here are some examples:

 precooking and parching of bulgur before cracking, not only enables its quick cooking, it reduces the phytates;

- steaming of breads and dumplings, widely practiced in parts of Asia, minimizes Maillard browning products and heat-produced compounds such as acrylamide;
- steaming of fermented flat breads such as "injera" from Ethiopia slightly increases the availability of lysine; and
- frying of wheat noodles to make "ramen" and "chow mein" noodles increases the fat and saturated fat content.

Spelt, kamut, and teff Most of the nutritional qualities of spelt and kamut are the same as their wheat parents and processing has the same impacts. However, the protein content of spelt was consistently higher (18–40%) than that of the hard red wheat; however, lysine content was lower. The use of spelt and kamut is common in some African populations. It is very limited in the USA although some in the health-conscious market are trying to bring it back.

Teff is used where it is grown in East Africa for the slightly spongy, slightly sour griddle bread called injera. Injera comprises approximately two-thirds of the diet in Ethiopia and Eritrea. Teff has very high calcium content, and contains high levels of phosphorus, iron, copper, aluminum, barium, and thiamine.

# Sorghum and Millet

Sorghum and millets are used as staples in parts of Africa and Asia. In some African countries, 45% of the total annual calorie intake from cereals comes from these gluten-free grains. Both millet and sorghum have tannins in the outer layers that can inhibit some protein utilization, but some sorghums contain amounts that can negatively impact nutrition status of those heavily relying on this staple. Removal of the outer layers by an abrasive mill increases storage, acceptability, and nutritional profile. However, the best nutritional quality occurs if only the outermost bran layers are removed retaining the germ and the aleurone layers intact.

Processing sorghum prior to use improves acceptability and digestibility. Soaking and cooking, especially in an acid medium, also aids cooking since the starch in these grains is slow to hydrate. Both processes improve digestibility. Other processes such as flaking, precooking, steaming, roasting, popping, or puffing making into "roti" or chapati improve the character of starch. The heat treatment not only imparts improved starch digestibility, it also contributes a highly crunchy texture and desirable flavor. Both the whole and polished grains and flours are used.

"Millet" is prized for its protein content, but not all varieties offer the same nutritional advantages. In India and parts of Africa, these differences may have dietary significance.

Pretreated millet grains are used for development of weaning and supplementary foods for school and preschool children. The pretreatment improves nutrition.

# **Barley**

Barley has been used as human food since Etruscan times as a porridge. It is still used by many cultures and has remained an ingredient in soups, particularly those from Scotland and Eastern Europe. Patent barley is used as a commercial thickener and as baby cereal. In many cultures wheat or other staples have replaced barley but its use remains in other cultures. For example, Tibetan monks prepare "tsampa," a porridge made with toasted barley and blended with yak butter and tea.

The rich nutrient contribution of barley fails to help most human populations because it is used much more often in animal feeding. There has been some effort to increase its contribution to Western diets because its high soluble fiber content lowers serum cholesterol and helps control blood glucose. In this case pearling has less impact than milling of other grains as the important  $\beta$ -glucan is distributed throughout the grain.

#### **Oats**

Oats is still widely used in Ireland and Scotland, Finland, and parts of North America. Hot and cold breakfast cereals with oats as a base form well over three-fourths of the uses. The remaining 15% are used as oat flour or in snack products such as granola bars, breads, muffins, and cookie mixes.

The milling of the oats into steel-cut oats, rolled flakes, quick and instant flakes, oat flour, and oat bran can affect the nutritional contribution of this cereal. The size of piece and the degree of processing can all impact the glycemic index of the food. For example, instant oats have a much higher glycemic index than steel-cut oats. Using the bran alone can concentrate the cholesterol lowering  $\beta$ -glucan making it easier for physiologically important levels to be eaten in a normal serving of food.

In Western countries, interest in oat products has increased recently because of their documented health properties including cholesterol lowering of the  $\beta$ -glucan, important phytochemicals, and plant estrogens. This has led to the increased introduction of a number of oat cereals and breakfast bars.

#### Rye

Rye (Secale cereale L.) is grown and used primarily in northern climates. It was central to peasant diets as breads in these regions until the eighteenth century and is still used in, for example, Scandinavia, especially Finland, Russia, Germany, Poland, Ukraine, Belarus, and other countries of the former Soviet Union, where traditional rye sourdough and pumpernickel breads were principally used as the staple. However, the actual amount of rye eaten has declined in many countries. Health movements in parts of Scandinavia have rekindled interest in rye flakes and other breakfast cereals and rye products. Rye crispbread continues to be popular.

Processing has an impact on the nutritional offerings of rye. Rye breads produced in northern Europe are usually whole grain or use a high rye flour extraction rate. In contrast, in the USA most rye bread uses a low extraction rate and molasses or other coloring agents. So there is great difference in the fiber and sterol contribution from rye in various countries. Only the higher extraction and whole grain breads will harvest some of the benefits of rye including:

- a feeling of fullness and satiety,
- protection of the cells of the colon,
- binding of bile acids to aid in lowering cholesterol,
- control of blood sugar,
- improving bowel function, and
- provision of lignans with their phytoestrogen activity.

#### Maize

Maize is a staple in parts of Central America, Africa, India, and China. It is either used alone or in combination with other foodstuffs. For example, in Indonesia, corn is consumed as a primary staple food for at least major parts of the year before the main rice harvest. In other parts of Asia maize is combined with rice or millet, taro, sweet potato, and wheat. In Africa, maize is combined with millet, sorghum, yams, and sweet potato. Eastern Africa's "ugali" and southern Africa's "mealie-meal," "nshima," and "sadza" are usually made from maize.

Maize continues to be important in the Americas and processing can have significant nutritional impacts. When corn is placed in a solution with wood ash and boiled, the product is hominy or, in Mexico, "pozole." It may also be dried and processed into various products such as grits or "masa," which ultimately end up in gruel or as a meal for such products like corn tortillas. This alkaline process destroys vitamin B, particularly thiamine.

Some corn is dry-milled and used to produce breakfast cereal and snack foods; corn meal is used in corn bread, hush puppies, and pancake, and bakery mixes; and masa flours form the basis of corn chips, tostadas, taco shells, tortilla chips, and other Mexican foods. The predominant form of corn meal is de-germed and so it does not offer the benefits of whole grain, whereas masa for Mexican products and chips is whole grain.

In 20 developing countries, primarily in Latin America and Africa, maize gruel is the main food mothers use to wean their babies, and maize is the single largest source of calories. But babies who subsist on maize can face a dangerous lack of protein during a critical stage of physical and mental development. The problem is that diets high in maize lack two essential amino acids needed to prevent malnutrition. Here processing can be important. New varieties have been developed that retain the taste of normal maize, but the nutritive value of their protein is nearly equivalent to cow's milk.

## **Unusual Grains**

Buckwheat is from seeds of a broad-leafed plant rather than a grass. It is popular in Eastern Europe and parts of the former Soviet Union. This whole grain product is sold both raw and toasted and cooked into a pilaf-like dish (called "kasha"). Buckwheat is rich in vitamin B, minerals, and the amino acid lysine. The toasted form is more subject to rancidity. It is processed into "soba" noodles in Japan.

Quinoa has the highest protein content of any cereal grain (20%) and is usually eaten as whole grain. Amaranth is also very high in protein and lysine and offers iron, calcium, and vitamin E.

## **Conclusions**

The role of grains as a source of carbohydrate, energy, dietary fiber, protein, essential fatty acids, vitamin B, and minerals varies depending on the ratio of unrefined and refined grain in the diet, the degree and method of milling and the actual grain used.

Methods that can be employed to improve the nutritive value of cereals include traditional genetic selection, genetic engineering, amino acid and other nutrient fortification, complementation with other proteins (notably legumes), milling, heating, soaking, germination, and fermentation.

The grain which is chosen as the staple, how it is used, what other foods are customarily consumed with it all affect human nutrition. Furthermore within any type of grain, the cultivar or variety can impact nutrition. More research is needed on the effects of

a number of processes on how the macro- and micronutrient and the phytochemical content are altered by processing.

See also: Cereals: Chemistry of Nonstarch Polysaccharides.

## **Further Reading**

- Baublis AJ, Lu C, Clydesdale FM, and Decker EA (2000) Potential of wheat-based breakfast cereals as a source of dietary antioxidants. *Journal of American College of Nutrition* 19(3 suppl.): 308S-311S.
- Fast RB and Caldwell EF (eds.) (2000) Breakfast Cereals and How They are Made, 2nd edn. Eagan, MN: AACC Press
- Jackson LS and Bullerman LB (1999) Effect of processing on Fusarium mycotoxins. Advances in Experimental Biology and Medicine 459: 243–261.
- Kerckhoffs DA, Brouns F, Hornstra G, and Mensink RP (2002) Effects on the human serum lipoprotein profile of beta-glucan, soy protein and isoflavones, plant sterols and stanols, garlic and tocotrienols. *Journal of Nutrition* 132: 2494–2505.
- Lampe JW (2003) Isoflavonoid and lignan phytoestrogens as dietary biomarkers. *Journal of Nutrition* 133(suppl. 3): 956S–964S.

- MacGregor AW and Bhatty RS (eds.) (1993) Barley, Chemistry and Technology. Eagan MN: AACC Press.
- Pamela JW and Lawrence AJ (eds.) (2003) Corn: Chemistry and Technology, 2nd edn. Eagan MN: AACC Press.
- Shayo NB, Laswai HS, Tiisekwa BP, Nnko SA, Gidamis AB, and Njoki P (2001) Evaluation of nutritive value and functional qualities of sorghum subjected to different traditional processing methods. *International Journal of Vitamin and Nutritional Research* 52: 117–126.
- Taylor JR and Dewar J (2001) Developments in sorghum food technologies. *Advances in Food and Nutrition Research* 43: 217–264.
- Thompson T (2003) Oats and the gluten-free diet. *Journal* of the American Dietetics Association 103: 376–379.

## **Relevant Websites**

http://www.wheatfoods.org.

http://hsc.csu.edu.au/food technology/nutrition. http://wbc.agr.state.mt.us/reference/nutrition. http://www.barleyfoods.org. http://www.grdc.com.au/consumers/main.htm. http://www.homebaking.org/hbamembers. http://www.kswheat.com.



## **ENZYME ACTIVITIES**

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## Introduction

Enzymes are biological catalysts that direct complex biochemical reactions at temperatures relevant to living organisms and the environments in which they exist. The proteinaceous nature of these molecules allows for a myriad of three-dimensional structures that will accommodate different substrate specificities, and respond to the presence of other "regulatory" molecules, changes in ionic environment, pH, temperature, and hydrophobicity. These interactions often result in alteration to the conformation of the enzyme protein that in turn impacts on binding of substrates, activators, inhibitors, and cofactors, or the efficiency of the catalytic activity. In addition to the mechanisms for controlling enzyme activity, the actual amount of enzyme protein in a cell can be controlled via a balance of synthesis and degradation, thus allowing for many tiers of regulatory control.

Cells in cereal grains contain a multitude of enzymes that are common to all living cells, including animals and microorganisms, albeit with minor variations in structure and specificity depending on the species. These enzymes, which are essential for general cellular metabolism and function, will not be discussed here and the reader is referred to any of the numerous plant biochemistry and general biochemistry texts that are readily available. Rather, this article focuses on some of the enzymes that are critical to the specific functions and structures of cereal grains, or those that influence the end use of cereal grains or the quality of cereal end products. One of the major functions of cereal grains is to synthesize and sequester starch, protein, and lipid in the endosperm tissue, which can subsequently be mobilized in the germinated grain to support growth of the developing seedling. The enzymes involved in the biosynthesis of these stored reserves are discussed in Lipid Chemistry. Protein Synthesis and Deposition. Starch: Synthesis. In this article, discussion of enzyme activities in cereal grains will be presented in the context of: synthesis and degradation of endosperm cell walls in relation to the specific functions of this specialized storage tissue; hydrolysis of stored endosperm reserves such as starch during germination and how in barley this has been utilized to advantage in the malting and brewing industries, whilst in wheat the same process presents a challenge to processing if the enzymes involved are synthesized prior to harvest; and finally, enzymes that are synthesized during grain development and retain activity in the mature grain where they influence quality, appearance, or color of end products such as noodles and pasta and storage quality in rice. Brief discussions of enzymes that are active in ripe, quiescent grains, and endogenous enzyme inhibitors will complete the article.

## Enzymes Involved in the Synthesis and Degradation of Endosperm and Aleurone Cell Walls

## **Cell Walls in Cereal Endosperm**

Cell walls are central to the growth and development of the endosperm of cereal grains and the remobilization of the stored reserves during germination. They are also important contributors to many quality characteristics of cereal grains. The enzymes responsible for the synthesis and degradation of cell-wall polysaccharides are therefore critical not only to the normal biological functions of the endosperm but also to the subsequent utilization of cereals by humans. The endosperm consists of two distinct tissues, the aleurone and the starchy endosperm, that have different roles. The starchy endosperm forms the bulk of the grain. It is the storage organ for starch, protein, and some lipids, and by the time grains are ripe and ready for harvest, it is a nonliving tissue incapable of synthesizing new enzymes. By contrast, the aleurone is the layer of cells that surrounds the starchy endosperm and responds to signals from the germinating

Table 1 Composition of cell walls in cereal endosperm

Cereal	Source of walls	Major polysaccharides
Barley	Aleurone	71% arabinoxylan 26% (1,3;1,4)-β-glucan 2% cellulose 2% glucomannan
	Starchy endosperm	75% (1,3;1,4)-β-glucan 20% arabinoxylan 2% cellulose 2% glucomannan
Wheat	Aleurone	65% arabinoxylan 29% (1,3;1,4)-β-glucan 2% glucomannan 2% cellulose
	Starchy endosperm	70% arabinoxylan 20% (1,3;1,4)-β-glucan 7% glucomannan 4% cellulose
Rice	Starchy endosperm	27% arabinoxylan 20% (1,3;1,4)-β-glucan 28% cellulose 3% pectin 15% xyloglucan/mannans

embryo to synthesize and secrete the key hydrolytic enzymes required to initiate degradation of the stored endosperm reserves. The aleurone remains a living tissue throughout development and long periods of storage and also retains some integrity during the early stages of germination to enable the programmed dissolution of the endosperm to proceed. Subtle variations in the fine structures of major wall polysaccharides can have a dramatic effect on the functional and rheological properties of cereal-based products. The major wall components in the endosperm of cereal grains are arabinoxylans and (1,3;1,4)- $\beta$ -glucans; other wall components include cellulose, protein, other minor polysaccharides, and some phenolic acids, chiefly ferulic acid (Table 1). Endosperm walls generally lack lignin. Although cell-wall components account for only 5-10% by weight of the grain, they can have a disproportionately large impact on grain technology, utilization, and nutrition.

## Biosynthesis of Cell-Wall Polysaccharides in Cereals

Given the central role played by cell-wall polysaccharides in cereal processing, there has been considerable interest in the enzymes that mediate their biosynthesis, particularly in the developing grain. Cellularization of the starchy endosperm early in grain development involves "atypical" growth of cell walls around individual nuclei in a multinucleate syncytium. The fertilized endosperm mother cell divides for  $\sim$ 70 h to form up to 2000 free, individual nuclei in the cytoplasm of the central cell. Walls subsequently grow between the free nuclei. After a lag phase, further expansion of the cellular endosperm occurs through meristematic activity of peripheral cells that eventually differentiate to form the aleurone. In barley, the major components of the starchy endosperm walls, (1,3;1,4)- $\beta$ -glucan and arabinoxylan, accumulate steadily between 13 and  $\sim$ 40 days postanthesis (dpa).

Despite the importance of cell-wall biosynthesis as a determinant of final cereal grain quality, polysaccharide synthases involved in wall synthesis have proved very difficult to purify by conventional biochemical procedures, mainly because they are membrane-bound enzymes and can be rapidly inactivated in vitro. A key breakthrough in this area occurred when cellulose synthase genes (CesA) were identified in higher plants; subsequently multiple CesA genes and multiple groups of cellulose synthase-like (Cs1) genes have been identified. It is highly likely that the genes encoding (1,3;1,4)- $\beta$ -glucan synthase and xylan synthase in cereals will be found in the cellulose synthase or cellulose synthase-like gene families, given the structural similarities between cellulose and both the (1,3;1,4)- $\beta$ -glucans and the xylan backbone of arabinoxylans.

## **Enzymic Hydrolysis of Wall Polysaccharides in Germinated Grain**

Most of the enzymes involved in (1,3;1,4)- $\beta$ -glucan and arabinoxylan degradation in the endosperm of germinated grain have been characterized in detail, particularly in barley, wheat, and maize. In each case, a battery of hydrolytic enzymes is required to completely depolymerize the polysaccharides. The enzymes responsible are generally synthesized de novo in the aleurone layer or the scutellum, and subsequently secreted into the starchy endosperm. Walls of the starchy endosperm are completely degraded during this process, but a thin inner layer of the aleurone cell walls appears to resist degradation, presumably to maintain the structural integrity of the aleurone cells while enzyme secretion is in progress.

The (1,3;1,4)- $\beta$ -glucans of endosperm cell walls are depolymerized by (1,3;1,4)- $\beta$ -glucan endohydrolases. The (1,3;1,4)- $\beta$ -glucan endohydrolases release lowmolecular-mass (1,3;1,4)- $\beta$ -oligoglucoside products that can be further hydrolyzed by exohydrolases such as  $\beta$ -glucan glucohydrolases and  $\beta$ -glucosidases. A second, distinct class of (1,3;1,4)- $\beta$ -glucan endohydrolase has been described in germinated barley and

in maize coleoptiles. The enzymes release (1,3;1,4)- $\beta$ -glucans with degrees of polymerization of 60-100, but the enzymes have not been characterized in detail. Their action patterns are consistent with a cellulase, but there remains some doubt as to their precise role in (1,3;1,4)- $\beta$ -glucan depolymerization in germinated grain.

Arabinoxylan depolymerization in germinated cereal grains also requires the concerted action of a range of different hydrolytic enzymes.  $(1,4)-\beta$ -Xylan endohydrolases hydrolyze glycosidic linkages in the (1,4)- $\beta$ -xylan backbone of the polysaccharide, and probably require a short, unsubstituted region of the (1,4)- $\beta$ -xylan chain for activity. Genes and cDNAs encoding barley (1,4)- $\beta$ -xylan endohydrolases have been isolated, and used to define the expression patterns and chromosome locations of the genes. There is another class of enzymes that can hydrolyze polymeric arabinoxylans from cereal cell walls. These enzymes hydrolytically remove single α-arabinofuranosyl substituents from the (1,4)- $\beta$ -xylan backbone of the polysaccharide and have been designated arabinoxylan arabinofuranohydrolases. They are probably responsible for the removal of arabinofuranosyl residues prior to (1,4)- $\beta$ -xylan endohydrolase action in germinated grain, but might also be involved in the modification of arabinoxylan fine structure during wall deposition, maturation, or expansion. Complete depolymerization of oligoarabinoxylosides released from cell-wall arabinoxylans by  $(1,4)-\beta$ xylan endohydrolase requires the action of  $\alpha$ -arabinofuranosidases and  $\beta$ -xylosidases, both of which have been detected in germinated cereal grains.

# Role of Enzymes in Malting and Brewing of Barley

Malt is used to give a distinctive flavor and color to a range of foods and beverages and whilst it can be prepared from a range of cereals, barley is the most common starting material. By far, the most widespread use of malt is as a source of fermentable sugars for the manufacture of beer and whisky. Malting equates to germination carried out under controlled conditions that are designed to synchronize the sequence of events and yield a uniform and consistent product. Production of malt has been discussed in detail in Barley: Malting, and involves steeping in water, germination or modification, and kilning. Steeping is designed to achieve sufficient grain moisture to initiate processes in the embryonic and aleurone tissues that lead to the synthesis and transport of hydrolytic enzymes into the starchy endosperm. During

germination or modification, the cell walls of the starchy endosperm and the protein matrix are degraded, leaving the starch exposed. Finally, the germinated grain must be dried, or "kilned," to reduce moisture to <5%, to stabilize the malt for storage and transport, and to generate a characteristic color, whilst carefully avoiding denaturation of enzymes that will be needed to degrade malt starch to fermentable sugars. In the early stages of the brewing process, the dried malt is milled, "mashed" in hot water for the degradation of starch to sugars, and an extract containing the sugars recovered by filtration.

# **Enzymes Released from the Aleurone during Modification**

Starchy endosperm cell walls are broken down by the combined action of enzymes discussed in the previous section. The keys to this process in barley are the (1,3;1,4)- $\beta$ -glucan endohydrolases since (1,3;1,4)- $\beta$ glucans represent up to 75% of barley endosperm cell walls and there is little or no glucanase activity in ungerminated barley grain. Similarly, disruption of the protein matrix that surrounds starch granules is initiated by endopeptidases (proteinases) that are synthesized *de novo* in the aleurone during germination. This group of enzymes degrades large storage proteins into smaller peptides that are susceptible to attack by exopeptidases, in particular carboxypeptidases, which are already present in the endosperm of ungerminated barley. During modification, as much as 50% of the storage protein may be mobilized. The remaining storage material, starch, is present as highly crystalline granules that can be attacked by  $\alpha$ -amylases, albeit very slowly at the temperatures used for germination. This enzyme is also synthesized de novo in the aleurone during germination and released into the starchy endosperm. Other enzymes that are present in ripe barley, or are synthesized during germination, and which can impact on malting and brewing include lipases, lipoxygenase, nucleases, peroxidases (PODs), phosphatases, and phytase.

#### **Kilning**

During kilning, the "green" malt is dried, whilst ensuring that appropriate enzymes retain activity and desirable flavors and colors develop. Drying is commenced using lower temperatures in order to protect the most heat-sensitive enzymes (glucan hydrolases, endopeptidases, limit dextrinase). As moisture is reduced, these enzymes appear to become more tolerant and temperatures can be increased. By comparison,  $\alpha$ -amylase is relatively stable at the temperatures used (up to  $75^{\circ}$ C) to kiln malt destined for use as a source of fermentable sugars. All enzymes are killed

in the more flavorsome, darker malts used in ale-type beer that are cured at 110°C. Kilning requires careful management, not only to retain the activity of important enzymes but also because these enzymes continue to act on substrates during kilning.

## Mashing

This process is an extension of malting during which milled malt is mixed with hot water to facilitate the degradation of starch, in particular, to oligosaccharides and glucose. Starch consists of amylose, linear chains of  $\alpha$ -(1,4) glucan, and amylopectin, a highly branched polymer which is composed of linear chains of  $\alpha$ -(1,4) glucan are interlinked via  $\alpha$ -(1,6) bonds. Starch hydrolysis proceeds very rapidly once the starch, present in cereal endosperm as highly crystalline granules, is solubilized or gelatinized and is initiated by α-amylase which catalyzes the hydrolysis of  $\alpha$ -(1,4) linkages in the interior of large starch molecules to release a mixture of small, linear, and larger branched dextrins. Small and linear dextrins are further hydrolyzed by  $\beta$ -amylase (already present in ungerminated barley) and probably  $\alpha$ -glucosidases, whilst limit dextrinase hydrolyses the  $\alpha$ -(1,6)-linkages in the branched dextrins releasing linear dextrins that can then be broken down by  $\alpha$ - and  $\beta$ -amylase. Limit dextrinase is of considerable interest since the branched limit dextrins containing  $\alpha$ -(1,4) and  $\alpha$ -(1,6)-linkages cannot be fermented by yeast during the brewing process. Synthesis of limit dextrinase lags some days behind synthesis of α-amylase during germination or malting of grains and represents a constraint to rapid and complete conversion of starch to fermentable sugars. This enzyme has consequently become the target of genetic and molecular manipulation in an attempt to further improve malting of barley.

## α-Amylases in Developing and Ripe Wheat Grains and their Impact on Processing

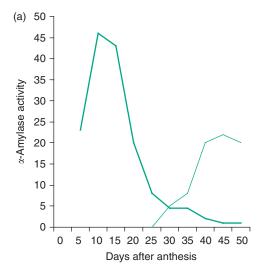
Starch constitutes up to 80% of the material in the endosperm of wheat grains and is stored in a highly crystalline form in granules that are laid down during development and ripening. α-Amylase is a critical enzyme in remobilization of the endosperm since it initiates hydrolysis of the starch granules, reducing the large, complex molecules of starch to smaller units as already described. Wheat grain tissues can synthesize two groups of  $\alpha$ -amylases, or  $\alpha$ -amylase isoenzymes, that differ in genetic control, temporal pattern of synthesis as well as physical and chemical properties. Perhaps the most important group in

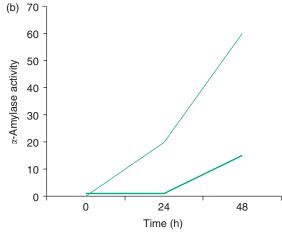
grains are the high pI group (germination or malt  $\alpha$ -amylases) that are products of the  $\alpha$ -Amy1 genes located on the group 6 chromosomes of wheat and that are normally only synthesized in the grains during the early stages of germination. The second group, referred to as low pI (pericarp or "green" α-amylases), are found in the pericarp of immature grains, at low levels in mature grains, and are also synthesized during the later stages of germination. The synthesis of the low pI isoenzymes is controlled by  $\alpha$ -Amy2 genes located on the group 7 chromosomes of wheat. In comparison with other cereal enzymes, the  $\alpha$ -amylases are relatively thermostable and remain active at temperatures above 60°C where the starch is gelatinized and becomes extremely sensitive to enzymic hydrolysis.

Under ideal growth conditions, ripe wheat grains contain a small amount of low pI α-amylase and no high pI α-amylase and, following milling, the flour can be used to produce a vast array of processed foods including pan breads, flat breads, noodles, cakes, and biscuits as well as industrial products. For leavened products such as western style pan breads, some  $\alpha$ -amylase is required to produce the fermentable sugars needed by the yeast. This  $\alpha$ -amylase, often microbial in origin, is normally added in measured amount during mixing particularly in the highly automated bread-making plants that require strict adherence to specifications to maintain quality and efficient production. Under nonideal growing conditions, or as a result of adverse genotype x environment interactions, variable amounts of either, or both, groups of  $\alpha$ -amylases may be retained or synthesized prior to harvest and as a consequence the grain may be unsuitable for processing. Wheat with unacceptably high levels of  $\alpha$ -amylase is commonly downgraded in quality, often to be restricted to animal feed, and attracts substantial financial dockages. Whilst some of the α-amylase is removed with the bran and germ during milling, at progressively higher levels of activity, more of the enzyme will have been transported into the endosperm and will be recovered in the flour. Excess levels of  $\alpha$ -amylase in wheat have a disastrous effect in long fermentation processes, which applies to most types of western style pan breads, and leads to sticky dough that is harder to handle, a sticky crumb, dark-colored crust and loaves with large holes that are difficult to slice and may jam slicing machines. During cooking or baking, the starch becomes increasingly susceptible to attack by α-amylase and even small amounts of enzyme can cause dramatic differences in starch viscosity and product texture. Flat breads, "chapatis," and sourdough breads are more tolerant to  $\alpha$ -amylase, whereas Japanese style ("Udon") noodles are very sensitive.

Irrespective of the end product, appearance and quality deteriorate as  $\alpha$ -amylase activity increases.

High levels of  $\alpha$ -amylase activity in ripe wheat grain can arise via a number of mechanisms. First, the low pI  $\alpha$ -amylase that is synthesized in the green (chlorophyllous) pericarp of immature grains may be retained rather than being gradually degraded as the grain matures. This takes place in parallel with the loss of starch granules from the chloroplasts in the immature seedcoat, as the seedcoat tissue desiccates and dies (Figure 1). Frost damage to partially





**Figure 1** Diagrammatic representation of *α*-amylase synthesis in wheat grains during development, ripening, and preharvest sprouting. Low pl *α*-amylase is synthesized in the pericarp during the early stages of grain development, then declines to very low levels at maturity in all genotypes (a), whilst new low pl *α*-amylase is synthesized by the aleurone later in grain germination or sprouting (b). High pl *α*-amylase is synthesized in the aleurone during later stages of grain development in LMA-prone cultivars (a) and in the aleurone during the early stages of germination of all cultivars (b). Low pl *α*-amylase (thick line), high pl *α*-amylase (thin line).

developed grains and conditions of high humidity and low-light intensity during ripening have been associated with this abnormal retention of enzymes. Second, a small number of wheat genotypes have a genetic defect referred to as late-maturity α-amylase (LMA) or prematurity α-amylase (PMAA) that is reflected in the synthesis of high pI  $\alpha$ -amylase in the aleurone tissue during the later stages of grain development (Figure 1). This may occur generally or be limited to situations where developing grains experience cool temperatures. LMA, unlike germination or sprouting, is not dependent on rain and does not appear to involve changes in, or signals from, the embryo. Finally, adverse weather conditions (rain accompanied by high humidity) at maturity and prior to harvest may trigger grains to commence germination whilst still in the spike. This phenomenon, known as preharvest sprouting (Figure 2), is relatively common in many parts of the world. The sequence of events that occurs during sprouting follows very closely those observed in isolated grain that is germinated under more controlled conditions. α-Amylase synthesis begins adjacent to the embryo or germ end of the grain and progresses in a wave towards the distal end. The system has been very widely studied by plant biochemists endeavoring to understand the intricacies of control of enzyme synthesis and the role of plant hormones. Initially, only high pI isoenzymes are synthesized, however, as germination progresses the low pI group are also produced. Whilst LMA and sprouting are readily distinguished both in genetic



Figure 2 Sprouted and nonsprouted spikes of wheat.

control and in the pattern of enzyme distribution within the grain, the  $\alpha$ -amylase isoenzymes involved are similar, as are the effects on the quality of end products.

## **Enzymes Involved in Determining the** Color and Appearance of Wheat End **Products such as Asian Noodles**

Large quantities of the wheat used in Southeast Asia, Japan, Korea, and China are consumed as noodles and increasingly these products are also finding a place on tables in western societies. As the markets for noodle products develop and become more quality-sensitive, greater emphasis has been placed on improving appearance, consistency of color, and color stability, because these characteristics strongly influence customer appeal. Asian noodles made from wheat flour come in a wide range of forms, although there are two basic recipes used for production. These are referred to as white salted noodles (WSN), made from flour, water, and varying amounts of salt, and yellow alkaline noodles (YAN), prepared using flour, water, and alkaline salts such as potassium and sodium carbonate. For both types, initial color should be clean and bright and there should be minimal darkening in the time between preparation and cooking. WSN vary from white to cream in color, whilst YAN have a yellow color that is often enhanced with artificial coloring agents or eggs.

## Synthesis of Flavonoids and Xanthophylls

The creamy color of WSN and a part of the yellow color of YAN is dependent on natural yellow pigments, xanthophylls (primarily lutein), present in the germ and endosperm of wheat grains. Lutein is a member of the C<sub>40</sub> terpenoids or carotenoid family of compounds that are found in all plants and are synthesized from isopentenyl phosphate via a biosynthetic pathway commencing with geranylgeranyl pyrophosphate synthase. The additional yellow color that develops in the presence of alkaline salts in YAN is attributed to flavonoid compounds such as flavone glycosides; these are found in the germ and seedcoat but not the endosperm. The starting point for synthesis of flavononoids is phenylalanine and the enzyme phenylalanine ammonia lyase, which leads into a biosynthetic pathway that produces a vast array of structures including flavones, cinnamic acids and lignins, anthocyanins, and tannins. Xanthophylls and flavonoids accumulate during the early to middle stages of grain development, depending on the activity of the enzymes in the respective biosynthetic pathways. In the case of xanthophylls, the levels decline markedly as the grain ripens. Lutein may also be converted to mono- and di-fatty acid esters during storage of ripe grain or flour. Degradation and esterification of lutein are almost certainly controlled by specific enzymes, but these have not been identified. The concentrations of xanthophylls and flavonoids in flour is a product not only of the balance of synthesis and degradation during grain development, but also the milling process, in particular the efficiency of separation of starchy endosperm from other grain tissues such as the germ and seedcoat.

## Lipoxygenase

Lipoxygenases (LOX) catalyze the oxidation of polyunsaturated fatty acids such as linoleic and linolenic acid to hydroperoxides. Fatty acid radicals produced during the intermediate steps in the reaction can initiate oxidative degradation of pigments such as lutein and  $\beta$ -carotene. As a result of this co-oxidation, lutein and lutein ester pigments may decline during dough mixing in preparation for production of WSN and durum pasta, leading to poor color stability. Loss of pigment can be reduced by addition of compounds such as  $\alpha$ -tocopherol that inhibit LOX activity, or by using durum cultivars that have been selected for zero LOX activity. In addition to its effects on color, LOX has been associated with the formation of compounds with undesirable odors and flavors. Loss of lutein and carotene is also undesirable due to the role these ompounds play in the health of our eyes, in iron uptake in the intestinal tract, and as general scavengers of peroxy-radicals that reduce oxidative damage to tissues.

## **Polyphenol Oxidase**

Polyphenol oxidases (PPO) catalyze the oxidation of phenolic compounds to produce brown pigments on the cut or damaged surfaces of fruits and vegetables, and can cause major economic losses. PPO located in the seedcoat of ripe wheat grains is able to hydroxylate certain phenols in the  $\sigma$ -position adjacent to the existing -OH group to form orthodiphenols. The orthodiphenols are oxidized further by PPO to  $\sigma$ -benzoquinones, which in turn polymerize nonenzymatically to melanins (brown pigments). Fortunately, most of the PPO in wheat grains is removed with the bran during milling, but sufficient enzyme is carried through in the flour of many wheat cultivars to present problems with the color stability of products that are not cooked immediately. Whilst the endogenous substrate of PPO in grains has not been identified, the role of PPO in darkening of WSN, YAN, and durum pasta is now clear. Addition of exogenous substrates such as tyrosine, catechol, and L-DOPA (L-3,4-dihydroxyphenylalanine) enhances



YAN + tyrosine



YAN + tyrosine + tropolone

**Figure 3** Noodle sheets prepared with a small amount of tyrosine (polyphenol oxidase substrate) or tyrosine plus tropolone (specific polyphenol oxidase inhibitor) demonstrating the darkening caused by excess amounts of this enzyme.

darkening, whereas darkening is greatly reduced in the presence of specific PPO inhibitors or in cultivars selected for low or zero grain PPO activity (Figure 3). Variation in PPO activity explains most of the genetic difference in noodle darkening between cultivars, but is only responsible for a portion of the total darkening that occurs. Physical effects and other unidentified oxidation reactions also contribute to the reduction in noodle brightness over time.

# **POD** and Hydrogen Peroxide Synthesis and Catalysis

PODs are oxidoreductases widely distributed in higher plants and have been shown to oxidize many compounds, including phenolics, in the presence of hydrogen peroxide. Oxidation of phenolic compounds can lead to the formation of brown pigments similar to those formed by PPO, and consequently PODs have also been implicated in browning or darkening of noodles and pasta. In the case of POD, the situation is complicated by the presence of a number of forms with different substrate specificity that are localized in embryo, endosperm, and seedcoat tissues of wheat grains, and by the interaction of POD with systems that either generate H<sub>2</sub>O<sub>2</sub> (superoxide dismutase, NADH oxidation) or catalyze (catalase) its destruction.

In addition to its possible role in product darkening, POD has been implicated in the development of symptoms of a grain quality defect known as "black point."

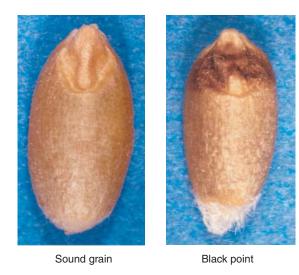


Figure 4 Sound and black point affected wheat grains.

Both wheat and barley are prone to this defect, which manifests as a dark stain in the seedcoat or husk, respectively, at the embryo end of the grain, and is associated with warm, humid conditions during ripening (Figure 4). Unfortunately this pigmented tissue fragments during milling and black specks are carried through into wheat flour or semolina. Whilst it has no effect on processing properties or texture, the specks are visible in noodle and pasta products and high levels of black point result in downgrading and substantial economic losses.

The enzymes discussed in this section all have specific functions within the tissues of developing grains, although in some instances the exact role has not been identified unequivocally. Their physiological functions are quite distinct from their effects on processing and end-product appearance. Whilst in many cases it is possible, or at least conceivable, to develop germplasm with extremes of variability for levels of these enzymes, dramatic changes may have adverse affects on plant growth, disease resistance, or grain yield.

# **Enzymes that are Active in Ripe, Quiescent Grains**

Despite the low-moisture content of ripe cereal grains and the death of the seedcoat and starchy endosperm tissues during grain ripening, it is clear that respiration and some other enzyme-catalyzed reactions continue at a slow rate during storage of harvested grain. Some of these activities result in the modification of the structure of grain constituents and secondary metabolites, whilst others may impact on the storage quality or the capacity to maintain freshness and palatability.

At maturity and depending on the genotype and growing conditions, wheat grains contain varying levels of xanthophyll (hydroxy carotene) present primarily as free lutein. During storage of wheat grain and wheat flour, and again depending on genotype as well as temperature and humidity, considerable proportions of the free lutein (up to 60%) may be converted to mono- and di-fatty acid esters. Fatty acids accumulate as a result of hydrolysis of grain lipids by lipase whilst the enzyme responsible for esterification has not been identified. Interestingly, esterification does not occur in some durum wheat and in a small number of bread wheat cultivars. Since adequate lutein intake is associated with a reduction in the incidence of glaucoma and macular degeneration, a major cause of blindness in elderly people, this modification may have implications for absorption, bioavailability, and attempts to improve eye health through dietary supplementation.

Enzymes have been implicated in the deterioration in quality of rice during storage. This deterioration may be linked to lipid peroxidation, peroxidative changes in polyunsaturated fatty acids that in turn are governed by the balance of production and dissipation of reactive oxygen species. POD is a key scavenger of reactive oxygen species and recent studies have indicated that the activity of this enzyme declines during storage of rice.

## **Endogenous Enzyme Inhibitors**

Cereal and legume grains contain inhibitors against a range of enzymes such as proteinase, α-amylase, endoxylanase, and limit dextrinase. Whilst some inhibitors are only effective against exogenous enzymes of microbial or insect origin, others are capable of inhibiting endogenous grain enzymes. The physiological function of these inhibitors is not certain but it has been suggested that they may be involved in plant defense mechanisms, protecting the stored reserves in the seed from invading microbes and insects, and/or play a regulatory role in plant development and metabolism. A role in the plant defense mechanisms seems plausible given the effectiveness of many endogenous inhibitors against hydrolytic enzymes from microbial and insect sources. Support for a regulatory role is less plausible and requires unequivocal evidence that the inhibitors reduce the activity of particular endogenous grain enzymes at physiological concentrations and that the inhibitors are located in the same tissue as the target enzyme. Most of the proteinase inhibitors that have been characterized to date are inhibitors of a particular class of protein hydrolases, the serine proteinases. As yet there has been no conclusive demonstration of serine proteinases of the chymotrypsin family or their genes in plants. In the case of the bifunctional α-amylase/subtilisin inhibitor (BASI) that is common in barley grains, there is clear evidence that it can significantly reduce the activity of endogenous high pI  $\alpha$ -amylase extracted from germinated or malted grain and ameliorate some of the deleterious effects of high  $\alpha$ -amylase on loaf volume and structure. However, recent studies of gene expression have indicated that the inhibitor is primarily located in the maternal seedcoat tissues of barley grains rather than endosperm. Similarly, whereas proteins of the "serpin" superfamily are localized in both vegetative and endosperm tissues of barley grains, they only inhibit specific mammalian serine proteinases. Limit dextrinase inhibitors isolated from barley inhibit the activity of endogenous limit dextrinase and appear to be ineffective against a number of other enzymes capable of hydrolyzing  $\alpha$ -1,6-linkages in dextrins. In theory at least, these inhibitors could interfere with the malting process although a role in commercial malting has not been demonstrated. Two groups of endoxylanase inhibitors (XIP-type and TAXI-type) have been isolated from cereal grains that are effective against fungal endoxylanases and both bacterial and fungal endoxylanases respectively. Microbial endoxylanases are commonly used in bread making to improve loaf volume.

Irrespective of possible roles in plant defense and regulation, significant numbers of inhibitors are active either against endogenous enzymes that are important in processing or against exogenous enzymes, usually of fungal or bacterial origin, that are added as ingredients to improve processing and quality of end products. In these instances, the inhibitor and the enzyme are brought into contact as a result of milling or deliberate physical admixture and there may be important implications for industrial applications.

## **Future Prospects**

Many cereal products and processes either depend on, or are adversely affected by, specific enzymes that are present in grains at maturity or that develop as the result of interactions between grains and the environment. As more of these enzymes are recognized, and their impact quantified, many opportunities will be presented to improve products and to develop new and novel products and processes. In many cases, there is already sufficient genetic variation within existing germplasm or near relatives for the selection of lines with enzyme levels more appropriate to the particular end-use process, whilst in other instances it may be possible via mutation, genetic engineering, or modification of milling techniques to extend

variation beyond that currently available. Genetic engineering, in particular, opens up the possibility of introducing genes encoding novel enzymes into cereal grains, overexpressing genes controlling synthesis of existing enzymes, or synthesizing enzymes in tissues such as the starchy endosperm where they are currently absent. As an example, identification of the genes should reveal potential strategies for the manipulation of cell wall  $(1\rightarrow 3, 1\rightarrow 4)$ - $\beta$ -D-glucan and arabinoxylan levels, for the enhancement of cereal quality and productivity. The three-dimensional structure of one of the barley (1,3;1,4)- $\beta$ -glucan endohydrolases has been solved and the structural information used to rationally design mutated forms of the enzyme with enhanced thermostability. The increased thermostability of this barley (1,3;1,4)- $\beta$ -glucan endohydrolase could have commercial potential through its ability to overcome filtration problems and other difficulties encountered in the malting and brewing industries. Increasingly sophisticated and high-throughput genomics technologies, coupled with rapid functional analysis systems, are under development. These new technologies can be confidently expected to identify more genes and reveal potential strategies for further manipulation of enzyme and grain constituent levels relevant to the grains industries in the near future.

See also: Cereals: Overview; Chemistry of Nonstarch Polysaccharides. Grain, Morphology of Internal Structure. Lipid Chemistry. Noodles: Asian Wheat Flour Noodles. Protein Synthesis and Deposition. Starch: Synthesis.

## **Further Reading**

- Bamforth CW (1994) *β*-Glucans and *β*-glucanases in malting and brewing: practical aspects. *Brewers Digest* 69: 12–16.
- Bode W and Huber R (2000) Structural basis of the endoproteinase-protein inhibitor interaction. *Biochimica et Biophysica Acta* 1477: 241–252.
- Champagne ET (ed.) (2003) Rice: Chemistry and Technology, 3rd edn. St Paul, MN: American Association of Cereal Chemists.
- Chen TF and Chen CL (2003) Analysing the freshness of intact rice grains by colour determination of peroxidase

- activity. Journal of the Science of Food and Agriculture 83: 1214-1218.
- Courtin CM and Delcour JA (2002) Arabinoxylans and endoxylanases in wheat flour bread-making. *Journal of Cereal Science* 35: 225–243.
- Fabriani G and Lintas C (eds.) (1988) *Durum Wheat: Chemistry and Technology*. St Paul, MN: American Association of Cereal Chemists.
- Fincher GB (1989) Molecular and cellular biology associated with endosperm mobilization in germinating cereal grains. *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 305–346.
- Fincher GB and Stone BA (1986) Cell walls and their components in cereal grain technology. *Advances in Cereal Science and Technology* 8: 207–295.
- Garcia-Olmedo F, Salcedo G, Sanchez-Monge R, Royo J, and Carbonero P (1987) Plant proteinaceous inhibitors of proteinases and α-amylases. Oxford Surveys of Plant Molecular and Cell Biology 4: 275–334.
- Jones RL and Jacobsen JV (1991) Regulation of synthesis and transport of secreted proteins in cereal aleurone. *International Review of Cytology* 126: 49–88.
- MacGregor AW and Bhatty RS (eds.) (1993) *Barley: Chemistry and Technology*. St Paul, MN: American Association of Cereal Chemists.
- MacGregor AW, Macri LJ, Schroeder SW, and Bazin SL (1994) Purification and characterisation of limit dextrinase inhibitors from barley. *Journal of Cereal Science* 20: 33–41.
- Mikola J (1987) Proteinases, peptidases and inhibitors of endogenous proteinases in germinating seeds. In: Daussant J, Moss J, and Vaughan J (eds.) *Seed Proteins*, pp. 35–52. London: Academic Press.
- Pomeranz Y (ed.) (1988) Wheat: Chemistry and Technology, 3rd edn. St Paul, MN: American Association of Cereal Chemists.
- Shirley BW (1998) Flavonoids in seeds and grains: physiological function, agronomic importance and the genetics of biosynthesis. *Seed Science Research* 8: 415–422.
- Stewart RJ, Varghese JN, Garrett TPJ, Hoj PB, and Fincher GB (2001) Mutant barley  $(1\rightarrow3,1\rightarrow4)$ - $\beta$ -glucan endohydrolases with enhanced thermostability. *Protein Engineering* 14: 245–253.
- Williamson PW (1997) Black point of wheat: *in vitro* production of symptoms, enzymes involved and association with *Alternaria alternata*. *Australian Journal of Agricultural Research* 48: 13–19.

## **EXTRUSION TECHNOLOGIES**

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## Introduction

Extrusion cooking technologies for foods and feeds grew out of a process developed by the Adams Company in the 1940s to manufacture snack foods from maize grits. They are derived from the earlier extrusion technologies used to form and extrude sausage meats and pasta. These processes involved conveying, mixing, and compressing of moist materials to form doughs that could be extruded as simple rods, or in the case of pasta, as well-defined shapes.

In extrusion cooking, a feedstock of moist powders is also conveyed, mixed, and compressed before shaping at a die (Figure 1). However, the dough temperature is raised to much higher levels compared to pasta making, in the range from 110-200°C on screws confined within a tubular barrel. This means that any water present is superheated within the dough but remains in the liquid state. The high temperatures also cause changes in the structures and form natural biopolymers, such as starch and soy globulins. Simple physical interactions, such as hydrogen and hydrophobic bonds that hold the subunits together, can be broken at high temperatures and the new forms of the polymer can be released and used to create novel structures for foodstuffs. A particular feature of extrusion cooking is the ability of the processing unit to manipulate these polymers at low moistures and to form doughs or fluid systems from them with the minimum of moisture.

The basis of the technology is the screw system, confined within a barrel that conveys the dough towards small openings at the end of the barrel called dies. In the confined space of the barrel, the dough is compressed and heated to high temperatures, while subjected to high pressures, before being extruded

through the dies into the atmosphere. An extrusion cooking process has a number of key features:

- the feeding devices supplying the raw material feedstocks,
- the design of the screw system and its barrel,
- the dimensions and number of the dies, and
- the devices that handle the extrudates.

Extrusion cooking is a continuous process in which the raw material feedstocks are metered into the screws at a constant rate and the machinery maintains a steady state equilibrium. This is achieved by balancing the forward flow produced by the screws against the back pressure caused by the resistance of the dies. It is a multivariate process depending on all the independent variables (those not affected by each other) of the machinery and the raw materials such as the screw speed, feed rate, and barrel temperature of the extruder, and the particle size, hardness, and composition of the raw materials. These variables produce a range of dependent variables (those determined by others) in the barrel, such as the material temperature, the pressure, and the mechanical energy input and a further range of dependent variables in the extrudates and products, such as the size, shape, crumb texture, and moisture levels. The consequence of varying one independent variable may be to change several of the product characteristics. Therefore, a good systems approach must be used to control such a process.

The range of products manufactured by extrusion cooking can be divided into two groups, food and industrial products. In the food sector, there are both animal products and human foods. The animal products themselves, include basic feeds for stock animals, marine feeds for freshwater and saltwater fish, shrimps, or prawns. They also include large markets for both dry and canned dog and cat foods and treats, and a number of products for other pets and for wild and tame birds.

Human foods include the original maize snacks and many new forms including modern versions of prawn crackers, potato chips, tortillas, and other traditional

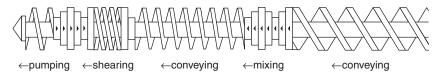


Figure 1 Diagram of the development of raw materials in a twin screw extrusion cooker.

snacks. There are many breakfast cereals products formed in balls, hoops, and flakes from all the major cereal flours and bran-rich recipes, which have large traditional markets in USA and UK and growing markets in many other areas. Similar expanded pieces of rice and maize are used in chocolate confectionery bars. These products called "bullets" of cereal are also used in other products where a crisp expanded foam particle is required, such as museli and granola bars, biscuits, and even in ice cream.

The range of extruded foods has been extended to coatings, such as breadings and flakes. These are used on fried foods, particularly frozen fish and chicken portions. Extruded products have replaced some of the traditional baked crumbs. They are made by expanding cereal extrudates and slicing them into crumbs. This process may be taken a step further in the manufacture of baby foods from extruded feedstocks of cereals and milk powders. For these products, the extrudates are ground to a fine powder, sieved, and used to replace the powders manufactured by the more expensive roller drying process.

Industrial products manufactured from grain include ranges of adhesives or glues and more recently, in biodegradable packaging materials such as expanded loose fill from wheat and maize derivatives and films of polylactic acid derived from maize.

## **Principles of Extrusion Cooking**

## **Overall Balance of Forces**

The extrusion cooking process is used to transform raw material into hot fluid dough and to continue to change the physical nature of this dough until it is forced out of the die. At that stage, the dough must have attained the ideal form to create a structure in the extruded product. This transformation of the native raw materials has an optimum range for most product types. For some products, only small changes in native biopolymers are required, whereas for others a very high degree of change must be achieved. For each individual product type, the process must be maintained in a balance between the independent input variables to obtain the ideal conditions within the barrel to achieve this level of transformation (Figure 2).

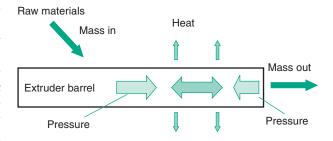
A good view of an extrusion cooking process is given by F Meuser and B van Lengerich. They showed that the relationships between the independent process inputs from the machinery and raw materials created a series of dependent variables within the barrel of the extruder. Most notable of these are residence time, temperature, pressure, moisture level, applied mechanical energy, and mass flow rate. Guy and

Horne showed that biopolymers in raw materials, such as starch, are affected by these variables so that their physical form changes and dominates the physical characteristics of the fluid dough. As the dough is extruded, the new forms of biopolymers influence the expansion process both in puffing and die swell. Thus, they influence the shape, extent of expansion, and the foam texture of the extrudates.

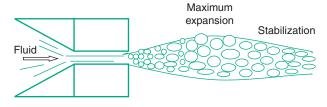
An important principle that must be understood for this is that the continuous extrusion cooking process is in a metastable steady state. Roberts and Guy showed that all extrusion cooking processes fluctuate as the balance is maintained between the independent inputs of the process. A fluctuation in an independent input that causes a perturbation of the equilibrium balance may either die away or grow and cause a larger fluctuation in the outputs and therefore in the product quality. Therefore, it is important to minimize fluctuations in the input variables, such as powder and liquid feed rates, raw material distribution, and quality.

## The Screw Design

The screws of an extrusion cooker are designed to convey a powder or viscous fluid from the feed port to the die and to force the material through the die (Figure 3). Single screws were used in the early machines for production rates of  $100-200\,\mathrm{kg}\,\mathrm{h}^{-1}$ , leading on from the pasta equipment, although twin screws were known and used by sausage makers. More recently, pairs of corotating intermeshing twin



**Figure 2** Extrusion cooking showing the balance between mass flow, pressure, and heat flow.



**Figure 3** Side view of screw system for a twin screw extruder used at CCFRA showing functions of the elements.

screw machine have been adapted from the plastics industry to become important machines for the food and feed industries. For conveying materials, the screws are designed with a pitch of 0.5–1 times its diameter and a small clearance between the leading edge and the wall. This design will provide a screw with excellent conveying power for viscous fluid and powders. However, its ability to process raw materials is very small.

The second feature for an extrusion cooking screw system is designed to apply high levels of mechanical shear to the feedstock being conveyed along its length. In the single screw, this is achieved by reducing the depth of the flights as the material approaches the die and by introducing cuts in the flights to increase backleakage.

In twin screw machines, special sections are added in which the flights are reversed to give a backpressure pump acting against the main flow. These sections are made weaker by cutting gaps in the flights, so that the main pumping screws overcome them, thus achieving a steady flow of the powders and melt fluids though the reversing section under shear. In general, the feed material entering the reversing section will be in the form of a moist or dry powder. This will be compressed and heated by mechanical energy plus barrel heating to a high temperature in a short distance. If the temperature is sufficient to melt the crystallites in the raw material it will become soft and form a fluid, otherwise it will remain as a powder and may be extruded without any expansion such as opaque compressed powder from the die. The fluids will lose the air bubbles before reaching the die and become translucent but have the ability to retain bubbles of steam and may expand to develop alveolar structures.

A special form of reverse section can be constructed from elliptical paddles placed on each shaft in a series. If successive pairs are placed at an angle to the upstream pair, a screw-like structure can be formed. This type of section acts on the fluid as it passes through, kneading the fluid against walls and the other elliptical paddles. An important feature of such a section is that it is set up as a reversing screw to ensure that all the paddles are full of fluid. If they are not full, they have little or no shearing effect on the fluid.

The final role of the screws is to push the fluid melt through the dies in a controlled manner. Normally, the extruder is run with the final sections completely full, so that pressure is transmitted from the main conveying screws to the dies. In those cases where reverse sections are used, there may be a pressure drop after the reverse section and a final section of conveying screw can be used to pump the fluid through the die. In most processes, where the extrusion melt

fluid has a temperature  $> 100^{\circ}$ C, the pressure in the die will need to be higher than the water vapor pressure. This will ensure that the fluid emerges from the die and expands in the air rather than in the die cavity. Normally, the pressure at the die for good control of hot melt extrusions will be > 40 bar.

The progress in the machinery has been remarkable and the largest machine currently can process  $10-30 \, \text{th}^{-1}$ , more than 100 times the rate of the early machines.

## The Transformation of Biopolymers

The main feature of extrusion cooking is the transformation of the biopolymers in a raw material feedstock. In 90% of the extruded products manufactured, the active biopolymer is starch, and for the remainder, it is a protein from oilseeds, such as soy, or wheat gluten.

Physical transformation is necessary to obtain a more suitable form of the biopolymer for structure creation in the postextrusion expansion processes (Figure 1).

Starch is found in many of the world major crops, cereals, such as wheat, rice and maize, and in important tubers such as potato and manioc. In all cases, it is found as partially crystalline granules, ranging from  $5-100 \, \mu m$  in maximum dimension. The polymers can be released from the granules (Figure 4) either by melting their crystalline regions and applying large shearing forces, or by gelatinizing (melting at  $60-75^{\circ}$ C and swelling) them in an excess of water (>30-35%). For extrusion cooking at low moistures, 15-20%, the melted starch must be dispersed purely by mechanical shear to obtain a melt fluid of partially, or fully dispersed aggregates. At moistures >30%, the granules swell and are readily dispersed.

The melting temperature for starch granules in a low moisture feedstock may be in the range of  $120-160^{\circ}$ C. Therefore, it is necessary to heat the feedstock to this temperature by the dissipation of mechanical energy and barrel heating. In systems with <25% moisture, the action of the screws on the powders being compressed to a density of  $1.4\,\mathrm{g\,ml^{-1}}$  will normally achieve the required temperature by frictional heating and the dissipation of mechanical energy from the drive motor.

The second part of the transformation of the starch is subtler. It has been shown that the major polymer in starch, amylopectin, is highly branched and has a molecular weight range of  $10^8-10^9$  Da. This size range is too large for good flow properties in fluids at the concentrations found in the melt fluids. It interferes with the expansion process and reduces the

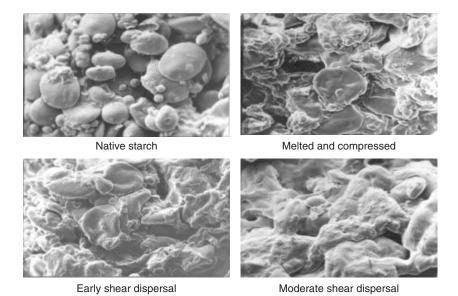


Figure 4 Stages in the transformation of starch in an extruder.

extensibility of the starch films in cell walls. However, the application of high shear inputs to the fluid using reversing screws and high compression ratios can degrade the starch to a much smaller range of  $1-5 \times 10^6$  Da. This material is much more extensible and gives a more expanded range of products.

The principle of extrusion is to transform the biopolymers by heat and shearing forces to new forms. This may be a completely dispersed form, in which the native granular structures have all been destroyed leaving the polymers to form a continuous fluid in water. Such processed starch would be suitable for forming films that can retain gases during expansion to form fine foams. In examples with proteins, the polymers may be dispersed in a fluid and then allowed to aggregate during laminar flow through long dies to form sheets or fibers in a texturization process.

The processing of starch may be set up to produce any type of starch structure from the native granules to the fully dispersed and degraded forms. If the range is divided into a number of stages as in Figure 4, the type formed at each stage may be associated with a different product in the list discussed earlier.

## **Miscellaneous Reactions and Physical Effects**

The extrusion cooker is a chemical reactor which can cause interactions between some of the ingredients such as amino acids and reducing sugars to take part in Maillard reactions and similar degradative and condensation reactions. These reactions produce color and flavor compounds and may also reduce amino acid and vitamin contents.

Extrusion cooking also has a unique action which serves to sterilize the materials being processed when their processing temperatures are  $> 100^{\circ}$ C. The time spent in the extrusion processing zone is short, only 45-120 s in most processes but the high shear fields and temperatures of  $140-160^{\circ}$ C in the shear zone help to reduce the viable cells and spore counts to low levels. Finally, the explosive release of superheated water from within cells rupture their structure and kills off the remaining viable cells.

# Extrusion Cooking Product Technologies

#### **Animal Feedstuffs**

The basic stock feeds are made with cookers and pelleting presses operating at  $20-50\,\mathrm{t}\,\mathrm{h}^{-1}$ . This does not gelatinize the starch and merely pasteurizes and compacts the material as loose pellets. The second level of processing is obtained with expanders that add steam to an open screw system feeding pelleting presses. This process also fails to gelatinize more than a small amount of starch, until a die is used and the equipment becomes a full extrusion cooker.

Feedstuffs with gelatinized and dispersed starch are made for special weaning foods and for all the marine feeds for fish and shrimps. In these products, the pellets are held together by the melted dispersed starch so that a strong structure is formed.

Extruders are also used to improve the nutritional value of oilseed and other protein stocks by denaturing proteins, which inhibit digestion such as lectins

and trypsin inhibitors in soybeans and dough ball forming elastic glutenins in wheat flours.

## **Pet Foods**

Dry pet foods are made from an extruded feedstock of cereals, fortified with protein-rich materials such as dried meat meal, chicken digest, and similar materials. They tend to be fairly dense products with a specific density of  $0.35-0.45 \,\mathrm{g \, ml^{-1}}$ . The structural material is normally starch, which is supplied by a cereal such as wheat or maize for standard products, and rice for special dietary feeds. There may also be higher levels of oils and fats and minerals to provide a balanced diet for the animals.

Many processes incorporate a preconditioning unit (Figure 2) in which the bulk of the feedstock is conveyed and mixed with oil and steam to produce a hot feedstock at 80°C for the extruder. This enables a higher throughput of materials to be processed by the extruder.

Processes containing high oil contents must be balanced with materials, which absorb oil and prevent slippage in the machinery. Alternatively, the oil can be sprayed onto the extrudates post extrusion.

#### **Snack Foods**

The snack foods are made in several forms by either direct expansion, or by the expansion of dense cooked pellets. In direct expansion, the feedstock may be formed from a cereal or potato base mix containing 75–85% starch. The extrusion process is high shear at low moisture to give highly degraded starch with no granular structure and a large amount of molecular degradation. This allows an expansion to a very low specific density of 0.14-0.20 g ml<sup>-1</sup>. Directly extruded snacks may be formed as tubes at the die and injected with a cream or jam through the center to manufacture a coextrusion or coextruded snack. The simple product is coated with oil and flavorings to complete the process.

For the pellet process, a similar feedstock is processed at low shear to melt the starch crystalline regions but to retain its granular structure. The cooked melted fluid is cooled to 95–100°C after cooking, before extruding as the final shape. This soft dense extrudate is cut into pellets and dried to < 12% moisture. As it cools to ambient temperatures, it becomes a glassy structure and is known as an intermediate pellet or half-product.

A snack is formed by heating the half-product for a few seconds in hot oil or hot air at 190°C. Recent developments have seen the production of such products in two layers with a half-product. On frying, this forms a large central cavity and has been called a 3D snack.

## **Breakfast Cereals**

A simple form of breakfast cereal is made from a cereal feedstock of wheat, maize, or rice flours and some small additions of sugar, salt, and proteins. The feedstock is processed at 20-25% moisture to a medium expansion at 150°C, to give a specific density of 0.22-0.28 g ml<sup>-1</sup>. The desired products are formed with a coarse cellular texture and thick cell walls, so that they remain crisp in milk for a few minutes. This is achieved by melting the starch granules and degrading  $\sim 50\%$  of the granular structures.

The recipes for directly expanded breakfast cereals may be varied to form single cereal varieties, such as crisp rice or corn pops, or to form multigrain cereals from blends of wheat, rice, maize, barley, and oats.

A unique form of the expanded breakfast cereal is the high-bran product in which wheat bran may be used at levels of 70-75%. In this product, a small amount of starch (15-20%) is used to bind the bran fibers together in a cellular structure. The starch must be melted and completely dispersed to achieve this

The second major form of extruded breakfast cereal is the flaked product, such as cornflakes or multigrain flake. This product is similar in its processing requirements to the half product snack in that it is made from a cereal feedstock at 120-130°C at low shear. The starch granules are melted, but not dispersed to any significant level. In the process, the cooked cereal feedstock is extruded as a dense fluid at 90°C and cut into small beads. Each bead forms the basis for a flake when rolled out to a thickness of 0.5-0.7 mm. This process occurs under temperature control at 40-60°C and is followed by a toasting process at high temperature of 240°C for a few seconds to blister and dry out the flakes.

The degree of processing of the starch is very important for the quality of the flakes when they are consumed in milk as a breakfast cereal. If the starch is sheared too much, the flakes take up water too quickly and become limp and flabby in the bowl.

#### **Breadings and Coatings**

Traditional breadcrumbs are manufactured by making loaves of bread, shredding, and drying their crumb to produce a small range of products. Several extrusion cooking processes have been set up to make similar products from the same materials and a wider range from other ingredients, which cannot be used in traditional bread.

The extrusion of a wheat flour feedstock at 28-35% moisture at temperatures of 110-120°C at low shear produces expanded extrudates with cellular textures similar to breadcrumb. Starch granules within the structure should be melted to lose their crystalline structure, but only dispersed to a small degree to give material for expansion. The extrudates may be cut while still warm and moist to form crumbs, and then dried to <10% to form the glassy structure required for coating crumbs. The recipe for coating crumbs may be based on wheat flour and modified with some chalk to increase the number of cells. Other cereals such as maize and rice may be used with, or instead of, wheat flour to give a variety of products. Maize adds an attractive yellow color to products, while rice gives whiter crumbs.

## **Baby Food**

Extrusion cooking has been used to produce expanded baby foods from rice and vegetable flours such as peas and carrots. These products are similar to snack foods in that the starch must be well dispersed to give good expansion and a light texture. The process must be controlled in terms of temperature to keep the mass temperature as low as possible <130°C during processing. The second form of baby foods is more important because it represents a larger amount of production. It was developed to match the rollerdried instant baby food, which is consumed as porridge, prepared by mixing water or milk with a dry instant powder. The extruded product is made by processing a feedstock of cereals with milk solids under conditions of medium shear input and controlled temperature. The aim is to melt the starch granules, but to retain their structures to a large extent. This gives a high paste viscosity when added to water or milk and produces a more attractive porridge.

It is also important to control the temperature of the fluid to reduce any browning reactions that would discolor the products and reduce the nutritional quality of the proteins.

See also: Animal Feed. Cereals: Breakfast Cereals. Pet Foods. Snack Foods, Processing.

## **Further Reading**

Blanshard IMV (1995) The glass transition, its nature and significance in food processing. In: Beckett ST (ed.)

- *Physico-Chemical Aspects of Food Processing*, pp. 17–48. London: Blackie Academic and Professional.
- Bouvier J-M (2001) Breakfast cereals. In: Guy RCE (ed.) Extrusion Cooking: Technologies and Applications, pp. 133–161. Cambridge: Woodhead Publishing.
- Bredie WLP, Mottram DS, and Guy RCE (1998a) Aroma volatiles generated during extrusion cooking of maize flour. Journal of Agricultural and Food Chemistry 46: 1479-1487.
- Bredie WLP, Mottram DS, Hassell GM, and Guy RCE (1998b) Sensory characterisation of the aromas generated in extruded maize and wheat flour. Journal of Cereal Science 28: 97-106.
- Bredie WLP, Mottram DS, and Guy RCE (2002) Effect of temperature and pH on the generation of flavour volatiles in extrusion cooking of wheat flour. Journal of Agricultural and Food Chemistry 50(5): 1118–1125.
- Frame ND (1994) The Technology of Extrusion Cooking. Glasgow: Blackie Academic and Professional.
- Guy RCE (1998) Measurement of the physical change in starch during extrusion cooking. In: Chang YK and Wang SS (eds.) Advances in Extrusion Technology: Aquaculture/Animal Feeds and Foods, pp. 243-252. Lancaster: Technomic Publishing.
- Guy RCE (2001a) Extruded snack foods. In: Guy RCE (ed.) Extrusion Cooking: Technologies and Applications, pp. 161–181. Cambridge: Woodhead Publishing.
- Guy RCE (2001b) Extrusion Cooking: Technologies and Applications. Cambridge: Woodhead Publishing.
- Guy RCE (2001c) Understanding extrusion. Food Engineering and Ingredients 26: 29-31.
- Guy RCE and Horne AW (1987) Extrusion cooking and coextrusion of cereals. In: Blanshard JMV and Mitchell JR (eds.) Food Structure-its Creation and Evaluation, pp. 331–350. London: Butterworths.
- Kazemzadeh M (2001) Baby foods. In: Guy RCE (ed.) Extrusion Cooking: Technologies and Applications, pp. 182-198. Cambridge: Woodhead Publishing.
- Kokini JL, Ho C-T, and Karwe VK (1991) Food Extrusion Science and Technology. New York: Marcel Dekker.
- Mercier C, Linko P, and Harper JM (1989) Extrusion Cooking, pp. 435-462. St. Paul, MN: American Association of Cereal Chemists.
- Meuser F, Gimmler N, and van Lengerich (1992) A systems analytical approach to extrusion. In: Kokini JL, Ho C-T, and Karwe VK (eds.) Food Extrusion Science and Technology, pp. 619-630. New York: Marcel Dekker.
- Roberts SA and Guy RCE (1987) Meta-stable states in a food extruder. Journal of Food Engineering 6: 103-112.
- Riaz MN (2001) Selecting the right extruder. In: Guy RCE (ed.) Extrusion Cooking: Technologies and Applications, pp. 29-49. Cambridge: Woodhead Publishing.



## **FERMENTATION**

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## **Origins and Applications**

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## Introduction

Fermentations are vital in utilizing many grains, pulses, and other seeds. This article will attempt to place fermentation in context with respect to its contributions to improving palatability, digestibility, and other desirable properties of foods derived from these concentrated, but sometimes, rather uninteresting raw materials. The raw materials from which the foods are derived may also contain toxins or "antinutritional factors" and fermentations can help to ameliorate the effects of these substances on the consumer. Archaeology shows that seeds were used as foods from the very beginning of the transition from hunter-gatherer lifestyles to the more settled communities that were needed to support the greater complexities of even the most primitive agricultural systems. Consideration of how fermentations developed to support consumption of seed-based foods is, necessarily, entirely speculative now.

It is known that the earliest civilizations had well-developed technologies for making staples such as bread and beer, and that they had taken significant steps in selective breeding to effect the transformation of (for example) wild grasses into more productive cereal crops. They surely lacked any conception of scientific breeding, microbiology, or any other, among the scientific disciplines which is in use in modern research, agriculture, and manufacture. A great respect for their powers of observation and *a priori* 

deduction must therefore be felt, leading to palatable and useful products, and to the start of food industries. Obviously the choice of seeds to use in these ways was heavily influenced by climate and other environmental factors.

Rice, wheat, and rye have become successively dominant crops from South to North in the northern hemisphere. The hardiness of rye and primitive barley strains (such as the bere barley still grown in the Orkney Islands and remote upland regions of Iran) means that these cereals are found in various regions where the environment is too harsh for more tender crops. This article will necessarily rely more on speculation than hard fact, but will make an honest attempt to identify some of the factors underlying the successes of fermentations in processing seed crops.

## The Beginnings of Food Fermentation

Although it cannot be known how or when the practice of fermentation in relation to food began, all available evidence suggests that it is a very ancient part of human development. In fact, fermentation can be seen as a subset of biodeterioration. All things that can serve as foods for humans (or any other animal) are, by their capacity to act as foods, more or less unstable. If they are food for mammals, then they will also be able to support the life of many types of bacteria, molds, etc. In practice, even such apparently stable materials as wood are open to attack by microbes, insects, etc. There is a powerful drive throughout nature to use the energy and nutrition present in organic materials, eventually mineralizing these materials back to carbon dioxide, water, and simple mineral salts.

Attacks by such microorganisms are seen as, primarily, a deterioration, or loss of desirable characteristics. Some such changes, however, turn out to have desirable characteristics. Such changes may produce improvements in flavor, odor, and texture. In addition, there is substantial evidence of improvements in nutritional status and value arising from controlled fermentations.

Various studies have shown that even simple fermentations of basic cereal grains with the indigenous (mainly lactic acid bacteria) microflora generate significant increases in the levels of some B-vitamins. There are strong suggestions that plant proteins, which are somewhat difficult for the monogastric digestive system to handle, can be made more digestible through the actions of proteolytic enzymes produced by bacteria and fungi. Many adults are intolerant to lactose, the principal sugar of milk, but fermentations such as those which produce yogurt, reduce or eliminate this problem.

Fermentations can also reduce or remove toxins and other "antinutritional factors." Perhaps the most important example of the former is the detoxification of cassava, while an example of the latter is hydrolysis of phytic acid, with removal of sequestering by it of divalent metals. There is also the prospect that fermentation increases the stability of the affected food, increasing its resistance to attack by other organisms. Before the advent of modern methods for food preservation, such as freezing, canning, etc., even a short extension in storage life would be precious. The prolonged storage possible for the main protein in milk (casein) consequent upon conversion to a hard cheese (such as Cheddar) would thus be of immense value.

It must also be remembered that availability throughout the year is a very modern phenomenon. In any given region (particularly in the temperate parts of the world) food supply is very seasonal. The idea that tomatoes, apples, "new" potatoes, would be always on sale, would have seemed unreasonable even a couple of decades ago. Now it is taken for granted throughout the industrialized world. In contrast to this prevailing situation with respect to foods rendered inherently unstable ("perishable") by virtue of their high water content, seeds were designed by evolution to remain stable and viable until conditions appropriate for germination were encountered. This made them excellent for storage during temperate and high latitude winters and the dry seasons of more tropical climates.

Obviously evolution did not design seeds to be palatable to animals because this would defeat their primary role as reproductive agents. It can be seen in, for example, the evolution of seed-eating birds, a competition between the birds' need to eat, and the seeds' need to survive to germination, and so to resist being eaten. Thus sorghum and millet have bitter-tasting tannins in their outermost layers, and attempts to breed low-tannin cultivars were more beneficial to the birds than to the farmers. Unfortunately, there are no fermentation strategies available at present that can remove such tannins, although it is conceivable that fungal enzymes could be harnessed for this purpose in the future.

On the other hand, antinutritional factors such as trypsin inhibitors are ameliorated by fermentation. Phytic acid presents an interesting situation. It is reasonable to see it as principally a phosphate, reserved for use as required by the developing seedling as germination progresses. However, its capacity to chelate divalent metal ions definitely gives it an antinutritional role, perhaps more particularly for monogastric animals. Also, the lack of phytase in the gastric juices of animals means that its valuable phosphate and inositol are denied to these consumers. Fermentations of "uncooked" seeds permit phytases present in the seed to effect at least a partial hydrolysis of the material, and this may be supplemented by phytases generated by microbes participating in the fermentation.

## An Example

The existence of small pits (perhaps a cubic meter, or even less) dug into the chalk of southern England, has long been known, but their purpose(s) remain matters for controversy. One suggestion has been that they would have been used to store grain. However, *a priori* this seems unlikely. In the ground, grain will be exposed to moisture. All seeds will take up available water as a preparation for germination, and will then become more easily attacked by microbes than the dry seeds would be. Chalk, being very porous, will readily conduct water. Thus grain storage in pits dug into the surface of chalk would seem to be an inappropriate method for conserving the material.

However, experiments showed an interesting sequence of events. Dry grain was packed into pits prepared for the purpose, and the pits were then sealed with a cap of clay or other impervious material. Initially, the grain closest to the clay took up water and was then colonized by a range of fungi and other microorganisms. Eventually this agglomeration of grains, hyphae, etc., formed an impervious layer, preventing further ingress of moisture. In addition, respiration by the stored grain and the microbes feasting in the outer layers of the stored material used up the available oxygen, replacing it with carbon dioxide. Thus, at the sacrifice of the outermost layer of the stored material, the bulk of it was preserved for a long time.

This does not correspond with normal ideas of a food-fermentation process. However, it has illustrations for a number of key points. The pit makers could have had no understanding of the processes at work in their pits. Despite this, the prevailing conditions favored growth of microbes in a beneficial way. There was some loss of stored material, but this was an acceptable price for the storage of the rest in useable condition. All of the foregoing is speculation; we can have no certain knowledge of how the pits were used by their makers. The grain storage hypothesis is nevertheless a valid one, and experiments show that the pits would operate as conservation devices.

## **Historical Background**

Very often an understanding of how fermentation began is speculation based on the interpretation of the modern process in the light of the knowledge of conditions in the past. Sometimes this is aided by ancient records, such as pictures from Egyptian tombs or continuing oral traditions, but in many cases such evidence is scanty or entirely absent. Thus, there is a great deal of guesswork in our attempts to reconstruct how particular fermentations originated.

In most cases this is unimportant, but it can be irritating when faced with a product such as "kefir." Here the fermentation relies upon structures called "grains." Modern analyses show that these grains are highly structured arrangements of yeasts and bacteria upon polysaccharide membranes. These organisms can be separately cultivated in the laboratory, but they do not reform into the characteristic kefir grains when cultured together. How did the grains arise? Was this a single event, or did it happen several times (kefir is widespread across Russia and Eastern Europe)? Clearly, it is unlikely ever that the answers to such questions will be known.

Even in simpler cases, such as bread making or the conversion of grapes to wine or grain to beer, there are no records showing the beginnings of such activities. Often there are myths showing such knowledge as gifts from gods or other superior intelligences. Also, foods such as bread and wine frequently have a place in sacramental activities. The giving and consumption of bread and wine, central to the Christian Eucharist, is also found in older traditions, such as those associated with the Roman god Bacchus. This infers the great importance of such activities at the change from hunter—gatherer economies to the beginnings of settled agriculture. The author suggests that this knowledge was an essential part of that change.

The ability to store foods enhanced survival overwinter and in other periods of hardship. Converting dry grains and other seeds into something more appetizing than a gruel must have made agriculture more attractive and valuable. Alcohol, despite its dangers, provided (and still provides), in reasonable moderation, a basis for social interaction. The apparent changes in the nature of consciousness affected by alcohol must have had spiritual significance, and the economic power possessed by those who control its production and distribution remains important to this day.

The vital part played by bread in the agrarian economies of Europe is made starkly plain by the draconian laws controlling the activities of medieval bakers, and that of beer by ordinances such as the German pure beer laws. The trade in fish sauces throughout the Roman Empire affords another example. Fish sauces is often associated with the Far East, but it is known that several fish sauces have been produced in the Mediterranean region since ancient times. Archaeological evidence indicates that these products were traded in substantial amounts throughout the Empire, even as far as Hadrian's Wall. Although it cannot be known for certain their nature, it seems clear that these were important condiments in the Roman kitchen.

The value of vinegar, as both a condiment and a preservative, has been recognized since the most ancient times. It also had importance in medicine and healing. While the earliest vinegars were, as the very name strongly suggests, produced by souring of wine, in more Northern climates, wine was probably too scarce and valuable to be used thus wastefully. Thus vinegars based on malted grains became economically significant. However, to achieve slow acetification, these were still produced by the traditional processes originally developed for wine vinegars (hence the name "Orleans Process" through association with the French town of that name). With the rapid transfer of the British population from a mainly rural existence to an increasingly urban one, consequent upon the Industrial Revolution, the enhanced demand for vinegar resulted in development of the "Quick" vinegar process to replace the slow Orleans method.

## Physical and Chemical Properties of Raw Materials for Fermentations

Appropriate fermentation techniques for given products will be strongly influenced by the physical and chemical properties of the raw material(s) to be used in its preparation. It is therefore useful to attempt a classification of raw materials. What follows will be entirely pragmatic, and is not intended as a formal classification scheme. Instead it will be a grouping by the characteristics most relevant to

fermentation procedures. In order to do this, it is first desirable to review the relevant physical and other characteristics.

Of the physical properties, probably the most important will be the availability of water, as this will strongly influence the types of organisms whose growth can be supported on a particular medium, and, in the extreme case, whether any organisms at all will grow on the material in question. Water availability can be limited by simple physical dryness, i.e., the actual absence of water, and by osmotic pressure creating a physiological drought. Salt and/or sugar in foodstuffs most commonly induce the latter. It should be noted that quite low concentrations of salt, well below that which might be expected to induce significant osmotic stress, can strongly influence the course of fermentations. This must have some significance in bread making with yeast, but becomes more significant in certain types of sourdough bread production, for example the German process called the Manheim salt-sour method.

This is a rather simple example of how the chemical composition of the fermentable material is of great importance in determining the types of organisms that can grow on it and the outcomes of fermentations which they affect. In some cases, there will be ample supplies of (for example) sugars to give desired levels of organic acids, B-vitamins to support growth of fastidious lactic acid bacteria, amino acids, etc. In many cases, however, there will be insufficient readily usable material. Then, organisms which can produce and export appropriate hydrolytic enzymes (amylases or proteases, for example) will be at an advantage. They may also prepare the way for later growth of less competent organisms such as yeasts and lactic acid bacteria.

The part played by chemicals in herbs, onions, garlic, etc. can be particularly interesting. These materials are valued principally as flavors in foods, but many of them also have long traditions as healing agents and medicines. There is increasing evidence that these traditions have a secure basis in scientific fact. The review by Sherman and Flaxman discusses claims that onions, garlic, and many culinary herbs are powerfully inhibitory against bacteria associated with food poisoning. Some baked foods use such herbs and spices, but of more evident importance is their role in foods such as continental sausages, where comminuted meat and cereal products are combined with such spices, and some of which use particularly high levels of garlic (e.g., salami). Sausages are harsh environments even without these inhibitors. Yet bacteria such as species of Carnobacterium and Micrococcus are present in these products, and are thought to be essential for flavor development. How selective is the toxicity of these plant products? There are clearly interesting biochemical challenges here, even if these claims were shown to be fully justified in the field, i.e., in actual food fermentations. It has been pointed out that, for example, salamis, although particularly heavily spiced with garlic, have been associated with serious illnesses due to Escherichia coli. Thus, claims of this type need to be assessed very carefully, with purely laboratory studies and robust trials in actual foods being run in partnership with each other, if the effects on food microflora of herbs and spices are to be fully understood.

The foregoing is not intended as a complete discussion of the issues involved here. Rather it is an indication of some of the factors that require consideration when attempting to understand the factors influencing the initial development and subsequent evolution of food fermentations associated with grains and other seeds.

## A Classification of Plant Raw Materials

The great diversity of plant materials means that any attempt at grouping into classes will be fraught by difficulties caused by overlaps and ambiguities. For example, tomatoes and cucumbers, although obviously fruit, are often placed among the vegetables, while the edible stems of rhubarb are placed alongside fruits. Thus, what follows is entirely arbitrary, reflecting the practical problems in fermenting plant foods.

## Leafy material

Grass and other forage materials are converted into silage on an enormous scale as an essential part of modern ruminant farming. Although not normally thought of as "food" fermentation, i.e., not part of the "human" diet, this is probably the most important among leaf fermentations in the wider context. Among materials for direct human consumption, the best known products are "kimchi" and "sauerkraut." The latter is a highly industrialized process in the major producing nations. Undoubtedly, it was developed principally as a storage process for conserving excess cabbage through the winter in areas where the severe winter weather would destroy cabbage heads left in the fields, while cut heads would quickly deteriorate in store if held as the unmodified material. The key factors for fermentation are provided for by adding  $\sim 2-3\%$  by weight of salt to shredded cabbage heads, then packing tightly into containers, where the sap withdrawn from the plant tissue by osmotic action of the dissolving salt quickly displaces remaining air from the plant material. Bacteria naturally present on the plant surfaces drive the lactic fermentation, although there is a tendency towards using selected cultures now. This simple process gave a product

that could be conserved without deterioration for many months, even in the absence of refrigeration.

Obviously, there is no need to continue this process as a means for conserving cabbage in industrialized societies, where advanced storage systems and transportation, which can deliver any food from any part of the world without regard to season, provide complete freedom from the old domination by the solar cycle. However, this has not reduced demand for the fermented product. On the contrary, those accustomed to it have taken its production with them when they have migrated to, and settled in other parts of the world. Consequently its production is, if anything, increasing. The technology is basically the same for the materials discussed in the next section, with the key difference being that they use salt in prepared brine, whereas here dry salt is added to the raw material.

#### **Fruits**

Cucumbers are perhaps the classical example here, although olives would be cited first in Mediterranean climates. Other examples include tomatoes. Small onions and certain types of radish roots, although not fruits, are processed in the same way. Again the technology is very simple, easily applicable on a village or domestic level, and originally a way to conserve seasonal surpluses of produce against winter shortages.

Essentially, the prepared material is immersed in a salt solution (brine) and a spontaneous lactic fermentation develops. In reasonably cool conditions, the products are stable during prolonged storage. The observations about the survival of sauerkraut technology, after the conservation need had passed, apply with equal force here, as do those on industrialization of the processes. Olives are remarkable in the market penetration that the fermented product has achieved outside its traditional consumers.

Another type of conservation of fruits relies on fermenting the released juices to produce stable liquids. This is a very pedestrian way to describe the delights of wines and the products from apple (cider; USA "hard cider") and pear (perry) juices, but is deliberately done to place them in a specific context. Undoubtedly, the capacity of the products to induce intoxication would have attracted attention as soon as they were discovered. Indeed, there is ample evidence that birds and mammals are attracted to fermenting fruits, and will manifest clear evidence of drunkenness, and even of addiction to ethanol. In many cases, the intoxication became associated with religious experiences among humans. Despite these complicating sociological factors, alcoholic fermentation can properly be seen as a preservation strategy, essentially indistinguishable from those presented in the preceding two sections.

Sweet fruits have a very short storage life, although strategies have been developed to permit storing some hard fruits (notably apples and pears) for extended periods. At the other extreme, grapes are so easily bruised that picking them for table-use demands great care; the yeasts always present on the grape skins quickly ferment the fruit juice at the slightest opportunity. The only practical alternative to fermentation is drying to raisins, currants, and sultanas.

## Roots, Bulbs, and Tubers

In general, root crops are stable enough for their storage in simple systems, and even for them to be left in the ground during winter until required. Thus, with minor exceptions such as the radishes referred to above, root crops are not fermented in temperate climates. Bulbs are also generally stable enough for winter storage, so fermentation in brine is probably done more for culinary than for conservation reasons.

The New World has contributed, among many other valuable crops, three novel starchy crops that have now become so indispensable that their origins now forgotten. Of these, one is a cereal (maize) but the other two are root crops, potatoes (Solanum tuberosum) and cassava (Manioc esculenta). Of these two, the author has no knowledge of potatoes being fermented, except in the very limited sense of their being a source of starch for alcohol production. Potatoes can produce various toxins as defenses against predators, but protecting the tubers from exposure to light easily prevents production of the most important of these, and the author is not aware of any evidence that fermentations will reduce toxicity of green tubers.

On the other hand, fermentation is absolutely essential for making cassava safe for use. Most varieties are extremely toxic, because of the presence of a cyanogenic glycoside. In the intact tuber this, and the enzyme that can hydrolyze it, are strictly segregated, but mechanical damage brings them into contact, thus releasing the highly toxic hydrogen cyanide. It is clear that even very primitive people from their native habitats had developed techniques for making the tuber comparatively safe to eat. These are not primarily fermentations, depending as they do on the enzymatic action described above to destroy the cyanogenic glycoside. However, the conditions under which the tubers are harvested and prepared for detoxification ensure that there is an abundant microbial flora associated with them.

Lactic acid bacteria, which do not use iron-containing enzymes, are resistant to cyanide, and their growth on the cassava portions produces acidic conditions that both favor the action of the relevant enzyme and help in removal of the cyanide as the unionized

gaseous form. Subsequent processing, such as drying, reduction to granular form and heat treatments such as (in the case of products such as the West African "gari") roasting. Unfortunately, even "garification" will not reliably remove the last traces of cyanide. Although the residual material is below the acutely toxic concentration, it can, by competing with iodide, produce the thyroid gland malfunction known as goiter.

#### Seeds

The plant products discussed so far have been characterized by high water content. This is true even of the firmest products, such as the root crops. This water content means both that any injury leaves the foodstuff liable to fermentation or other microbial attack, and that even the undamaged item has a restricted storage life because eventually water loss will reduce it to a flaccid and uninviting state. However, some vegetables can be made suitable for storage by deliberate, controlled dehydration.

With seeds, as already noted their evolutionary development resulted in a very dry, tough, long-lasting product. Seeds are also packed with nutrients placed there by the parent plant to support initial growth of the new plant whose embryo the seed contains. Thus a seed has many characteristics, which make it potentially desirable as a human or animal food. From the human viewpoint however, seeds often have significant disadvantages as foods. As harvested, they are difficult to eat. If processed in the obvious way, by soaking and/or boiling in water, the product is apt to be rather bland and unappetizing. Indeed, of the various grains and seeds harvested by humans, only rice is primarily consumed in this way. Comminution by crushing or grinding can effect some improvements, but the resulting gruels or porridges are still of limited appeal. Much the same is true if the material is formed into a paste and baked or roasted.

On the other hand, even the simplest fermentations can effect considerable improvements. For example, a common breakfast dish in Africa is a maize porridge in which the ground maize is left overnight in water, and then boiled next morning, ready for eating. Overnight there is substantial acidification through the action of lactic acid bacteria. Although this is a fairly minor change, the result is a much more appetizing porridge, especially when a little crude sugar is added just before consumption of the cooked material. Most such mixtures of flours and water will develop substantial populations of lactic acid bacteria, alone or associated with yeast cells.

Microbial gas production will cause some expansion of the paste or dough, and give a tastier and more appealing product upon cooking. The most obvious example of this type is of course sourdough bread. It seems reasonable to believe that this is a very ancient product, and archaeological evidence is emerging to support this view. At the most basic level leaving the dough overnight will produce a leavening effect. A simple improvement would be to add a part of a good ferment to a fresh mixture of flour and water. This process, sometimes called "back slopping" remains the basis for much sourdough bread production to the present. Not only does it provide a more reliable fermentation, but also the increased level of microbes present, and the fact that they are already in active fermentation will tend to give enhanced leavening of the bread over that which could be expected from a newly started fermentation.

A more dilute mixture of flour and water would give a crude alcoholic drink. The really important development here was the discovery that germinating the seeds greatly increased the alcoholic content, and the general quality of the resulting drink. Beer production in the West further modifies the properties of the drink by drying and heat treatment of the germinated seeds (barley malt). This also permits production of the malt in quantity greater than is immediately required, and its storage for future use. African sorghum beer does not require this added refinement, and it seems reasonable to think that the earliest barley fermentations would have used "green" (undried) malt. Again the fermentations would have used a mixture of lactic acid bacteria and yeasts. It is clear that from fairly early times residual yeast from beer and wine fermentations were used for bread making. Equally, "kvass" fermentation from rye bread and water uses a portion of sourdough bread ferment to start a new brew going, although once started, it is maintained by "feeding" the ferment with additions of bread and water.

Rice flour mixed with legume seed flour and water will ferment with leavening provided by carbon dioxide generated by heterofermentative lactic acid bacteria. In parts of India, this simple process yields products such as "idli" and "dosa."

It seems that rice will not malt well, although the author is not aware of any specific reason why this should be so. Certainly it is not fermented by processes involving germination. Instead "koji" processes similar to those used for soy sauce and other legume seed fermentations have long been employed in preparatory stages for subsequent conversion of rice into alcoholic beverages, vinegar, etc. Essentially strains of the koji mold Aspergillus oryzae producing high levels of amylolytic enzymes are employed to saccharify the rice starch, followed by alcoholic fermentation under conditions such that the fugal enzymes remain active during it, so providing a steady supply of fermentable carbohydrates.

Thus, seeds rich in starch are converted to many foods and drinks by a range of fermentations that depend on yeasts and lactic acid bacteria, alone or in associations. Fermentations of protein-rich seeds, particularly the soybean (Glycine max) and the ground nut (peanut, monkey nut, Arachis hypogea), of tropical and subtropical origin, together with the seeds of tropical leguminous trees, have provided both staple foods (such as "tempeh" and "oncom" ("ontjom," "onjom," etc.) and flavoring materials (soy sauce, "miso," etc.) throughout the tropics and subtropics for many thousands of years. The driving force for such developments was probably the rather bland nature of the starting materials, but it is now known that fermentations can increase the digestibility of constituents of the seeds, particularly the proteins, enrich the products with extra B-vitamins, decrease concentrations of some toxic or antinutritional factors, and increase the availability of essential mineral nutrients. These fermentations are frequently multistage, using mold fungi to manufacture a range of digestive enzymes, then yeasts and lactic acid bacteria to complete the process. The origins of these fermentations are lost in time, but it is known that soy sauce and miso have existed for at least 3000 years. Legume seeds tend to be rather well supplied with antinutritional factors, and detoxification during the fermentation is a significant benefit of the process. Perhaps the most remarkable example of seed detoxification however, concerns the castor bean (*Ricinus* spp.). This seed contains a wealth of toxic materials, including ricinoleic acid (a powerful purgative), strongly allergenic proteins, ricin, etc. Despite this, in parts of Nigeria the seeds are collected, boiled, de-hulled, wrapped in leaves and allowed to ferment, then used as a food flavoring, apparently without any ill effects on its consumers.

## Types of Fermentations

It is worth noting the range of fermentation procedures developed for dealing with foods. The simplest are probably the liquid fermentations used in making drinks such as beers, kvass, wines, and some of the milk products such as vogurt and buttermilk. These are sometimes called suspension cultures because the microbial cells float suspended in the surrounding liquid. Another name is stirred tank reactor fermentations. Fermentations involving molds usually require free access to the air, both to meet the organism's need for abundant oxygen, and to remove the products of the fermentation, principally carbon dioxide and heat. In one case (tempeh), however the process requires a very limited exposure to oxygen, traditionally achieved by wrapping the substrate in banana or

teak leaves, although plastic bags pierced with a pattern of fine holes now often replace these. Such fermentations, which do not require free liquid water, are described as solid substrate processes. Some lactic fermentations, for example, of starchy or farinaceous materials, might also be placed here, as might sausage fermentations. When a solid substrate is in free water, it appears that this is just a type of suspension culture, but there is evidence to suggest that the active microbes attach themselves to the solid material to a considerable extent, thus producing some characteristics of a solid substrate process. The kefir grain provides an interesting problem. Attachment of the cells to the polysaccharide membrane suggests solid substrate process, but analysis of the fermentation shows that there are large numbers of cells suspended in the liquid, quite free of the grains, and that the proportions of bacterial species in suspension are radically different from the proportions within the grains. In Orleans vinegar production, the acetification is effected by a layer of bacterial cells growing as a film or skin at the interface between water and air, so exhibiting some characteristics of a solid substrate process. The "Quick" process features a layer of cells on the surface of the wood shavings, irrigated by the recycling liquid, but also supplied with abundant oxygen, thus showing an even closer relationship to solid substrate process. Citric acid production by Aspergillus used to require static growth on the surface of a liquid layer, rather like the Orleans vinegar process. Some processes were described using growth on bamboo supports and a liquid recycle like the "Quick" vinegar process, but it is not clear if these were applied on a production scale.

## The Future for Traditional **Fermentation Processes**

Some very ancient fermentation processes continue to flourish and develop on a very large scale. Soy-sauce production almost seems capable of expansion without limit, as does cheese. Yogurt has been a remarkable success story, with market penetration far beyond its traditional homelands. Vegetable fermentations, such as sauerkraut and olives also seem to grow, although they perhaps do so more slowly than some of the other cases cited. The sourdough bread story is another remarkable one, with the product now on offer at (it seems) every airport in the USA. There does not seem to be any fear for the future of these and a selection of other products. It is also noteworthy that cereal-based products, notably bread and beer, are effectively penetrating markets (such as South-east Asia) where they did not have a traditional role before colonial times.

See also: Barley: Malting. Beverages: Distilled. Breads. Consumer Trends in Consumption. Cultural Differences in Processing and Consumption. Fermentation: Foods and Nonalcoholic Beverages. Fuel Alcohol Production. Milling and Baking, History. Nutraceuticals from Grains. Nutrition: Effects of Food Processing. Soybean: Soy-Based Fermented Foods.

## **Further Reading**

Bushell ME (ed.) (1984) Progress in Industrial Microbiology: vol. 19, Modern Applications of Traditional Biotechnologies. Oxford: Elsevier.

Campbell-Platt G (1987) Fermented Foods of the World: A Dictionary and Guide. London: Butterworths.

Dirar HA (1993) Indigenous Fermented Foods of the Sudan. Oxford: CAB International.

Hesseltine CW and Wang HL (eds.) (1986) Indigenous Fermented Foods of Non-Western Origin: Mycologia Memoirs, No. 11. New York: New York Botanical Garden.

Huang HT (2000) Science and Civilisation in China, 6(v): Fermentations and Food Science. Cambridge: Cambridge University Press.

Pederson CS (1979) Microbiology of Food Fermentations, 2nd edn. Westport, Connecticut: AVI.

Reddy NR, Person MD, and Saluniche DK (eds.) (1986)

Legume-Based Fermented Foods. Boca Raton: CRC

Press.

Rose AH (ed.) (1982) Economic Microbiology, vol. 7, Fermented Foods. London: Academic Press.

Sherman PW and Flaxman SM (2001) Protecting ourselves from food. *American Scientist* 89(2): 142–151.

Steinkraus KH (ed.) (1989) Industrialization of Indigenous Fermented Foods. New York: Marcel Dekker.

Steinkraus KH (ed.) (1996) Handbook of Indigenous Fermented Foods, 2nd edn. New York: Marcel Dekker.

Sudarmajdji S, Suparmo, and Raharjo S (eds.) (1998) Reinventing the Hidden Miracle of Tempe. Jakarta: Indonesian Tempe Foundation.

Wood BJB (ed.) (1998) Microbiology of Fermented Foods, 2nd edn. Glasgow: Blackie.

# Foods and Nonalcoholic Beverages

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#### Introduction

Fermented foods (including beverages) can be defined as "food substrates that are invaded or overgrown by edible microorganisms, whose enzymes and metabolic processes modify the substrate producing nontoxic flavors, aromas, and textures attractive to the human consumer." As grains are the world's most important foodstuffs there are several categories of fermented grain foods and beverages. This article, however, will only deal with cereal-based products that are produced with a predominantly lactic acid bacterial fermentation. For others, such as: mold fermentations of vegetable meat substitutes, *see* Beverages: Distilled. Soybean: Soy-Based Fermented Foods; Soymilk, Tofu, and Okara; and for yeast leavened breads, *see* Breads.

This article will examine the lactic acid bacteria responsible for lactic acid fermentation and the fermentation process itself, the safety and nutritional aspects of lactic acid fermented cereal foods and nonalcoholic beverages (LAFCFANAB), the different types of traditional LAFCFANAB, production processes for specific examples where commercialization has already taken or could easily take place, and lastly the potential for further development of LAFCFANAB.

## **Lactic Acid Bacteria**

Lactic acid fermentation is carried out by several different genera of bacteria, collectively referred to as lactic acid bacteria (LAB). They are with some exceptions, gram-positive, catalase-negative, nonsporeforming spheres and rods. Currently the LAB group comprises 11 genera: Carnobacterium, Enterococcus, Lactococcus, Lactobacillus, Lactosphaera, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Vagococcus, and Weissella. The Lactobacilli are probably the most important in LAFCFANAB. LAB can carry out respiration without oxygen. Carbohydrates such as glucose are partially oxidized with the release of energy in the absence of any external electron acceptors. The final electron acceptors are organic compounds, specifically lactic acid, produced directly from the breakdown of the carbohydrate. Although the process releases much less energy than aerobic respiration, it confers considerable competitive advantage to the LAB in that an anaerobic, low pH environment is created in which LAB can flourish.

LAB can be conveniently divided into two groups: homofermentative and heterofermentative. Homofermentative types produce lactic acid as the major or sole product of glucose fermentation, whereas the heterofermentative types produce equal molar amounts of lactic acid, carbon dioxide, and ethanol. Heterofermentative types also produce more of the generally desirable flavor compounds such as acetaldehyde and diacetyl. The genera *Pediococcus*, *Streptococcus*, *Lactococcus*, and *Vagococcus* are all exclusively homofermentative, whereas the important *Lactobacillus* genus comprises homofermentative and heterofermentative species. The other LAB genera are all heterofermentative.

## **Factors Affecting Fermentation**

Most LAFCFANAB are produced using a natural, mixed microbial culture. The original source of microorganisms is the environment and the cereal food or beverage itself. Some products are produced with a so-called "spontaneous fermentation" where the product or intermediate is simply incubated at

a suitable temperature for up to 72 h. However, in most cases a culture is maintained and optimized by the process known as "back-slopping." A portion of the food/beverage or intermediate that has undergone a successful fermentation is kept back and used to inoculate a new batch. Although apparently crude, back-slopping is highly effective. This is because the environmental conditions of the fermentation favor the growth of the desired bacteria strains.

Several intrinsic and extrinsic factors affect the intensity and particular type of fermentation. Intrinsic factors include the pH, moisture, redox potential, and nutrient content and composition of the food or beverage. For example, yeasts grow over a wide pH range, although a pH 4-4.5 is favored, whereas LAB will grow at low pH (< pH 4) and through the production of lactic acid, the pH can be lowered further. In contrast, most bacteria, especially many spoilage bacteria and all pathogenic bacteria, do not grow at such a low pH. This has important consequences with regard to the shelf life and safety of LAFCFANAB. Moisture, or more strictly water activity  $(a_{w})$ , which is an expression of water availability in the food system, controls the rate of microbial growth. Most bacteria require a minimum  $a_{\rm w}$  of 0.9 for growth, whereas yeasts have a somewhat lower minimum  $a_w$  0.88. Microorganisms are sensitive to redox potential (Eh). Aerobic microorganisms require positive Eh for growth, whereas anaerobes require negative values. The presence of, for example, reducing sugars such as glucose maintain the reducing conditions required for the growth of anaerobes. Oxygen itself also plays an important role in determining the nature of the fermentation. The anaerobic environment, in which LAB will grow, prevents the growth of obligate aerobes.

The nutrient content and composition of the food influences the particular type of fermentation. Despite the fact that mixed cultures are used to produce these fermented products, there is generally a clear distinction between those that are alcoholic and those that are sour and substantially nonalcoholic. This is primarily as a result of the fact that yeasts tolerate much higher levels of sugars than bacteria. Hence, under high sugar conditions, alcoholic fermentation is favored.

External factors, particularly temperature, also have an influence. The LAB fermenting cereals tend to grow best between 20°C and 32°C. At such temperatures, the fermentations are invariably heterofermentative. However, a few species, notably *Lactobacillus leichmannii* (*delbrueckii*) which is homofermentative, will grow at much higher temperature, 48-50°C.

These various factors, particularly in traditional fermented products where spontaneous fermentation or

back-slopping are relied upon, generally result in a sequence of different organisms responsible for the fermentation. Many lactic acid fermentations are initiated by spherical bacteria such as Leuconostoc mesenteroides and Pediococcus. The rod-shaped bacteria such as Lactobacillus plantarum and L. brevis then take over and lower the pH to  $\sim 3.6$ . Alternatively, a yeast alcoholic fermentation may initiate or follow the LAB fermentation. The complexity of such systems, especially as conditions invariably vary between producers and even between batches, poses great challenges to the food microbiologist attempting to elucidate the microorganism(s) of importance in a particular product.

## **Food Safety and Nutritional Implications**

People worldwide enjoy the sour, sharp, refreshing taste of lactic acid in foods, not just in cereal foods, but in vegetables such as sauerkraut and dairy products like yogurt. In addition, the lactic acid fermentation has effects on the food that are perhaps more important than sensory.

## **Food Safety**

LAB fermentation of porridge to a pH of 4.0 or below will inhibit the growth of Enterobacteriaceae, including pathogenic bacteria such as Salmonella typhimurium. It appears that the growth of gramnegative intestinal pathogenic bacteria such as enterotoxigenic Escherichia coli, Campylobacter jejuni, Shigella flexneria, and S. typhimurium is strongly inhibited by the low pH caused by the formation of lactic and acetic acids. In slight contrast, the inhibition of the gram-positive Staphylococcus aureus is possibly also due to the formation of bacteriocins, antibiotics produced by the LAB. The inhibition of food-borne pathogens by LAB fermentation is of great benefit in developing countries, where even today many people do not have access to safe water. The implications of this are profound. The World Health Organization (WHO) estimates that annually there are  $\sim$ 1500 million episodes of diarrhea in children under the age of five and over 3 million children die as a direct result. In many areas of the world, cereal gruels and porridges are generally used as weaning food for infants. Hence, the WHO is promoting lactic acid fermentation, together with hygienic food preparation practices, as a way of helping to ensure the safety of food.

It has also been reported that LAB fermentation of aflatoxin-contaminated maize or sorghum to produce porridge, reduces the level of aflatoxin by more than 70%. Notwithstanding this apparent benefit, the

preparation of food from mycotoxin-contaminated grain is not a practice that should be encouraged.

A further real advantage of LAB fermentation is that the low pH slows down the rate of microbial spoilage of the foodstuff due to other bacteria. The growth of spoilage bacteria is inhibited by the environmental conditions created by the LAB. Hence, LAB fermentation can be considered as a way of extending the safe storage life of foods and beverages. This is of particular importance in the less-developed world where the vast majority of households do not have refrigerators.

## **Nutritional Implications**

There is evidence that LAB fermentation can reduce the viscosity of porridges. The extent of viscosity reduction is affected by factors such as the pH attained and whether the organisms involved possess amylase activity to hydrolyze the cereal starch into dextrins and sugars. Viscosity reduction is greatly enhanced if fermentation is combined with the addition of malted (sprouted) cereal flour that contains high levels of amylase enzymes. Viscosity reduction is especially important in cereal weaning porridges for infants. Porridges where the starch is unmodified can have very high viscosity making them unpalatable. If they are diluted to reduce viscosity, there is a concomitant reduction in energy and nutrient density. Reduction in viscosity with fermentation and malt addition, as is done in the porridge "togwa" from Tanzania, can improve palatability and enable a porridge of higher energy and nutrient density to be produced without it having excessive viscosity.

LAB fermentation has also been found to increase *in vitro* carbohydrate availability. Although the quantity of protein present in cereals is little affected by fermentation (Table 1), the quality in terms of both lysine availability and relative nutritional value is in general significantly improved. These improvements are probably brought about by a combination of the low pH and bacterial proteolytic action, which modifies the structure of the cereal proteins. In sorghum, where cooking to make the porridge reduces protein digestibility, fermentation appears to counteract this effect, improving *in vitro* protein digestibility.

With regard to micronutrients, there is some indication that B-vitamins, particularly thiamine are increased in LAB fermentations. However, the findings are not clear-cut, presumably because of differences in the extent and type of fermentation. The quantity of minerals in cereals cannot be affected by LAB fermentation. However, there could be some improvement in their availability since the quantity of phytate (myo inositol hexaphosphate), a powerful chelator of divalent metal ions such as calcium, magnesium, and iron

**Table 1** Effect of spontaneous fermentation for 6 days<sup>a</sup> on the protein content, available lysine and relative nutritive value of various cereals

Cereal	Crude protein (%)	Available lysine (mg/g N <sup>b</sup> )	Relative nutritive value (%) <sup>c,</sup>
Barley			
Nonfermented	9.75	19.0	70
Fermented 22-25°C	9.62	75.6**	85 (15)*
Fermented 37°C	9.81	96.3**	84 (14)*
Maize			
Nonfermented	9.94	17.4	67
Fermented 22-25°C	9.81	47.4*	80 (13)**
Fermented 37°C	9.69	51.8*	80 (13)**
Millet (species not specified)			
Nonfermented	11.56	3.0	65
Fermented 22-25°C	12.31	36.0*	79 (14)**
Fermented 37°C	12.00	45.0*	81 (16)**
Oats			
Nonfermented	10.75	15.0	85
Fermented 22-25°C	10.31	104.6**	88 (3)
Fermented 37°C	9.81	113.0**	88 (3)
Rice			
Nonfermented	7.87	5.0	63
Fermented 22-25°C	7.75	46.0*	85 (22)*
Fermented 37°C	8.25	61.0*	82 (19)*
Wheat			
Nonfermented	10.75	23.3	72
Fermented 22-25°C	9.14	64.2**	86 (14)*
Fermented 37°C	9.26	79.8**	86 (14)*

<sup>&</sup>lt;sup>a</sup>The pH fell to pH 3.6-4.1 within 2 days.

which occurs in grains, is substantially reduced by fermentation.

LAB fermentation may have negative nutritional effects. Some lactic acid bacteria, generally mesophiles (bacteria which grow at ambient temperatures), which are the norm in traditional fermentations, produce both optical isomers of lactic acid: L(+) and D(-). Since the latter cannot be metabolized by humans, excessive intake may result in acidosis, a disturbance of the acid-alkali balance in the blood. WHO recommends that young babies should not be fed with foods containing DL or D-lactate.

## Types of LAFCFANAB

LAFCFANAB can be categorized into flour/meal, pancakes, doughs/dumplings, cakes/sweetmeats, thick porridges, thin porridges, and beverages (Table 2). This classification is inevitably arbitrary. For example, the difference between thick and thin porridges, and between thin porridges and beverages is often simply an issue of how much water is in them, which may be a matter of personal taste. Other products such as "koko," "ogi," and "pozol" are prepared as a dough but consumed as thin porridge or beverages. Another dough product "mawe" is even consumed in a wide variety of including dumplings, steamed bread, fritters, couscous, and thick porridges.

It can be seen from Table 2 that LAFCFANAB are widely distributed throughout the world, occurring in south-east Europe, right across Asia especially in the Indian subcontinent, throughout Africa, and in northern Latin America. The list is by no means complete and the impression given that the largest number of such products occurs in Africa may not be entirely correct. However, it is probably significant that there are many LAFCFANAB in Africa. Africa is the only continent that straddles both tropics and the warm ambient temperature, facilitates the natural proliferation of lactic acid bacteria in foods left to stand, as for example mesophilic Lactobacilli have a temperature optimum in the range 28-32°C.

An example from each of the LAFCFANAB categories will now be examined.

#### Flour/Meal

In northern Namibia, pearl millet is the staple and is generally eaten in the form of porridge. The traditional process of milling pearl millet involves a steeping (fermented) step. Since the country's independence in 1990, fermented pearl millet flour ("uusila" or "oufila") has started to be produced commercially in small mills as a convenience product (Figure 1a), particularly for urban dwellers and institutional feeding. The flour is also used to make a beverage ("oshikundu"), porridges, cakes, and cookies.

The steeping (fermenting) vessels and drying floor of a pearl millet mill in northern Namibia are shown in Figures 1b and 1c, respectively. The process is described in detail in Millet: Pearl. In brief, it involves abrasively de-hulling the grain, then steeping it overnight. The steep is also a lactic acid fermentation with a culture being maintained by back-slopping. The bacteria responsible are not known, but are probably heterofermentative Lactobacilli. The steeped grain is partially dried in the sun, then hammer-milled into a flour and then fully sun-dried. The fermented flour has a pH between 4 and 5, and a slight acidic taste, which is favored by consumers. The lactic acid fermentation has a number of other positive effects. It lightens and brightens the flour as a result of the low pH

<sup>&</sup>lt;sup>b</sup>Determined by *Pediococcus cerevisiae* procedure after enzyme hydrolysis.

 $<sup>^</sup>c$  Determined by *Tetrahymena pyriformis* procedure. Nutritive value relative to casein.

<sup>&</sup>lt;sup>d</sup>Numbers in parentheses are increase over nonfermented.

<sup>\* =</sup> Increase significant at P < 0.05.

<sup>\*\* =</sup> Increase significant at P < 0.01.

Reproduced with permission from Hamad AM and Fields ML (1979) Journal of Food Science 44: 456-458

 Table 2
 Examples of traditional lactic acid fermented cereal foods and nonalcoholic beverages

Flour/meal	Pancakes	Doughs/dumplings	Cakes/sweetmeats	Thick porridges	Thin porridges	Beverages
Africa Uusila/Oufila (PM) Namibia	Galettes (PM, S) Burkina Faso Injera (FM, S, T) Ethiopia Kisar (S) Chad Kisra (S) Sudan	Kenkey (M) <i>Ghana</i> Maasa/Massa/ Waina (S, PM) <i>West Africa</i> Mawe (M) <i>Benin</i>		Aceda (S) Sudan Koko (M) Ghana Banku (M) Ghana Ting (S) Southern Africa	Nasha (S) Sudan Ogi (M, PM, S) Benin, Guinea, Nigeria Togwa (M, PM, S) Tanzania Ugi/Uji (FM, M, PM, S) East Africa	Hulu mur (S) Sudan Kunun-zaki (S) Nigeria Mageu/aMahewu/ Magou (M) Southern Africa Motoho oa mabela (S) South Africa Munkoyo (M) Zambia Oshikundu (PM, S) Namibia
Europe, Middle-East, Asia	Dosa (R, S + black gram) <i>India</i>	Khanomjeen (R) <i>Thailand</i>	Anarshe (R) India Dhokla (R + Chickpea) India Idli (R + Black gram) India Jalebi (W) India Putu (R + Sugar) Philippines			Boza (B, M, O, R, W) Bulgaria, Turkey
Latin America		Jamin-bang (M) <i>Brazil</i>		Atole (M) Mexico	Pozol (M) Mexico	Agua-Agria (M) <i>Mexic</i>

 $B = barley, \ FM = finger \ millet, \ M = maize, \ O = oats, \ PM = pearl \ millet, \ S = sorghum, \ R = rice, \ T = teff, \ W = wheat.$ 







**Figure 1** Manufacture of fermented pearl millet flour (uusila/oufila) in northern Namibia: (a) bags of flour, (b) steeping (fermentation) vessels, and (c) drying steeped (fermented) de-hulled grain.

decreasing the color of the pearl millet polyphenolic pigments and also leaching some out. The fermentation also facilitates the grain being reduced into a soft flour (particle size  $< 500 \, \mu \text{m}$ ) during milling, which enables baked goods of reasonable quality to be produced from it, despite the fact that pearl millet does not contain gluten.

## **Pancakes**

"Injera," a large (50 cm diameter) leavened pancake is a staple food of Ethiopia. Injera is sour in taste, flexible and spongy in texture with a characteristic honeycomb-like structure (Figure 2a). It is served at most meals, generally with a spicy meaty relish. Injera is almost exclusively home-produced and is preferably made from teff and/or sorghum flour, although flour of other cereals may also be added. Injera is thus rather unusual in that it is a leavened bread-like product made from nongluten containing cereals.

The process for making injera is described in detail in Teff. In brief, it involves two fermentations. In the first, a starter culture ("irsho") from a previous injera batter is added to the flour dough. The fermentation takes up to 72 h, during which time the pH falls to  $\sim$ pH 3.8. A portion of the dough,  $\sim$ 20%, is then diluted with water and cooked to gelatinize the starch. This product is called "absit." The absit is then combined with the fermenting dough and a second fermentation of  $\sim$ 2 h takes place, during which time gas is generated. The dough is thinned to a batter and then poured in a circular motion over a hot clay griddle ("mitad") (Figure 2b). The "injera" is then covered and baked and steamed for 2-3 min. During this time, the starch gelatinizes setting the batter, and gas bubbles expand and escape creating the honeycomb-like structure.





Figure 2 Injera: (a) sorghum injera showing honeycomb-like structure and (b) removing from the clay griddle (mitad).

The microbiology of the injera fermentations is complex. The first fermentation is believed to be initiated by Enterobacteriaceae, then LAB including Leuconostoc mesenteroides, Streptococcus faecalis, Pediococcus cerevisiae, Lactobacillus brevis, L. plantarum, and L. fermentum are involved. Yeasts of the genera Saccharomyces and Torulopsis are involved in the second fermentation that produces the leavening gas.

#### Cakes

"Idli" is a small fermented and steamed cake with a characteristic sour taste and leavened texture, made from rice and black gram. It is widely consumed in southern India and Sri Lanka, where it is a breakfast food. Idli is consumed with chutney, pickles or curry. The combination of a legume and a cereal makes idli highly nutritious with a good essential amino acid balance. As can be seen, the idli making process (Figure 3) has some similarities with that of injera. However, for idli it is known that the main fermentation organism is Leuconostoc mesenteroides. This heterofermentative bacterium produces both lactic acid and carbon dioxide. During fermentation the pH of the batter decreases from pH 6.0 to 4.3 and the volume increases by 1.6-3.1 times. It is believed that mucilaginous polysaccharides and globulin proteins from the black gram stabilize a gas-holding foam. This enables the formation of a leavened cake. Other bacteria found in the idli fermentation include: Lactobacillus delbrueckii, L. fermentum, L. lactis, S. faecalis, and P. cerevisiae. Several types of yeasts have also been implicated in the fermentation including species of Geotrichum and Torulopsis.

As an alternative to steaming, the thick fermented batter can be diluted to a thin batter and seasoned and then fried to produce a pancake called "dosa."

## **Dumplings**

"Kenkey," a staple food in Ghana, is an orange-sized dumpling produced from fermented white maize with a solids content of  $\sim$ 35%. It is produced in the home and by street vendors. Kenkey is eaten at main meals, served with a relish often made with oily fish such as herring.

It can be seen that there are in fact two fermentations in kenkey making, the first while steeping the whole maize and the second a fermentation of the dough (Figure 4). After the second fermentation, the pH should be ~pH 4. The main LAB found in the fermenting dough are *Lactobacillus plantarum*, *L. confusus*, *L. brevis*, and *Pediococcus pentosaceus*. As the pH decreases and titratable acidity increases

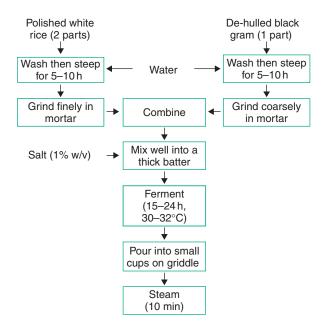


Figure 3 Process for making idli.

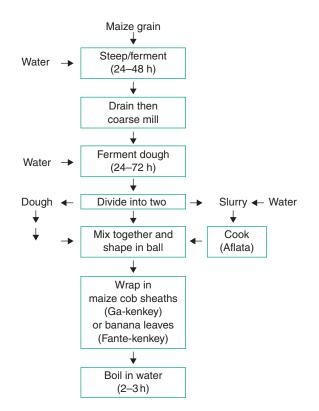


Figure 4 Process for making kenkey.

the heterofermentative types are succeeded by the more acid-tolerant homofermenters.

As in injera making, part of the dough is cooked to gelatinize the starch and hence bind the dough into a cohesive mass. However, in kenkey this dough is not





Figure 5 Ting: (a) cooked ting showing its firm consistency and (b) maintaining the culture (letlhabego) by back-slopping.

leavened by a subsequent fermentation, but simply cooked in water.

Research has been undertaken to simplify the kenkey making process for potential industrial manufacture. Developments include accelerating the fermentation process by using a starter culture, and producing dried fermented flour (like the pearl millet flour) and pregelatinized fermented flour.

## **Thick Porridges**

"Ting" is a firm, fermented sorghum porridge with a solids content of  $\sim 15\%$  and pH in the range 3.6—4.3 (Figure 5a). It is widely produced in the home in Botswana and northern South Africa. Ting is eaten as breakfast porridge and is often served at celebratory functions.

In South Africa, ting is generally produced from coarse sorghum meal, industrially manufactured for the purpose from red, condensed tannin-free sorghum. In Botswana, meal from white sorghum is generally preferred, which may be produced industrially or by small village mills. A ting-making process used in South Africa is shown in Figure 6. To start the process from scratch, a spontaneous fermentation is carried out at room temperature. The meal sours rapidly with some gas production. The fermented meal, called "pediso," can be used to inoculate more sorghum meal to produce a culture known as "letlhabego" or directly to make ting. Many households maintain a letlhabego, also known as a "yeast," by back-slopping, the use of which shortens the process of ting making (Figure 5b). As the letlhabego is so useful, it is also traded in townships. The LAB Lactobacillus delbrueckii, L. curvatus, and Leuconostoc mesenteroides have been found to be the predominant species

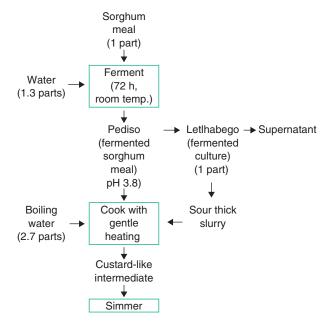


Figure 6 Process for making ting.

in the *letlhabego*. To prepare ting, the pediso or letlhabego is added to boiling water and cooked as shown. In South Africa, industrially produced ting made from maize and sorghum has been developed. It is marketed in polythene sachets and has a shelf life of several weeks, due to its low pH. The product, however, has not been commercially successful on account of its high price.

#### Thin Porridges

"Ogi" (north west Nigeria) or "akamu" (eastern Nigeria) is a thin, sour porridge of solids content

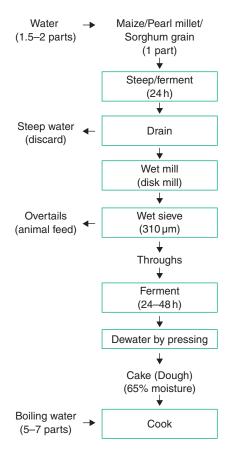


Figure 7 Traditional process for producing ogi.

between 5% and 8% or higher, depending on the desired consistency. It is a staple food in Nigeria, eaten as breakfast porridge or as a food for infants and the infirm. It is served hot, often accompanied by bean cake.

The traditional production process starts with steeping the grain (a first fermentation), wet milling, and sieving to remove coarse material (Figure 7). The throughs are then fermented by a spontaneous fermentation. The precise microorganisms responsible are not known. However, Lactobacillus, Enterobacter, and Leuconostoc species have been found to be present in sorghum ogi fermentations, with the latter being replaced by Corynebacterium species later in the fermentation. The final pH should be pH 4.1-4.5. Traditionally, ogi slurry is pressed into a dough (known as a cake), which is sold commercially by street vendors. To prepare ogi, the dough is made into a smooth slurry with cold water and added slowly to boiling water and cooked for ~2 min. It may be sweetened if so desired.

Since the dough has a shelf life of only two days, research had been conducted by the Nigerian Federal Institute of Industrial Research to produce shelf-stable products. A long-life powder has been produced by

spray drying. However, this product still requires to be cooked. An "instant" powder produced by roller drying has been developed. As it is precooked, it only requires the addition of hot water to prepare the ogi. A further development is a more nutritious composite instant ogi powder incorporating full-fat soya flour. This nearly doubles the protein content, increasing the percentage contribution to the recommended dietary allowance (RDA) of an infant from 25% to 48%. Additionally, the ogi biological value, protein efficiency ratio (PER), and digestibility are dramatically improved. Soy-ogi has become a popular product, sold in supermarkets.

## **Beverages**

"Mageu," also commonly known as "amahewu" or "magou," is a southern African maize-based fermented beverage or gruel (**Figure 8**). It is produced in the home and in institutions such as hospitals and mine hostels. It is also produced on a large industrial scale in South Africa and Botswana. Industrially produced mageu has a solids content of  $\sim 8\%$  with an energy content of  $1595 \, \text{kJ} \, \text{l}^{-1}$  and a pH of  $\sim 3.5$ . Mageu is regarded as a nutritious, energy food, ideal for those doing hard manual work.

The traditional method of making mageu simply involves preparing a maize meal porridge of 8-10% solids, allowing it to cool to room temperature and then adding a small quantity of wheat flour. The porridge will then undergo a lactic acid fermentation. The wheat flour acts both as a starter and as a source of enzymes, in particular  $\beta$ -amylase which produces a small amount of maltose which is utilized in the fermentation.

A typical modern industrial process is shown in Figure 9. The major improvements from the traditional process are the introduction of thermophilic LAB cultures which only produce lactic acid and extension of the shelf life of the product by pasteurization and/or chemical preservation. It has been stated that the LAB is *L. delbrueckii*, although there is a strong indication that the cultures are mixed and also contain *Thermobacterium* as they are very heat- and acid-tolerant. The industrial product also differs from the traditional mageu in that it is often artificially flavored with flavors such as banana, cream, pineapple, and guava (Figure 8).

Another development in mageu manufacture is using pregelatinized maize meal. The use of this so-called powder mageu is ideal for institutional feeding as it obviates the need for porridge cooking equipment. The pregelatinized maize meal is normally produced by "dry-cooking," either high-temperature, short-time extrusion, or gun puffing.



Figure 8 Commercial mageu with maize meal, the raw material, in the background.

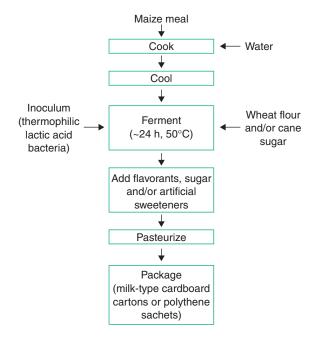


Figure 9 Industrial process for making mageu.

## **Potential for Development**

Traditional LAFCFANAB seem to be at crossroads. Although remaining very popular as home or small-scale commercially produced products, industrialization has been limited. Further, there are some indications that with rising incomes and changing lifestyles, people in developing countries turn away from traditional fermented foods. For example, in South Africa over a period of 5 years from 1996 to 2001,

industrial mageu production fell from 109 million liters (Ml) to only 76 Ml. Some scientists view this trend with great concern. It has been stated that if consumers in developing countries abandon traditional fermented foods for "smart" products popularized in Europe and America, such as cola, it could have a significant negative impact on their daily nutrition.

Perhaps this is overstating the case, but LAFCFA-NAB do have some unique functional and nutritional properties that food scientists should exploit through the development of innovative products, which satisfy the needs of today's consumers in both the less-developed and more-developed world. For example, injera seems ripe for development into a commercial product as it is a leavened bread produced from non-gluten containing cereals such as sorghum. Thus, injera could be very suitable for countries in the semi-arid tropics where wheat cannot be produced economically and it could be attractive to people who are allergic or intolerant to wheat products. Additionally, injera could make a great fast-food as it can be used as a "wrap" for meat or vegetable fillings.

A nutritional aspect of fermented cereal foods that is already being exploited in Europe is as Functional Foods, foods that have a health-promoting properties over and above their basic nutrient content. In Finland, a range of oat-based, yogurt-like products called oat Vellie is being produced (Figure 10). Three aspects of oat Vellie are novel. First, it is entirely cereal and thus is an ideal alternative to yogurt for people who are allergic or intolerant to dairy products. Second, it is rich in oat fiber which has a cholesterollowering property, and third, in addition to LAB, it



**Figure 10** Oat Vellie. (Reproduced with kind permission of Bioferme oy.)

contains the probiotic bacterium *Bifidobacterium lactis*. Many benefits have been claimed for *Bifidobacteria*, but perhaps the most important, especially for the "at risk" in developing counties, is that they are protective against gastrointestinal infections.

See also: Beverages: Distilled. Breads. Millet: Pearl. Soybean: Soy-Based Fermented Foods; Soymilk, Tofu, and Okara. Teff.

## **Further Reading**

Chavan JK and Kadam SS (1989) Nutritional improvement of cereals by fermentation. *CRC Critical Reviews in Food Science and Nutrition* 28: 349–400.

Hamad AM and Fields ML (1979) Evaluation of the protein quality and available lysine of germinated and

fermented cereals. *Journal of Food Science* 44: 456–458.

Hard NF, Odunfa SA, Lee C-H, et al. (1999) Fermented Foods. A Global Perspective. Rome: Food and Agriculture Organization of the United Nations. Also at www.fao.org.

Murty DS and Kumar KA (1995) Traditional uses of sorghum and millets. In: Dendy DAV (ed.) *Sorghum and Millets: Chemistry and Technology*, pp. 185–221. St. Paul, MN: American Association of Cereal Chemists.

Steinkraus KH (ed.) (1989) Industrialization of Indigenous Fermented Foods. New York: Marcel Dekker.

Steinkraus KH (2002) Fermentations in world food processing. Comprehensive Reviews in Food Science and Food Safety 1: 23–32.

Taylor J and Dewar J (2000) Fermented products: beverages and porridges. In: Wayne Smith C and Frederiksen RA (eds.) Sorghum: Origin, History, Technology, and Production, pp. 751–795. New York: Wiley.

Wood BJB (ed.) (1998) Microbiology of Fermented Foods, 2nd edn. London: Blackie Academic and Professional.

World Health Organization (1996) Fermentation: Assessment and Research. Report of a Joint FAO/WHO Workshop on Fermentation as a Household Technology to Improve Food Safety. WHO/FNU/FOS96.1. WHO, Geneva.

## **Relevant Websites**

http://www.fao.org — Various information, including Hard NF, Odunfa SA, Lee C-H, *et al.* (1999) *Fermented Foods. A Global Perspective*.

## FOOD SAFETY THROUGH THE PRODUCTION CHAIN

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## Introduction

Contaminated food is one of the most widespread public-health problems of the contemporary world and it causes considerable morbidity and mortality. Globally, millions of people are affected by foodborne disease. Food poisoning can be very serious in vulnerable groups such as the elderly, infants, young children, pregnant women, and immuno-compromised individuals. The proportion of vulnerable groups such as the elderly and immuno-compromised is increasing in industrialized countries.

Looking at food consumption broadly, recent highprofile food-safety emergencies and scares have shaken consumer confidence in the production of food, focusing attention on the way food is produced, processed, and marketed. These incidents have included an epidemic of bovine spongiform encephalitis (BSE) in cattle, possible links between BSE and

Table 1 Risks to food safety throughout the grain production and processing chain, and possibilities for risk minimization

Stage of grain production	Possible risks	Risk-minimization strategy
Breeding	Plant and grain diseases may leave residual contaminants	Select genotypes that are resistant or tolerant to diseases
Breeding	Antinutritional compounds may be present in mature grains	Select for genotypes that do not contain significant amounts of antinutritional components
Pure-seed production	Toxic seeds may be contaminants in harvested grain	Eliminate contaminating seeds from seed for sowing
On-farm production	Toxic seeds may be contaminants in harvested grain	Appropriate preparation of ground before sowing
On-farm production	Agricultural chemicals may remain in harvested grain	Minimize use of chemicals, especially those that persist to the mature grain
Harvesting	Spoilage organisms affecting mature grain at harvest	Harvest grain when dry, before there is opportunity for molds, etc., to attack
Grain receival	Contaminated grain is combined with sound grain	Test grain at delivery for defects and contaminants; eliminate contaminated grain from grades for human consumption
Storage	Pesticide residues may remain in grain above minimum levels	Avoid methods of grain protection involving persistent residues
Transport	Grain may be contaminated by materials in transport containers	Ensure cleanliness and sealing of transport containers
Milling	Microorganisms and filth as contaminants	Remove dust, very small grains and nongrain material before milling
Processing	Contaminants become incorporated into food products; inappropriate additives may be used as processing aids	Use best-practice procedures to ensure absence of contaminants; minimize the levels of food additives
Marketing	Consumers may be uncertain about additives and possible contaminants	Use labeling to provide assurance about maximum levels of relevant contaminants and additives

variant Creutzfeldt-Jakob disease (CJD) in humans, and the discovery of dioxin-contaminated animal feed entering the food chain. New food-borne pathogens such as *Escherichia coli* O157:H7, *Cyclospora cayentanensis*, and *Listeria monocytogenes* have also emerged. Infection with *E. coli* O157:H7 can be fatal in young children, the elderly, and the immunocompromised, and since the 1990s, serious outbreaks have occurred in the USA, Scotland, and Japan. *L. monocytogenes* can result in meningoencephalitis and/or septicemia in newborns and adults and abortion in pregnant women. Parasites such as *C. cayentanensis* and *Cryptosporidium parvuum* have emerged as a serious health threat for immuno-compromised individuals, particularly AIDS patients.

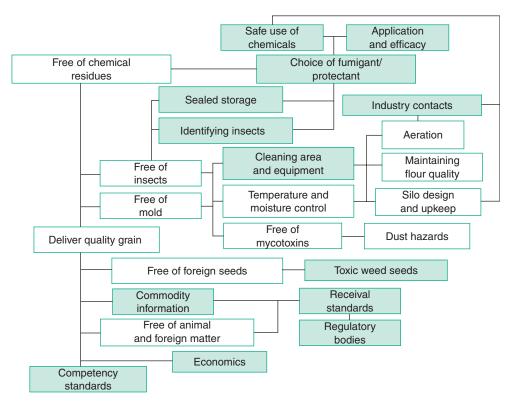
Most of these highly publicized cases are not related directly to the grains industry, but there is significant potential for food safety risks to arise at any of the various stages of grain production and processing (Table 1 and Figure 1). In addition to risks of biological origin, chemicals in foods have also caused concern, including herbicides and grain protectants, with the possibilities of residues remaining in grain-based foods. Specific chemicals are also added to grain-based foods during processing in the form of improvers, nutrients added as part of food fortification, flavorings, colors, and various other food additives. Well "downstream" of grain production,

grain-based foods come into close interaction with the full range of foods, opening up the complete range of risks relevant to the food industry in general. For example, traditional grain-based foods like bread and pastry become subject to all the risks associated with meat products when they are consumed as ham sandwiches and meat pies.

Food safety is a sensitive political and economic issue as well as an important public-health issue. Many consumers and consumer lobby groups continue to express concerns about the long-term impact of chemical and biological contaminants in food, in addition to potential chronic and acute effects on vulnerable groups. This article describes food-safety issues relating to the grains industry, as well as expanding its scope to include the wider issues in the food-processing industry. Integral to these considerations are the biological, chemical, and physical hazards in food, and possible control and prevention strategies for industry.

# Food-Safety Hazards in Grain Production and Processing

Most food-safety hazards arise from contaminating agents. The term "contaminant" covers harmful substances or microorganisms that are not



**Figure 1** A summary diagram coordinating the many aspects of food safety and quality control required in the grain-production industry. (Reproduced with permission from the Value-Added Wheat CRC, Sydney, Australia.)

intentionally added to food. Contaminants may enter the food accidentally during growth, cultivation, transport, or preparation. They may accumulate in food during storage, form in the food through the interaction of chemical components, or may be concentrated from the natural components of the food. Several risks associated specifically with the grains industries are listed in Table 1, together with strategies for limiting these hazards. Figure 1 summarizes the many aspects of food safety and quality control that must be coordinated in grain production. Most of the risks can be summarized under three headings, namely, those due to contaminants of biological, chemical, and physical nature.

#### **Biological Hazards**

Broadly in food safety, there is a wide range of biological agents of concern to public health. These include pathogenic strains of bacteria, viruses, helminthes, protozoa, and algae, and certain toxic products they may produce. In the case of grain-based foods, most of these are likely to be introduced via the nongrain components of the food. In such cases, food-borne infections are caused when microorganisms are ingested. Microorganisms may be introduced directly from infected raw materials, from

workers, from other foods, or from the environment during the preparation or processing of food. Poisonous substances may also be produced by the growth of bacteria and molds in food. Table 2 lists pathogenic organisms of public-health importance, which may be transmitted through contaminated food.

An obvious source of toxicity for harvested grains is contamination with weed seeds that are either toxic or at least unpleasant due to their contribution of taint. Furthermore, seeds with a dark seedcoat are likely to cause dark specks in the flour. Table 1 indicates this risk, plus the likely remedies of ensuring that seed for sowing is free of contaminating weed seeds and that the ground where the seed is to be sown must be correctly prepared to avoid the risk of these weed seeds germinating and growing together with the crop seeds.

Further biological hazards of grains arise due to defects that occur during growth. For example, for wheat and barley, these include infections such as ball smut (also bunt or stinking smut), caused by *Tilletia caries* or *T. foetida*, which replace the endosperm of the grain by black spores with an unpleasant odor.

A classical disease of cereals, especially rye, is caused by ergot (*Claviceps purpurea*) infecting the flowers of the cereal grain, producing an ergot body in place of the grain. These ergot bodies are recognizable in grain samples as black bodies (sclerotia) larger

Table 2 Examples of major biological hazards in foods

Bacteria (spore-forming) Clostridium botulinum C. perfringens Bacillus cereus	Viruses Hepatitis A and E Norwalk virus group Rotavirus
Bacteria (nonspore-forming) Brucella abortis B. suis Campylobacter spp. Pathogenic Escherichia coli (E. coli O157:H7, EHEC, EIEC, ETEC, EPEC) Listeria monocytogenes Salmonella spp. (S. typhimurium, S. enteritidis) Shigella (S. dysenteriae) Staphylococcus aureus Streptococcus pyogenes Vibrio cholera V. parahaemolyticus V. vulnificus Yersinia enterocolytica	Protozoa and parasites Cryptosporidium parvum Diphyllabothrium Entamoeba histolytica Giardia lamblia Ascaris lumbricoides Taenia solium T. saginata Trichinella spiralis

Source: FAO (1998) Food Quality and Safety Systems. A Training Manual on Food Hygiene and the Hazard Analysis and Critical Control Point (HACCP) System. Rome: Food and Agricultural Organization of the United Nations

and longer than the normal grain length, but they break up easily, and of course, they disappear during milling. Ergot may also be present in grain consignments due to the infection of contaminating grains, especially ryegrass. Ergot is a source of toxic alkaloids (Table 3), so there are strict limits on the maximum levels permissible in grains for human or animal consumption.

A range of microorganisms may risk the food safety of grains as early as at harvest time. Microbiological contamination is not uncommon in harvested grain; contaminants may include a wide range of species, including *Bacillus* species, coliforms, molds, and yeasts. These organisms cannot readily be removed from the grain at harvest, so they may continue through milling and processing. Nevertheless, the levels of microbiological contamination are generally low for dry sound grains of cereals, for example.

On the other hand, grain that is harvested very moist, e.g., over 17% moisture, may suffer from the very serious condition of the production of mycotoxins, produced by fungi of the genera *Aspergillus*, *Fusarium*, and *Penicillium*. The specific mycotoxins produced by these fungi are listed in Table 3. Mycotoxins are active at very low concentrations, even at a few ppb. They are not always produced in moldy grain, but the risk is obviously best avoided by keeping grain dry and free from molds. Immuno-assay kits are now available for the determination of various mycotoxins in "field" situations within minutes, providing

 Table 3
 Important mycotoxins of grains produced by infecting fungi

9.		
Genus and species	Mycotoxin	Disease symptoms
Claviceps purpurea Aspergillus flavus A. parasiticus A. nominus	Ergot alkaloids Aflatoxin	St Anthony's fire Carcinogenic
A. ochraceus A. carbonarius Penicillium verrucosum	Ochratoxin	Neurotoxic, nephrotoxic, immunotoxic
Fusarium culmorum F. equisetti F. graminearum	Type B trichothecenes (Vomitoxin)	Acute toxicity, immunotoxic
F. poae F. sporotrichioides	Type A trichothecenes	Acute toxicity, immunotoxic
F. culmorum F. equisetti F. graminearum F. semitectum	Zearalenone	Estrogenic effects, infertility, still births, abortion
F. avenaceum F. graminearum F. culmorum	Moniliformin	Cardiotoxic

Source: Waalwijk C (2003) Detecting mycotoxin contamination of cereals. In: Cauvain SP (ed.) *Bread Making: Improving Quality*. Cambridge, UK: Woodhead Publishing.

the opportunity of checking for this risk "on-thespot," in contrast to the need some years ago of reliance on laboratory-based analysis taking some days for results.

Excessive grain moisture is likely to cause deterioration on storage and during transport, due to increased risk of attack by insects and molds. For this reason, there are critical limits for the maximum moisture for grain that may be received at the grain elevator or mill. This maximum varies between graingrowing regions and between grain species, but it is generally in the range 12–14.5%. Artificial drying is thus a requirement in many grain-growing regions.

At the stage of flour milling, there is the possibility of reducing the bacterial count for wheat. This is because the highest levels of contamination by microorganisms are found in the light (low-density) fraction of raw wheat and in the grain dust. Therefore, special attention must be paid to the removal of this material prior to milling. For infected grain, it has proved possible to reduce the bacterial count greatly by applying the various cleaning methods in a selective manner (removal of impurities, aspiration, classification, and scouring).

Genetically modified (GM) grains are considered as a "hazard" by some consumers. As a result of the controversy about the new genetics, there has been the need to identify GM varieties of some grain species, especially maize and canola, so that these are kept separate throughout grain receival, storage, transport, and processing, so that the food products can be labeled appropriately (Table 1).

# **Chemical Hazards**

Food-borne toxicants can be categorized according to their chemical nature. Some food-borne toxicants are inorganic, e.g., lead and arsenic, whereas others are organic, e.g., polychlorinated biphenols (PCBs). Major categories of man-made chemicals that occur in foods are shown in Table 4. Chemical hazards may result from pollution arising from industrial and other human activities (e.g., lead, mercury, cadmium, and PCBs), from agricultural practice (e.g., pesticides, fertilizers) or from food processing and packaging (e.g., nitrosamines and certain polycyclic aromatic hydrocarbons). These contaminants may present a potential hazard for human health if exposure exceeds tolerable levels.

Most obviously for the grain industry, there is the risk of residues from agricultural chemicals occurring at levels above the specified minimal residue limits. These grain protectants are used justifiably to avoid another hazard, namely, insect infestation. The value of chemicals for this purpose is the residual protection of grain during extended storage and transport. However, there are alternatives, including fumigation with compounds that leave virtually no residue, or storage in an enriched atmosphere of carbon dioxide.

Some grains contain natural chemical hazards, such as a variety of antinutritional components. Some of

Table 4 Examples of chemical hazard in foods

Industrial/environmental pollution	Food additives
Polychlorinated biphenyls (PCBs)	Vitamins and minerals
Agricultural chemicals	Contaminants
Pesticides	Lubricants
Fertilizers	Cleaners
Antibiotics	Pest-control chemical
Growth hormones	Coatings
Inorganic chemicals	Paints
Lead	Chemicals migrating
Tin	from packaging
Mercury	Plasticizers
Zinc	Vinyl chloride
Cadmium	Printing/coding inks
Arsenic	Adhesives
Cyanide	Lead
Additives	Tin
Feed additives	
Veterinary drugs	
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Source: FAO (1998). Food Quality and Safety Systems. A Training Manual on Food Hygiene and the Hazard Analysis and Critical Control Point (HACCP) System. Rome: Food and Agricultural Organization of the United Nations

these are naturally occurring inhibitors of digestive enzymes. In such cases, there may be the need for various pretreatments to overcome the antinutritional effects of such compounds. There is the added possibility that suitable application of plant-breeding methods may be able to select for genotypes that lack the potentially toxic components (Table 1). Celiac disease is a specific case of a naturally occurring "toxin" that affects certain people; in this case, the problem compound is the gluten—protein complex. These gluten-like proteins are present in wheat, rye, triticale, barley, and possibly oats.

# **Physical Hazards**

Examples of physical hazards are foreign matter, including dirt and glass that accidentally get into food. They can be the result of environmental contamination during production, processing, storage, packaging, and transport, or from fraudulent practices. Further examples that are specifically relevant to grains include materials of biological origin, such as rodent hairs and insect pieces. The identification of these fragments may involve microscopical examination, plus reference to catalogues of commonly found contaminating materials. Simple tests are available as backup for cases of identification, such as the use of ultraviolet examination for the presence of rodent urine.

The potential for ionizing radiation to have long-term health effects, not only on the people living nearby but also the health of the ecosystem, is considerable. Following the nuclear reactor failure at Chernobyl (Ukraine) in 1986, food contaminated with radionucleides with long half-lives, such as cesium 137, is the major source of exposure for people living in this area. Table 5 lists examples of physical hazards in food.

Table 5 Examples of physical hazards

Material	Sources
Glass	Bottles, jars, light fixtures, utensils, gauge covers, etc.
Wood	Field sources, pallets, boxes, building materials
Stones	Fields, buildings
Metal	Machinery, wire
Insulation	Building materials
Bone	Improper processing
Plastic	Packaging, pallets, equipment
Personal effects	Employees

Source: FAO (1998) Food Quality and Safety Systems. A Training Manual on Food Hygiene and the Hazard Analysis and Critical Control Point (HACCP) System. Rome: Food and Agricultural Organization of the United Nations.

# **Ensuring Food Safety**

The ultimate objective of the food industry and government regulators is to insure that food reaching the consumer is safe and wholesome. Feed manufacturers, farmers, and food operators have the primary responsibility for food safety. Government regulators and consumer associations also have a role to play. However, food-safety emergencies over the last 2 decades have exposed weaknesses in the food industry from farm to fork. They have shown that considerable progress is needed to come to grips with the preventive aspects of food-borne disease.

# **World Trade and International Standards**

The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have complementary food-safety mandates to protect the health of consumers, to prevent the spread of disease and to ensure that the procedures followed in food trade are fair. The Codex Alimentarius Commission (CAC), which manages the joint FAO/WHO food-standards program, sets international standards for food. It was established in 1961–62 by the FAO and WHO, and it aims to protect the health of consumers and ensure fair practices in food trade.

The World Trade Organization (WTO) rules provide a framework for the application of food-safety measures in international trade. The standards and guidelines of the CAC are recognized by the WTO as the international benchmark standards, guidelines, and recommendations to be used as the basis of harmonizing food safety measures affecting human health and world food trade.

International standards influence trade as well as food safety, and national governments are under pressure to ensure that their regulatory programs are comprehensive and conform to these global standards. Owing to the complexity of the food-production chain, the traditional role of government as the food-safety inspector has changed, with the food industry taking more responsibility for the implementation of quality-assurance programs throughout food production, distribution, and retail. However, a strong national food-control agency is required to bring about cooperation between government departments and industry, and to set standards and targets for health protection.

# **Risk Analysis and Food Safety**

The concept of risk analysis is gaining acceptance internationally as a very important component of national food-control programs, and its principles are being integrated more fully into the work of

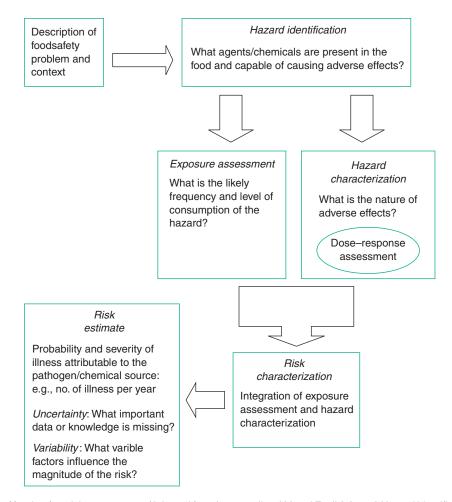
food safety regulators and policy-makers. This principle consists of three components: risk assessment, risk management, and risk communication.

#### **Risk Assessment**

Risk assessment is a science-based investigation that can provide an estimate of the probability and impact of adverse health effects attributable to potentially contaminated foods. The aim is to identify, in some quantitative or comparable way, the relationship between hazards and actual exposure to harm. The detail and complexity of a given risk assessment will vary, depending on the availability of time, resources, and scientific information. A full risk assessment may not always be carried out, because data may be incomplete, and resources may be inadequate. However, decisions that are made by well-focused assessments, using whatever data are available, will usually be more reliable and transparent than subjective judgment. Risk assessment links into the other two components of risk analysis, the others being risk management and risk communication. The stages of the risk-analysis process are closely linked and cannot be separated easily. Consideration of the ways in which a risk might be managed is necessarily part of the assessment and communication.

Risk assessment consists of four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization (Figure 2). In hazard identification, the association between disease and the presence of a pathogen or contaminant in food is documented. Hazard characterization involves obtaining quantitative information about the hazard, including, where possible, information on doseresponse relationships. Exposure assessment estimates the intake or exposure to a chemical or pathogen, in terms of its magnitude, duration, and frequency, for the general population, for subgroups or for individuals. Risk characterization is the integration of hazard identification, hazard characterization, and human intake/exposure assessment. It assesses the likelihood of a particular event. This is the framework adopted by the CAC, the international standard setting body for foods in international trade.

Risk assessment is already fairly well developed for chemical hazards. The estimation of chemical health risks often relies on data obtained in the customary rodent-feeding assays using standardized safety margins. Such assessments call for an extrapolation of response data derived from animal studies to humans. The setting of advisory standards, such as acceptable daily intakes (ADIs) for food additives, pesticides, and veterinary residues in food, is a key component in the overall risk assessment of many chemicals subject to



**Figure 2** Steps of food-safety risk assessment. (Adapted from Lammerding AM and Fazil A (2000) Hazard identification and exposure assessment for microbial food safety risk assessment. *International Journal of Food Microbiology* 58: 147–157.)

regulatory control. Quantitative risk assessment is an emerging tool in the field of microbial food safety. Estimation of microbiological hazards presented by foods is more complex, because of biological diversity and variability. However, such estimations are usually more accurate than estimates on adverse effects from chemicals in foods. This is because incidents are frequent, in sharp contrast to those resulting from exposure to chemicals. This allows for a better risk assessment.

# **Risk Management and Risk Communication**

Food scares such as salmonellae in eggs, *E. coli* O157 in beef and BSE have all damaged consumer confidence in regulatory agencies. Such problems were compounded when government departments had the dual role of promoting farming and the food industry as well as protecting the consumer. Such roles resulted in a conflict of interest. A regulatory agency should ideally be independent of trade and economic

interests and have consumer interests as its primary function. Although it is the primary responsibility of the food industry to comply with the laws and regulations, the regulatory agency should provide an integrated inspection system from farm to fork. This may best be achieved by establishing a national food standards agency that has responsibility for all sectors of the food chain. Many countries have established such agencies and coordinate the work of all government departments involved in food safety. Such coordination overcomes the problems of duplication of work and conflicts of interest.

A food-borne disease surveillance program is also an essential part of a food-control program. Surveillance is necessary to identify food-borne contaminants, their sources, and their modes of transmission in order to determine the best short- and long-term control measures. Interpretation of trends and investigation of outbreaks can help identify the mechanisms by which contamination and disease transmission occurred. When an outbreak occurs or a chemical

Table 6 Seven principles of the hazard analysis and critical control point (HACCP) system

Principle 1

#### Conduct a hazard analysis

Prepare a list of steps in the process where significant hazards occur and describe the preventive measures. Assess the likelihood of occurrence of the hazard(s) and identify the measures for their control.

Principle 2

#### Identify the Critical Control Points (CCPs) in the process

Determine the points, procedures, or operational steps that can be controlled to eliminate the hazard(s) or minimize its (their) likelihood of occurrence

Principle 3

Establish critical limits for preventative measures associated with each identified CCP

Principle 4

#### **Establish CCP monitoring requirements**

Establish procedures from the results of monitoring to adjust the process and maintain control.

Principle 5

Establish corrective actions to be taken when monitoring indicates a deviation from an established critical limit

Principle 6

Establish effective record-keeping procedures that document the HACCP system

Principle 7

Establish procedures for verification that the HACCP system is working correctly

Reproduced with permission from Mortimore S and Wallace C (1994) HACCP: A Practical Approach. London: Chapman and Hall.

contamination incident is identified, it is vital to learn from the investigation what went wrong in order to devise strategies to prevent similar events in the future.

# **Quality Assurance via HACCP Principles**

Food quality and safety can only be insured through the application of quality-control systems throughout the entire food chain. They should be implemented at farm level with the application of good agricultural practices, good manufacturing practice at processing, and good hygiene practices at retail and catering levels. One of the most effective ways for the food sector to protect public health is to base their foodmanagement programs on hazard analysis critical control point (HACCP).

HACCP consists of seven principles that outline how to establish, implement, and maintain a quality-assurance plan for a food establishment. It is a systematic approach to the control of potential hazards in a food operation and aims to identify problems before they occur. Control is proactive, since remedial action can be taken. The HACCP principles have international recognition, and the seven principles are listed in Table 6.

HACCP plan adoption has greatly improved the food industry's ability to design programs to insure the safety of food. It was introduced in the early 1970s and has become the premier system for preventing food-borne hazards, particularly those of microbiological origin. The basic process in creating HACCP plans is that the main hazards associated with a food

product are determined, and key steps in the production, processing, distribution, marketing, and preparation of food are controlled within pre-established limits to insure the safety of food. A program of monitoring, verification, and record-keeping is then implemented to insure that the system functions correctly.

The Codex General Principles of Food Hygiene recommend a HACCP-based approach to enhance food safety. This management tool is internationally recognized as essential to ensure food safety and is recommended by governments, industry, and consumers.

# Conclusion

Food safety in the early twenty-first century is an international challenge requiring close cooperation between countries in agreeing standards and in setting up transnational surveillance systems. The lessons of recent decades are plain to those engaged in the food industry. No longer can farmers grow just what they want or use technical aids to farming without taking into account the effect on the quality of the food produced. Food will always present some risk, and it is the task of the food industry to keep the level of risk to the minimum, which is practicable and technologically feasible. It should be the role of official bodies and the food industry to use risk analysis to determine realistic and achievable risk levels for food-borne hazards and to base food-safety practices on the practical application of the results of these analyses, thus continuously improving the safety of food and thereby lowering the disease risk. By providing effective

food-control, countries not only provide public-health benefits, but also enable themselves to participate in international food trade with greater confidence.

The lasting solution to the problems, which have shaken the food industry in recent years, can only be overcome if all those involved – farmers, food processors, wholesalers, transporters, retailers, caterers, scientists, regulators, and government - work together towards common goals.

See also: Cereals: Grain Defects; Grain Diseases. Chemicals for Grain Production and Protection. Consumer Trends in Consumption. Contaminants of Grain. Cultural Differences in Processing and Consumption. Fortification of Grain-Based Foods. Genetically Modified Grains and the Consumer. Labeling of Grain-Based Foods. Nutraceuticals from Grains. Nutrition: Beriberi. A Deficiency Related to Grains; Guidelines for Grain-Based Foods; Effects of Food Processing. Organic Growing of Grains. Stored Grain: Handling from Farm to Storage Terminal; Invertebrate Pests; Physico-Chemical Treatment. Appendix: Foods for Celiac Diets.

# **Further Reading**

- Bryan F (1992) Hazard Analysis Critical Control Point. A Guide to Identifying Hazards and Assessing Risks Associated with Food Preparation and Storage. Geneva: WHO.
- Commission of the European Communities (1999) White Paper on Food Safety. Com, 719 Final. Brussels.
- Dillon M and Griffith C (1996) How to HACCP, 2nd edn. London: M.D. Associates.
- FAO (1998) Food Quality and Safety Systems. A Training Manual on Food Hygiene and the Hazard Analysis and Critical Control Point (HACCP) system. Rome: Food and Agricultural Organization of the United Nations.
- FAO (1999) Report of the Conference on International Food Trade Beyond 2000: Melbourne, Australia. 11-15 October 1999. Rome: Food and Agriculture Organization of the United Nations.
- FAO/WHO (1995) Application of Risk Analysis to Food Standards Issues. Report of the Joint FAO/WHO Expert Consultation. Geneva: WHO.
- Jones JM (1992) Food Safety. St. Paul, MN: American Association of Cereal Chemists.
- Lammerding AM and Fazil A (2000) Hazard identification and exposure assessment for microbial food safety risk

- assessment. International Journal of Food Microbiology 58: 147–157.
- Leaper S (1997) HACCP: A Practical Guide, Technical Manual No. 38. UK: Campden and Chorleywood Food Research Association.
- Miller JD and Trenholm L (eds.) (1994) Mycotoxins in *Grain:* Compounds Other than Aflatoxin. St. Paul, MN: American Association of Cereal Chemists.
- Mills R and Pedersen J (1990) A Flour Mill Sanitation Manual. St. Paul, MN: American Association of Cereal Chemists.
- Mortimore S and Wallace C (1994) HACCP: A Practical Approach. London: Chapman and Hall.
- Posner ES and Hibbs AN (2003) Wheat Flour Milling, 2nd edn. St. Paul, MN: American Association of Cereal
- Tothill IE (2003) Rapid and On-line Instrumentation for Food Quality Assurance. Cambridge, UK: Woodhead
- Unnevehr LJ (2000) Economics of HACCP: Costs and Benefits. St. Paul, MN: American Association of Cereal Chemists.
- Waalwijk C (2003) Detecting mycotoxin contamination of cereals. In: Cauvain SP (ed.) Bread Making: Improving Quality. Cambridge, UK: Woodhead Publishing.

# **Relevant Websites**

http://www.aaccnet.org - American Association of Cereal Chemists.

http://www.awb.com.au - AWB Ltd, Melbourne, Australia.

http://www.campden.co.uk - Campden and Chorleywood Food Research Association.

http://www.cgc.ca - Canadian Grain Commission, Winnipeg.

http://www.fsis.usda.gov - Codex Committee on Food Additives and Contaminants.

http://www.wheat.pw.usda.gov - Graingenes.

http://www.icc.or.at - International Association for Cereal Science and Technology.

http://www.crop.cri.nz - New Zealand Institute of Crop & Food Research.

http://www.sgrl.csiro.au - Stored Grain Research Laboratory, Canberra, Australia.

http://www.usda.gov - United States Department of Agriculture.

# FORTIFICATION OF GRAIN-BASED FOODS

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# Introduction

Fortification is a way of providing different nutrients using staple foods as agents, with the aim of supplementing the diet through the intake of these nutrients. Such supplements could be an important tool in combating nutrient deficiencies. In this article, the following aspects concerning fortification will be considered:

- criteria for fortification:
- cereals as vehicles for food fortification;
- processes for cereal fortification;
- additives used as a nutrient source; and
- legislation concerning cereal fortification.

# **Definition**

Fortification can be defined as the addition of one or more vitamins and/or minerals to a food, regardless of its usual content in the food. Additions are carried out based on generally accepted scientific knowledge of the role of vitamins and minerals in attaining good health. Foods are fortified for the following reasons:

- to prevent or correct a demonstrated deficiency of one or more vitamins and/or minerals in the population or specific population groups; and
- to improve the nutritional status of the population and dietary intakes of vitamins or minerals due to changes in dietary habits.

Enrichment is a term usually interchanged with fortification. The term "enriched flour" was coined during the Second World War when the American government decided to improve the soldiers' diet by including different nutrients in bread. Initially, the enrichment consisted of the addition of the three major B-vitamins (thiamine, riboflavin, and niacin) and iron, despite the scarce scientific information concerning the real nutritional improvement obtained with that proposal. Later, numerous scientific studies have been conducted for verifying the utility of those measures.

Ideally, enrichment should be equivalent to restoration. This means that the addition of vitamins and

minerals, present in the edible portions of the food, will restore the levels lost during manufacturing, storage, and handling.

The other relevant term in this subject is "nutritional equivalence," which means "being of similar nutritive value in terms of quantity and bioavailability of vitamins and minerals." Since fortification is a broader term, it will be used here while referring to the addition of nutrients.

# **Criteria for Food Fortification**

Minerals and vitamins may be added to foods only for the purpose of (1) restoration of nutrients lost during processing; (2) nutritional equivalence of substitute foods; (3) fortification; and (4) ensuring the appropriate nutrient composition for a special purpose food.

Some considerations should be taken into account, prior to addition of any nutrient to foods:

- The nutrient added should be present at a level that will not result in either an excessive or an insignificant intake, considering the amount provided from other dietary sources. The maximum safe amount to be added should be set considering the levels established by generally accepted scientific data and bearing in mind the diverse degree of sensitivity shown by different consumer groups.
- Nutrients should not be added to fresh products including meat, fish, fruits, and vegetables, neither to beverages containing more than 1.2% by volume of alcohol.
- The nutrient added should be biologically available from the food and should not interfere in the metabolism of any other nutrient.
- The nutrient added should be sufficiently stable during packaging, storage, distribution, and end use, and should not affect both the sensory characteristics and the shelf life of the food.
- Technology and processing facilities should be available to permit the addition of the nutrient and also methods of measuring and controlling the levels of nutrient added.
- Fortification should not be used as a tool for providing nutrients in substitution of the natural nutrient sources, as this would lead to a change in the dietary patterns and thus jeopardize good dietary practices.

It is clear that the policy for food fortification has a completely different objective when considering developed and developing countries. In developed countries, the social changes (industrialization, high per capita income, consumption of fast food outside the home) over the years have led to increasing consumption of more highly processed foods. This has been used to justify the necessity of food fortification for ensuring the adequate intake of nutrients, mainly vitamins and minerals. However, there is no general consensus about the extent to which food fortification should be allowed. There are differing opinions that manufacturers could use fortification as a promotional tool resulting in an excessive intake of certain nutrients that would represent a risk for consumer health.

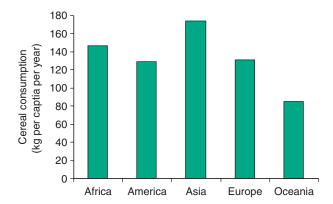
There are different considerations involved in the establishment of food fortification programs in developing countries compared to those in the developed ones. Food fortification is one approach towards solving nutrient deficiencies in developing countries, and so far the implementation of these programs has been very successful in correcting nutritional deficiencies within a very short period. In the developing countries, care should be taken that the food selected as a medium for the nutrient addition is stable and consumed by the population at risk, and that the amount of nutrient added is sufficient to correct the possible deficiency.

# Cereal-Based Products as Vehicles of Minerals and Vitamins

One of the most critical decisions concerning food fortification is the selection of the appropriate food to act as a carrier of the nutrient. The basic criteria for identifying the food products for fortification include selecting a food that is commonly eaten by the target population group, affordability, and availability all through the year.

Cereals and cereal-based products constitute a major component of diet around the world. Cereals have been used as a basis of human diet from the ancient times. Nowadays, cereal production is estimated to be 1862 million tons (Mt), out of which  $\sim$ 950 Mt is consumed by people. In fact, cereal consumption is  $\sim$ 150 kg per capita per year (Figure 1), with the highest consumption in Asia, in contrast to Oceania, where that value drops to 85 kg per capita per year. These values reflect the significance of cereals to the human diet.

Globally, cereals account for 48% of total energy intake. When the contribution of the cereals to the daily energy intake is analyzed continent-wise (Figure 2), it is evident that cereals provide the maximum percentage of calories in Asia and Africa, and that their contribution decreases to 32% in Europe and America. Slight changes to that trend are



**Figure 1** Cereal consumption (kg per capita per year) in different continents. (Data from FAOSTAT (2003).)

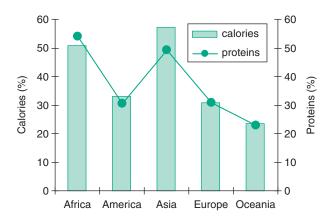
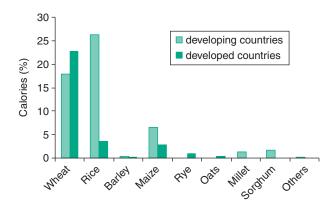


Figure 2 Percentage of calories and proteins contributed by cereals to human diet. (Data from FAOSTAT (2003).)

observed when analyzing the contribution of cereals to the percentage of protein intake. Cereals are a cheap source of proteins compared to animal proteins. In Africa, cereals supply up to 33 g of proteins per person daily; that means 54% of the daily protein intake. Similar values are found in Asia, where cereals provide 51% of the daily protein intake, while that value drops to 20% in the developed countries.

When the cereal contribution is analyzed (Figure 3), it can be observed that rice accounts for 26% of the total energy intake in the developing countries, and for only 4% of the total energy intake in the developed countries. Wheat provides 23% of the total energy intake in developing countries, while that value decreases to 18% in the developed countries. The other cereals have a minor contribution to the energy and protein intake; only maize is an important supplier of energy in the developing countries.

These data illustrate the popularity of cereals in the diet worldwide, and therefore the importance of cereals, mainly rice and wheat, as a staple medium for fortification in both developed and developing countries.



**Figure 3** Percentage of calories provided by different cereals to the diet. (Data from FAOSTAT (2003).)

# Vitamins and Minerals Added in Cereal Fortification

Once the food to be fortified is selected, the nutrient deficiencies affecting the population must be determined. Nutrient deficiencies are different in the developing and developed countries. While vitamin A, iodine, and iron are found to be deficient in the former, in the developed countries B-vitamins, iron, calcium, and folic acid are currently added to wheat flour to counter deficiency. There are only certain vitamins and minerals that may be added to foods (Table 1).

In general, all the rice-consuming countries have a vitamin A deficiency, which is associated with corneal lesions that can lead to partial or total blindness, and with reduced resistance to infectious diseases. This would, in consequence, lead to an increase in morbidity and mortality. Another deficiency associated with rice-consuming countries is anemia due to iron deficiencies. This has been linked to reduced resistance to infection and also affects the cognitive development and physiological functions in children and, in severe cases, causes maternal deaths. Iodine constitutes the third major deficiency in rice-consuming countries. This mineral is necessary for proper fetal development and also for normal physical and mental activities in adults.

In the developed countries, iron supplementation has been a measure adopted in some countries to reduce the prevalence of iron-deficiency anemia. B-vitamins (thiamine, riboflavin, and niacin) and calcium are also supplemented in developed countries to ensure the adequate intake of those nutrients. Recently, some studies, confirming the role of folates in congenital malformations (neuronal tube defect) and the development of chronic diseases in elderly people like Alzheimer's, have recommended the supplementation of folic acid in cereal-based foods. However, the effectiveness of this fortification program in

Table 1 Vitamins and minerals that may be added to foods

Vitamins	Minerals
Vitamins  Vitamin A  Vitamin D  Vitamin E  Vitamin K  Vitamin B <sub>1</sub> Vitamin B <sub>2</sub> Niacin  Pantothenic acid  Vitamin B <sub>6</sub> Folic acid  Vitamin B <sub>12</sub> Biotin  Vitamin C	Minerals  Calcium Magnesium Iron Copper Iodine Zinc Manganese Sodium Potassium Selenium Chromium Molybdenum Fluoride
Vitariiii C	Chloride Phosphorus

the decrease of homocysteine concentration and its relationship with a reduction in the incidence of cardiovascular disease will not be available for several years.

# **Methods for Cereal Fortification**

Cereals are not directly consumed as grains; processing is generally required to transform the grain prior to its consumption. That process results in a substantial reduction of different nutrients, as B-vitamins and iron, mainly concentrated in the outer layers and germ. Cereal-fortification methods have been developed to restore the nutrients that have been removed during milling and to improve the nutrient intake level of specific population.

# **Rice Fortification**

Although rice and wheat are the most appropriate cereals for food fortification, rice requires special methods for fortification as it is commonly consumed as a whole grain and is extensively washed prior to cooking or strained after cooking.

There are different methods for rice fortification; the earliest one was the production of parboiled rice. In the parboiling process, rice is steamed under pressure to gelatinize the starch within the kernel; this process allows the transference of nutrients from the bran layer to the starchy endosperm.

Other methods currently used are the powder and grain enrichment methods. In the powder enrichment method, a powder preblended with mixtures of vitamins and minerals is added to the rice. For white parboiled rice, the blend is added immediately after milling because the heat and moisture content of the grain surface at that moment facilitates the

adherence of the powder mixture. However, the nutrient supplementation in this method can be washed off if the grains are rinsed before cooking.

The grain enrichment method involves the addition of a powdered mixture of vitamins and minerals and a subsequent coating of the grain with a waterinsoluble substance. Usually grains enriched by this method contain a high-nutrient concentration and they are mixed in a proportion of 0.5% with normal milled rice, to finally obtain an enriched product that meets the required standard levels. Different variations of these methods have been developed through the years. For instance, the powdered mixture can be sprayed onto the rice and then fitted by the application of water-insoluble coating like ethanol, isopropanol, palmitic acid, or cellulose derivatives. This method has been successfully used for enriching rice with niacin, thiamin, pyridoxine, vitamin A, vitamin E, folic acid, iron, and zinc by adding alternative layers of nutrients and coatings.

Recently, an alternative method consisting of the development of fortified simulated rice has been recommended. It involves the production of synthetic rice by extrusion of rice flour in the presence of vitamins and minerals to a rice kernel shape. The fortified simulated grains are then mixed with normal milled rice. The disadvantage of this method is that the consistency of the resulting grains after cooking is different from that of the natural product. In addition, the consumer often notices an off-type texture and physically removes those grains before cooking. This is obviated by dyeing them a distinct color, so the consumer clearly knows that it should be present.

#### Flour and Pasta Fortification

Fortification of flour is usually carried out in mills, where the mixture of the required nutrients is added to the flour. The addition of B-vitamins, iron, and calcium is a common practice in some developed countries. However, the nutrient added must have good stability during storage.

In pasta-consuming countries, pasta and noodles are used for fortification. The production of fortified pasta or noodles requires only the addition of the nutrient at the mixing stage prior to the dough-extrusion process, or instead previously fortified flour can be used for this purpose. Some vitamin losses are produced during the drying process, although the extent of that loss depends on the temperature reached during drying.

# **Breakfast-Cereal Fortification**

The fortification of ready-to-eat breakfast cereals is a well-established practice. In fact, this plays an important role in ensuring the adequate intake of nutrients by the US population.

Vitamins are added to the basic recipe prior to processing when they are heat stable, like niacin and riboflavin, or sprayed onto the processed breakfast cereal in the case of heat-labile vitamins (vitamin A, C, and thiamine), ensuring the uniformity of the coverage. Hydrophobic vitamins, e.g., vitamins A and D, need special consideration as emulsions need to be sprayed on the cereals. In these products, the packaging material is very important, as the moisture content should be below 5% to avoid the texture modification of the product.

# Compounds Used as a Source of Vitamins and Minerals

Three different requirements should be considered when selecting the source of nutrients: the chemical compound (1) should be stable during packaging, storage, distribution, and end use, (2) should not modify the sensory characteristics (appearance, texture, taste, and aroma), and (3) should ensure the bioavailability of the nutrient. Different chemical compounds are used as a source of vitamins and minerals depending on their use (Tables 2 and 3).

**Table 2** Compounds that may be added to cereals as a source of vitamins

Vitamins	Chemical compound
Vitamin A	Retinol
	Retinyl acetate
	Retinyl palmitate
	Beta-carotene
Vitamin B <sub>1</sub>	Thiamin hydrochloride
	Thiamin monohydrate
Vitamin B <sub>2</sub>	Riboflavin
	Riboflavin 5' phosphate
Niacin (B <sub>3</sub> )	Nicotinic acid
	Nicotinamide
Pantothenic Acid (B <sub>5</sub> )	D-pantothenate, calcium
	D-pantothenate, sodium
Vitamin B <sub>6</sub>	Pyridoxine hydrochloride
	Pyridoxine 5' phosphate
	Pyridoxine dipalmitate
Vitamin B <sub>12</sub>	Cyanocobalamin
	Hydroxocobalamin
Vitamin C	Ascorbic acid
	Sodium ascorbate
	Calcium ascorbate
	Ascorbyl 6-palmitate
Vitamin E	Tocopherol
	Tocopheryl acetate
	Tocopheryl acid succinate
Folic acid	Pteroylmonoglutamic acid

Nutrient stability is very important in the case of vitamin fortification. Vitamins are sensitive to heat, oxidizing and reducing agents, light, and other physical and chemical stress. For instance, vitamins are stable when added to flour but the combination of high humidity and temperature adversely affect the stability of the vitamin A. This vitamin is labile to high temperature, and unstable in the presence of oxygen and light, due to its susceptibility to oxidation. Therefore, when vitamin A is added, a protection provided by packaging materials and appropriate conditions of storage should be used in order to reduce the vitamin loss. Encapsulation of this vitamin in a more hydrophilic coat is a common practice in order to obtain a more water-dispersible product.

The supplementation of B-vitamins to flour is faced with the problem of low stability of these compounds

**Table 3** Compounds that may be added to cereals as a source of minerals

Minerals	Chemical compound
Calcium	Calcium carbonate
	Calcium chloride
	Calcium salts of citric acid
	Calcium gluconate
	Calcium lactate
	Calcium hydroxide
Copper	Cupric carbonate
	Cupric citrate
	Cupric gluconate
	Cupric sulfate
Iodine	Sodium and potassium iodide
	Sodium and potassium iodate
Iron	Ferrous carbonate
	Ferrous citrate
	Ferric ammonium citrate
	Ferrous gluconate
	Ferrous fumarate
	Ferrous lactate
	Ferric diphosphate
	Elemental iron (carbonyl + electrolytic +
	hydrogen reduced)
	Hydroxocobalamin
Magnesium	Magnesium acetate
	Magnesium carbonate
	Magnesium chloride
	Magnesium salts of citric acid
	Magnesium oxide
	Magnesium sulfate
Manganese	Manganese carbonate
	Manganese sulfate
	Manganese chloride
	Manganese citrate
Phosphorus	Calcium salts of orthophosphoric acid
Zinc	Zinc acetate
	Zinc chloride
	Zinc oxide
	Zinc sulfate
	Zinc carbonate

during storage. Hence, higher effectiveness is obtained when B-vitamins are added at the bakery rather than at the mill.

The bioavailability of added nutrients, particularly iron, varies widely depending on the iron compound used for fortification. The physical and chemical properties of the food to be fortified and the iron form used determine which iron compound would be suitable for the purpose. The selection of the iron form is based on organoleptic considerations, bioavailability, cost, and safety. Iron powders are extensively used in flour fortification, but they are poorly absorbed and their absorption is highly dependent on the meal served (phytates, calcium, and polyphenols decrease the bioavailability of iron). For instance, ferric orthophosphate is often the chosen form for rice fortification due to its light color and water insolubility, but it has a very poor absorption. Ferric pyrophosphate is also used, as it does not affect the appearance, texture, aroma, and flavor of the cooked rice. Ferrous sulfate is also used because of its high bioavailability but it often leads to unpleasant colors and flavors due to reactions with other components of the food matrix. Some of these interactions with other food components can be avoided by coating the iron form with hydrogenated oils or ethyl cellulose. Electrolytic iron has also been used for its increased bioavailability.

# **Legislation Concerning Cereal Fortification**

Although cereal fortification can be an effective means of improving micronutrient status, there are some barriers to the general implementation. These include concerns related to a proliferation of the fortified foods if the fortification is voluntarily practiced by manufacturers, which would lead to a simultaneous replacement of the nonfortified foods in the diet. The success of fortification programs always depends on good control, so they should be set up, regulated, and enforced by the national governments. An uncontrolled food fortification program could result in excessive intakes of certain nutrients creating nutrient imbalances, and in consequence representing a risk to the health of consumers.

The addition of nutrients is generally practiced by manufacturers either voluntarily or because it is compulsory under national rules. In general, vitamins and minerals may be added in conformity with the legislation of the country in which the product is being sold. The Economic and Social Department of FAO has elaborated a review about the legislation status concerning rice and wheat flour fortification (Tables 4 and 5).

Table 4 Summary of voluntary and mandatory fortification of rice

Country	Law status	Legislation	Known nutrient deficiencies
Angola		No	Vit. A, I, Fe
Australia	Prohibited		
Canada	Voluntary	B <sub>1</sub> , B <sub>3</sub>	
Costa Rica	Voluntary	No	I, Fe
Cuba	Voluntary	No	Fe, Vits. B <sub>1</sub> , C, A
El Salvador	·	No	Vit. A, I, Fe
Ethiopia		No	Vit. A, I, Fe
Finland	Prohibited	Restoration allowed	
Gambia		No	I, Fe, Ca, folic acid, Vit. B <sub>2</sub>
Haiti		No	
Hungary	Voluntary	1 serving must contain 1/3 of RDA	
Mauritania	•	No	I, Fe, folic acid
Morocco	Voluntary	No	Vit. A, I, Fe
New Zealand	Prohibited	In process of reviewing the legislation	
Norway	Prohibited		
Peru	Voluntary	No	
Philippines	Voluntary	Legislation for B <sub>1</sub> , B <sub>3</sub> , Fe	Vits. A, B <sub>1</sub> , B <sub>2</sub> , I, Fe
Sweden	,	May be allowed with special permission	, ,, _, ,
Tanzania		No	Fe, I, folic acid, Vit. A
Turkey	Voluntary	In accordance with Codex	Fe, I, Ca, Vits. B <sub>2</sub> , B <sub>6</sub> , C, D
UK	Voluntary	Must be safe	
Uruguay		No	Fe, I, Vit. D
USA	Voluntary	B <sub>1</sub> , B <sub>2</sub> , B <sub>3</sub> , folic acid	
Vietnam	Voluntary	No	Vit. A, I, Fe

Table 5 Summary of voluntary and mandatory fortification of wheat flour

Country	Law status	B-vitamins	Vitamin E	Folic acid	Iron	Zinc	Magnesium	Calcium
Australia	Voluntary	B <sub>1</sub> , B <sub>2</sub> , B <sub>3</sub> , B <sub>6</sub>	+	+	+	+	+	_
Canada	Mandatory	B <sub>1</sub> , B <sub>2</sub> , B <sub>3</sub>			+			
	Voluntary	B <sub>5</sub> , B <sub>6</sub>		+			+	+
Chile	Mandatory	$B_1, B_2, B_3$			+			
Costa Rica	Mandatory	$B_1$ , $B_2$ , $B_3$			+			
Dominican Republic	Mandatory	$B_1, B_2, B_3$			+			
Ecuador	Mandatory	$B_1, B_2, B_3$		+	+			
El Salvador	Mandatory	$B_1, B_2, B_3$			+			
Finland	Prohibited							
Guatemala	Mandatory	$B_1, B_2, B_3$		+	+			
Honduras	Mandatory	$B_1, B_2, B_3$			+			
Hungary	Voluntary							
Malta	Voluntary	$B_1, B_2, B_3$			+			
New Zealand	Prohibited							
Nigeria	Mandatory	$B_1, B_2, B_3$			+			
Norway	Prohibited							
Panama	Mandatory	$B_1, B_2, B_3$			+			
Philippines	Voluntary	+			+			
Saudi Arabia	Mandatory	$B_1, B_2, B_3$			+			
Sweden	Voluntary	$B_1, B_2, B_3, B_6$			+			
Switzerland	Voluntary	$B_1, B_2, B_3$			+			
UK	Mandatory	$B_1$ , $B_3$			+			
Uruguay		$B_1$ , $B_3$			+			+
USA	Mandatory	$B_1$ , $B_2$ , $B_3$		+	+			
Venezuela	Mandatory	$B_1, B_2, B_3$			+			

Rice fortification is voluntary in USA; however, most of the rice sold is fortified, and a recent regulation requires that rice to which folic acid has been added be labeled as enriched rice. In Canada, fortification of rice is also voluntary, and when labeled as enriched, it has added vitamin B<sub>1</sub>, vitamin B<sub>3</sub>, and iron. Moreover, in Canada the addition of vitamins B<sub>5</sub> and B<sub>6</sub>, and folic acid is optional. Fortification of rice in Philippines has a long tradition, and there is a mandatory regulation for the addition of vitamin B<sub>1</sub>.

There are currently 14 countries with legislations or regulations that mandate the fortification of wheat flour, whereas in countries such as Finland, New Zealand, and Norway law prohibits the addition of nutrients to wheat flour. In United States, the Food and Drug Administration approves the supplementation of four B-vitamins (thiamine, niacin, riboflavin, and folic acid) and iron to the wheat flour, obtaining "enriched flour," although this practice is regulated by Federal law. Recently, the USA government has passed a mandatory regulation concerning folic acid fortification. In the United Kingdom, it is required by law that the nutrients removed with the bran during milling be replaced in all types of flours, with the exception of whole meal. In addition, white and brown flour must have thiamine, niacin, iron, and calcium. From 1997, the Organización Panamericana de la Salud (OPS) is supporting the supplementation of iron to wheat flour due to its low cost and extensive consumption. In the previous years, different countries from South and Central America have implemented this measure for reducing the incidence of irondeficient anemia. The levels of nutrients to be added are also regulated by national governments.

# **Future Prospects**

Currently, in the developed countries the term "functional foods" or "nutraceuticals" seems to be old-fashioned, and the cereal-based products surely may be one of the best carriers of different compounds related to physiological functions. Therefore, the near future in nutrition could be the use of cereal-based products as carriers of  $\omega$ -fatty acids and probiotic compounds such as fructo-oligosaccharides, inulin, etc.

See also: **Nutrition**: Beriberi, A Deficiency Related to Grains; Guidelines for Grain-Based Foods.

# **Further Reading**

Baurenfiend JC and DeRitter E (1991) Foods considered for nutrient addition: cereal grain products. In: Baurenfiend JC and LaChance PA (eds.) *Nutrient Additions to Food*, pp. 143–209. Connecticut: Food and Nutrition Press.

FAO/WHO (1995) Report of the 9th Session of the Codex Committee on Cereals, Pulses and Legumes. Rome: FAO.

Hoffpauer DW and Wright SL, III (1994) Iron enrichment of rice. In: Marshall WE and Wadsworth JI (eds.) *Rice Science and Technology*, pp. 195–204. New York: Marcel Dekker.

Hurrel R (1999) The Mineral Fortification of Foods. Surrey: Leatherhead International Ltd.

Hurrell RF (1996) The role of the food industry in iron nutrition. Iron intake from industrial food products. In: Hallberg L and Asp N (eds.) *Iron Nutrition in Health and Disease*, pp. 341–346. England: John Libbey and Company Ltd.

Ranum PM (2000) Cereal enrichment and nutrient labelling. In: Kulp K and Ponte JG (eds.) *Handbook of Cereal Science and Technology*, 2nd edn., pp. 697–704. New York: Marcel Dekker.

Rosell CM (2003) The nutritional enhancement of wheat flour and related products. In: Cauvain S (ed.) *Bread-Making: Improving Quality*, pp. 253–269. Cambridge: Woodhead Publishing.

Whittaker P and Dunkel V (1995) Iron, magnesium, and zinc fortification of food. In: Berthon G (ed.) *Handbook of Metal-Ligand Interactions in Biological Fluids*, vol. 1. New York: Marcel Dekker.

# **Relevant Websites**

http://www.fao.org — FAO (2002), Economic and Social Department. This web site includes the report of an FAO technical meeting held in 1995 related to Food Fortification: Technology and quality control. http://apps.fao.org — This web site provides statistics on commodities, food supply, food balance sheets, food aid, population, and the Codex Alimentarius.

# **FUEL ALCOHOL PRODUCTION**

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# Introduction

The production of ethanol or ethyl alcohol ranks among one of the oldest technologies starting with fruit and honey, and finally grain as fermentation media. Several centuries later, sugar cane, corn dry milling, corn wet milling, and ethylene are playing dominant roles in alcohol production.

The production of ethanol by the US corn refining industry began before the Second World War. It was used as a processing aid in the manufacture of rubber and as a fuel extender. After the war, the necessity of producing ethanol faded, but it got its first boost as a gasoline extender during the oil embargo of the 1970s when it offered a more economical alternative to high oil prices. Thus, fuel alcohol began as "gasohol," a popular gasoline extender during this period of oil shortage. Later when oil prices dropped, it helped fuel companies solve an environmental problem by replacing lead as an octane-enhancing fuel ingredient. In recent years, as urban centers struggle to achieve cleaner air, ethanol serves as a fuel oxygenate to control carbon monoxide problems and as an additive in reformulated gasoline to reduce smogforming emissions. This article summarizes world ethanol production data, production processes, and ethanol's enhanced and diverse utilization in the fuel industry.

# **Global Ethanol Production Statistics**

Ethanol is considered by far the largest fermentation end use worldwide. Global ethanol production increased from 28.7 billion liters (7.6 billion gallons) in 1993 to 33.3 billion liters (8.8 billion gallons) in 1998. Brazil accounted for most of the increase in production, with the US showing an erratic trend and EU's production remaining static in recent years. Table 1 shows that Brazil is the largest producer with 49% of global output followed by the United States (17%), China (8%), and European Union (7%).

# **Ethanol Production in Brazil**

Brazil has the largest bio-ethanol industry worldwide. It began its fuel ethanol program in 1975. In the mid-1980s,  $\sim$ 75% of all the new cars sold ran on pure ethanol. Like the US, its growth in ethanol production has been driven by government policy aimed at reducing national dependence on oil and also at supporting farm incomes.

Ethanol is produced directly from sugar cane juice as well as from molasses that are a by-product of sugar processing. Brazil's choice of sugar cane as feedstock is likely to be copied by other countries like India, Thailand, and possibly Mexico. One of the significant changes in Brazil is the liberalization by the government of its domestic alcohol market, which followed the earlier liberalization of the sugar sector. The sugar cane industry consists of  $\sim$ 250 mills. About one-third of those mills produce only ethanol, and nearly all of the remaining mills can process both sugar and ethanol. There is considerable flexibility among the mills to produce either sugar or ethanol, depending on the relative return from these two commodities. Alcohol demand has been relatively flat in recent years due to gradual scrapping of vehicles powered solely by alcohol. All petrol sold still contains 26% ethanol, a mixture on which standard petrol engines can run without adaptation.

#### **US Ethanol Production**

Ethanol production in the US received a major boost in 1990 with the passage of the Clean Air Act Amendments providing for the establishment of oxygenated fuel program and reformulated gasoline program in an attempt to control carbon monoxide and ground-level ozone problems. Both programs require certain oxygen levels in gasoline: 2.7% by weight for oxygenated fuel and 2.0% by weight for reformulated

Table 1 Global shares of ethanol production in 1998

Country	Percent share
Brazil	49
United States	17
China	8
European Union	7
Russia	4
India	4
Thailand	1
Saudi Arabia	1
South Africa	1
Others	8

O'Connor J and Cropley J (1998) Recent ethanol developments: production, policy, and switch capacity. In: Starch and Fermentation Analysis June 1998. New York, NY: LMC International Ltd.

gasoline. Combined ethanol demand in 2000 for reformulated gasoline and oxygenated fuel programs was estimated at 650 million gallons per year.

Seventy-four ethanol manufacturing plants with yearly capacity of 10.2 billion liters (2.7 billion gallons) of ethanol used diverse feedstocks during production with corn being the dominant feedstock (Table 2). Majority of ethanol is produced in Illinois, Indiana, Iowa, Kansas, Minnesota, and Nebraska. About 90% of ethanol output is derived from corn. Several new plants opened in 2003 bringing the yearly production capacity to 3.7 billion gallons. The US ethanol industry is expanding, boosted by the growth prospects for cleaner burning fuels.

About 690 million bushels of corn in 2001 were processed to produce ethanol (Table 3). The amount of corn utilization for ethanol production increased from 1991 to 1994, and then dipped in 1995. Since then, the amount of corn usage has been increasing steadily from a low of 396 million bushels to a high of 690 million bushels.

**Table 2** Feedstocks used in ethanol manufacturing plants in the United States

Feedstock	Number of manufacturing plants
Corn	54
Corn/milo	4
Cheese whey	3
Beverage waste	3
Corn/wheat starch	2
Potato waste	2
Corn/barley	1
Corn/milo/wheat starch	1
Brewery waste	1
Sugars and starches	1
Milo	1
Waste beer	1

Reproduced with permission from Renewable Fuels Association (2002). Growing *Homeland Energy Security: Ethanol Industry Outlook 2002*. Washington, DC: Renewable Fuels Association.

 Table 3
 Corn utilization for ethanol poduction during 1991–2001

Year	Corn usage (million bushels)
1991	398
1992	426
1993	458
1994	533
1995	396
1996	429
1997	481
1998	526
1999	566
2000	628
2001	690

# **EU Ethanol Production**

The total ethanol output in EU was ~2.3 billion liters (608 million gallons) in 1997. France was the single largest producer followed by Germany and the United Kingdom. In France, ethanol is produced by fermentation of sugar beet and wheat. Ethylene-derived ethanol is also available from these three EU countries. In Italy, Spain, and Portugal, a substantial amount of alcohol is produced from distillation of surplus wine.

# The Fuel Ethanol Industry

To produce fuel-grade ethanol, anhydrous ethanol is denatured in accordance with the regulations of the US Bureau of Alcohol, Tobacco, and Firearms. Typical denaturants are unleaded gasoline or natural gasoline. The minimum denaturant level is 2 gallons for every 100 gallons of ethanol. Since the denaturant is less expensive than ethanol, the maximum permitted level of 5 gallons of denaturant are typically added for every 100 gallons of ethanol. Specifications for denatured fuel ethanol are defined in ASTM D 4806 (Table 4). In the US, fuel-grade ethanol is currently blended into conventional gasoline, reformulated gasoline, and suboctane gasoline with the most common blend level being 10% v/v ethanol. Blends of 5.7% v/v ethanol (equating to 2% by wt. of oxygen) and 7.7% v/v (equating to 2.7% by wt. of oxygen) are produced to achieve regulatory compliance levels. At 10% v/v blend level, ethanol is usually added to 87 octane regular to make a mid-grade product, and to a mixture of 87 octane regular and 92 octane premium to make a premium product. When ethanol is blended to suboctane gasoline with 84 or 84.5 octane, the finished blend is 87 octane.

**Table 4** ASTM D 4806 standard specification for denatured fuel ethanol for blending with gasoline for use as automotive spark-ignition engine fuel

Ethanol, vol.%, min.	92.1
Methanol, vol.%, max.	0.5
Solvent-washed gum, mg/ml, max.	5.0
Water content, vol.%, max.	1.0
Denaturant content, vol.%, min.	1.96
vol.%, max.	4.76
Inorganic chloride content, mass ppm (mg l <sup>-1</sup> ), max.	40 (32)
Copper content, max, mg kg <sup>-1</sup>	0.1
Acidity (as acetic acid CH <sub>3</sub> COOH) max, mass% (mg I <sup>-1</sup> )	0.007 (56)
рНе	6.5-9.0
Appearance	Visibly free of suspended or precipitated contaminants (clear and bright)

Ethanol is sold as an oxygenate or high-octane fuel that delivers improved vehicle performance and reduced engine knock while reducing harmful emissions and improving air quality. It is used to replace the fuel oxygenate, methyl tertiary butyl ether, which is being phased out as it is known to cause ground and surface water contamination and is resistant to microbial degradation. Ethanol is nontoxic and is quickly biodegradable in surface water, ground water, and soil. It contains 35% oxygen, and adding it as an oxygenate to gasoline improves the efficiency of fuel combustion resulting in reduced tailpipe emissions of volatile organic compounds and fine particulates that pose a health threat to humans.

Ethanol dramatically reduces carbon monoxide emissions, which are responsible for as much as 20% of urban smogs. It also reduces greenhouse gases emitted from vehicles, including carbon dioxide, methane, and other gases that contribute to global warming. A full-cycle analysis by the US Argonne National Laboratory concluded that per gallon of ethanol used in E-10 fuel (10% v/v blend of ethanol with gasoline) reduces greenhouse gas emissions by 12–19%, petroleum use by 90–93%, and fossil energy use by 40%. Ethanol serves as an excellent fuel additive in reducing pollution from off-road vehicles, such as motorcycles, all-terrain vehicles, and snowmobiles, which lack pollution control devices. As an alternative fuel, ethanol reduces a country's reliance on imported crude oil and gasoline, provides new market and adds value to agricultural feedstocks, and promotes rural development.

In addition to the traditional use of ethanol as a low level blend component in conventional or reformulated gasoline, other possible transportation fuel uses of ethanol like E-85, oxydiesel, aviation fuel, and fuel cells have varying degrees of potential. The term E-85 is used to denote fuels containing 75–85% v/v of denatured ethanol, with the remainder of the blend comprised of either conventional or reformulated gasoline, which is in the early stages of commercialization. Currently, the highest profile alternative fuel use for ethanol is the use of E-85 in flexible fuel vehicles. Per gallon use of ethanol in E-85 fuel reduces greenhouse gas emissions by 17–24%, petroleum use by 92–95%, and fossil energy use by 44%.

Oxydiesel is in the experimental or demonstration phase. It consists of a blend of traditional diesel fuel blended with up to 15% denatured fuel-grade ethanol and special additives. It can be used in existing diesel engines without modification. In a truck demonstration test performed in 1998, the performance characteristics of oxydiesel were very similar to No. 2 diesel.

Ethanol's application in aviation fuel has been examined with primary focus on piston-powered aircraft

(spark ignition/internal combustion), but minimal amount of work has been done on turbine-powered aircraft (jet engines). Recently, an aviation-grade E-85 (AGE 85) has been developed. AGE 85 has 88% ethanol, and was certified by US Federal Aviation Administration for use in Cessna 180 and Cessna 182 engine-airframe combinations.

Ethanol, which contains 13% hydrogen, also has applications in fuel cells. Fuel cells work by combining hydrogen and oxygen in a chemical reaction to create electricity, without the noise and pollution of internal combustion engines. In principle, a fuel cell operates like a battery, but unlike a battery a fuel cell does not run down or require recharging. As long as fuel is supplied, it will produce energy in the form of electricity and heat with low emissions, which primarily consist of water and steam. Because ethanol has relatively simple molecular structure and is easy to transport and store, it lends itself to fuel reforming (i.e., the chemical process for extracting hydrogen from fuel) with relative ease. The primary benefits that ethanol provides in fuel cells include the following: liquids with high-energy density store well in vehicles; delivery through existing fuel infrastructure; less toxic than methanol and gasoline; does not pose a threat to the environment in the event of a spill; easier to reform than gasoline, hydrocarbons, and most alternative fuel options; reduction in greenhouse gas emissions; and reduced reliance on imported fossil fuel energy.

# **Feedstocks**

Unlike any other liquid fuel alternative, ethanol is derived from a wide range of renewable feedstocks by fermentation technology. The major carbohydrate sources for ethanol fermentation are: (1) simple sugars from sugar cane, molasses, sugar beet, sweet sorghum, fodder beets, and fruits; (2) inulin, a fructose polymer, from Jerusalem artichoke, dahlia, and chicory; (3) starch from cereal grains (corn, milo, wheat, rye, sorghum, barley, triticale, and rice) and tubers (potatoes, sweet potatoes, and cassava); (4) cellulosic plant materials from wood, crop residues (corn stover, coconut husk, and wheat straw), and forages (alfalfa and sudan grass); and (5) by-product carbohydrates from food processing (whey, cannery waste, vegetable waste, sugar cane bagasse, and fruit waste) and paper production (waste sulfite liquor, wood waste, and pulp mill waste). The predominant feedstock is corn in the US and sugar cane in Brazil, but many agricultural crops, by-products, or process waste have been sourced for fermentable carbohydrates (Tables 2 and 5). The US, China, EU, Brazil, and Mexico are the top five corn-producing countries in the world (Table 6) (see Grain Production and

Table 5 Raw material usage for ethanol production among different countries

Country	Raw material
Brazil	Sugar cane
US	Corn
	Molasses
	Synthetic
France	Sugar beet
	Synthetic
	Wheat
	Molasses
	Wine
UK	Synthetic
	Potatoes
Germany	Grains
	Synthetic
	Molasses
Italy	Molasses
Spain	Wine
Portugal	Synthetic
Saudi Arabia	Synthetic
India	Molasses
China	Molasses
	Variety of agricultural products

O'Connor J and Cropley J (1998) Recent ethanol developments: production, policy, and switch capacity. In: *Starch and Fermentation Analysis June* 1998. New York. NY: LMC International Ltd.

**Table 6** World corn production in 2000-01

Country	Production, million bushels		
Country			
US	9 507		
China	4 252		
EU	1 530		
Brazil	1 417		
Mexico	709		
Argentina	453		
India	445		
South Africa	354		
Romania	331		
Canada	323		
Hungary	287		
Others	3 360		
Total	22 967		

Consumption: Overview). Of the roughly 23 billion bushels of corn produced in the world, 9.5 billion bushels representing 41% of total was produced in the US. The share of China, EU, Brazil, and Mexico represents 19%, 7%, 6%, and 3% of the world's total corn production, respectively.

Ethanol can also be manufactured from ethylene, a petroleum-derived chemical, via synthesis. The manufacturing process consists of combining ethylene with water vapor at elevated temperature and pressure, and passing over the surface of a catalyst support impregnated with phosphoric acid.

# **Conversion of Feedstocks to Sugars**

To produce monomeric, fermentable sugars, starchy feedstocks undergo a traditional cooking process to gelatinize the starch followed by liquefaction with  $\alpha$ -amylase and saccharification with glucoamylase. The efficiency of liquefaction is a function of temperature, residence time, rate of heat-up, uniformity of heat distribution, and the amount and heat stability of liquefying enzyme. Saccharification depends on enzyme level, pH, temperature, and residence time. Modifications of the process evolved with respect to choice of processing conditions appropriate to a particular plant facility. Other technologies have emerged in succeeding years. For example, sulfuric acid hydrolysis of starch wastes was practiced in a potato chips industry. Another technology is steam explosion pretreatment of potato at high pressure, which hydrolyzed it into low-molecular-weight liquid

Cellulosic feedstocks require a more elaborate conversion process to generate fermentable sugars, and more often would involve pretreatment processes because of the intractable nature of the raw material. Some of these pretreatment processes involve ammonia-fiber explosion, steam explosion, supercritical carbon dioxide explosion, ultrasonic, acid-catalyzed steam, and alkaline hydrogen peroxide. Primarily, the processes are designed to enhance the susceptibility of treated feedstocks to enzymatic hydrolysis, thereby increasing the yield of fermentable sugars. Typical technological applications in diverse cellulosic materials include ammonia-fiber explosion of corn fiber; steam explosion or alkaline hydrogen peroxide treatments of sugar cane bagasse; supercritical carbon dioxide explosion of sugar cane bagasse and recycled paper mix; and ultrasonic and acid-catalyzed steam treatments of waste office paper.

# **Fermentation Processes**

Yeasts (Saccharomyces cerevisiae) are facultative anaerobes, which can ferment (see Fermentation: Origins and Applications) industrial sugars derived from any feedstocks (e.g., starch, cellulose, inulin, or sugars) to produce alcohol even in the presence of small amount of oxygen in the mash. The mash is fermented under anaerobic conditions except for the initial small amount of air present during the inoculation step. Fermentation of simple sugars by yeast goes through four characteristic phases: (1) the lag phase when the yeast cells become acclimated to their new environment; (2) the exponential growth phase when the yeast cells propagate rapidly; (3) the stationary phase; and (4) the death phase when alcohol

concentration is high and available sugar for yeast metabolism is low.

Yeasts use the glycolytic or Embden-Meyerhof pathway to break down sugars into energy, several intermediates which the yeast requires for cell growth, and large amounts of the major end products, ethanol and carbon dioxide, which the cell excretes. The efficiency to produce ethanol is influenced by the strain of yeast, pH, temperature, sugar concentration, nutrients, and microbial infection. The theoretical yield of ethanol can be calculated using stoichiometry. Every 1000 pounds of glucose (originating from hydrolysis of 901 pounds of starch) fermented by yeast produce 511 pounds of ethanol and 489 pounds of carbon dioxide. Thus, the yield of ethanol based on glucose is 51.1% (w/w) and, if calculated based on starch, the yield of ethanol is 56.7% (w/w). Actual yields of ethanol are generally slightly lower than the theoretical yield because  $\sim$ 5% of the sugar is utilized by the yeast to produce new yeast cells and minor products such as glycerol, acetic acid, lactic acid, and fusel oils. Fermentation efficiency can be expressed as the ratio of the actual weight of alcohol produced and the theoretical weight of alcohol produced from glucose multiplied by 100.

The production of ethanol from grain feedstock involves two processes: dry milling and wet milling. In dry milling (see Maize: Dry Milling), the entire kernel from corn or other grains is first ground into flour (meal) and slurried with water to form the mash. The mash is processed in a high-temperature cooker, cooled and treated with liquefying and saccharifying enzymes to convert starch to glucose. The converted mash is further cooled and transferred to batch fermenters where yeast is pitched. For continuous fermenters, the time of enzyme and yeast addition is adjusted for a simultaneous saccharification and fermentation process. Fermentation of glucose to ethanol and carbon dioxide begins, and generally takes  $\sim$ 40–50 h to complete. After fermentation, ethanol is stripped from the beer, and the remaining stillage is centrifuged to separate the coarse grain from the solubles. Concentration of the solubles by evaporation yields the syrup or condensed distillers solubles, and co-drying of the coarse grain and syrup yields the dried distillers grains with solubles. The ethanol stream is concentrated to 190 proof by distillation and then dehydrated to 200 proof by ternary azeotropic distillation or by molecular sieve technology.

In wet milling (see Maize: Wet Milling), the kernel of corn is steeped in water containing dilute sulfurous acid for 24–48 h. The steeped kernels are ground to separate the germ. The remaining fiber, gluten, and starch components are separated using centrifuges, screens, and hydrocyclones. Steeped liquor is concentrated by

evaporation, and co-dried with the fiber component to yield corn gluten feed. The gluten component is filtered and then dried to produce corn gluten meal. The fermentation of starch slurry to ethanol is conducted similarly as the dry milling process described above with respect to cooking, enzyme conversion, and yeast fermentation. Current corn wet-milling technology produces 2.5 gallons of ethanol per bushel of corn, while dry-milling technology produces 2.7 gallons of ethanol per bushel of corn. Increased fermenter capacity and reduced production cost can be realized by recovering corn fiber ("quick fiber process") and germ ("quick germ process") before fermentation.

One method to achieve increased production of ethanol without building new facilities is through the adoption of high gravity fermentation. In the fuel alcohol industry, normal gravity mash concentrations between 20 and 24 g dissolved solids per 100 g of mash are fermented to produce ethanol ranging from 9% to 12% v/v. Mash concentrations ranging from 25 to 38 g dissolved solids per 100 g of mash are termed very high gravity, and during fermentation can yield as high as 24% v/v ethanol. The technology of very high gravity fermentation had been employed in the production of fuel alcohol using barley, oat, wheat, rye, or triticate as feedstock. The benefits of very high gravity fermentation technology include: considerable saving of water; reduced distillation costs; reduced capital costs; lower energy cost per liter of alcohol; reduced risk of bacterial contamination; and higher ethanol production with a given plant capacity and labor costs.

#### **Biomass Fermentation**

Cheap and renewable feedstocks for ethanol production are readily available worldwide, in the vast quantities of agricultural residues (biomass). Using waste plant materials such as corn stover, coconut husks, wheat straw, or wood waste as a renewable energy source makes good sense. For example, waste disposal problems may be converted into profits as ethanol or other products. Disposal costs in landfill or as sewage are reduced. In addition, it is a solution to air pollution problems in some areas that are sensitive to clearing of harvested fields by burning.

The potential yield of ethanol in liters/hectare/year from cellulosic biomass ranks second to sugar cane, but higher than sweet sorghum and corn. Biomass contains cellulose, hemicellulose, and lignin, which pose difficult challenges during fermentation into alcohol. Hydrolysis yields not only six-carbon sugars (hexoses), but also five-carbon sugars (pentoses) that are not readily fermented by yeast to alcohol.

A Canadian company developed the bioethanol process, which has advanced technology to produce ethanol from biomass. The bioethanol process involves pretreating plant fibers, enzyme hydrolysis, fermentation, and alcohol distillation. The yield of ethanol is  $\sim 300 \, \text{lt}^{-1}$  of fiber.

Microorganisms from several species are capable of transforming cellulosic feedstocks from different sources into ethanol. Recombinant Klebsiella oxytoca strain P2 was utilized in simultaneous saccharification and fermentation of acid-treated bagasse to yield ethanol. The same *Klebsiella* strain produced ethanol by fermentation of sugar beet pulp. Commercial feasibility of ethanol production from bagasse is possible using cellulase from mutant strains of Trichoderma reesei and continuous fermentation with immobilized Saccharomyces cerevisiae AD-3. Pichia stipitis CBS 5773 and a genetically engineered xylose-fermenting yeast produced ethanol from bagasse hydrolyzates.

Thermophilic strain of Bacillus stearothermophilus fermented lignocellulosic wastes from wheat straw and sugar cane bagasse into ethanol under anaerobic conditions. Some species of anaerobic and thermophilic Clostridium can also ferment plant wastes from sugar cane bagasse, corn stover, and forestry residues into ethanol. Fermentation of bagasse, corn stover, and corn hulls plus fiber by genetically engineered Escherichia coli strain KO 11 was substantially complete within 48 h. Genetically engineered E. coli KO 11, E. coli SL 40, E. coli FBR 3, Zymomonas mobilis CP 4 (pZB 5), and S. cerevisiae 1400 (pLNH 32) fermented hydrolyzates of corn fiber to ethanol. Other feedstocks such as rice hull and mixed-waste office paper were also successfully fermented by recombinant E. coli KO 11.

# **Pentose Sugar Fermentation**

One of the drawbacks of fermenting waste cellulosic materials is the inability of common fermentative organisms to utilize pentose sugars such as xylose and arabinose. Mainly fungi and nontraditional yeasts are capable of fermenting pentose sugars to ethanol. Rhizopus strains have the ability to ferment glucose, xylose, and arabinose as well as complex substrates such as cellulose, oat-spelt xylan, corn fiber, and corn germ pressing. A cellulytic fungus, Aspergillus terreus, was able to ferment glucose and other hexoses, pentoses, and disaccharides to ethanol. Two fungi, Candida entomaea NRRL Y-7785 and Pichia guilliermondii NRRL Y-2075, preferentially utilized glucose > xylose > arabinose from mixed substrates and produced ethanol, xylitol, and arabitol. Using a mixture of glucose, xylose, and arabinose in ratios typical of corn fiber hydrolyzates, cultures of *Pichia* 

guilliermondii strain NRRL Y-12723 preferentially utilized glucose and only slowly metabolized xylose and arabinose.

Candida auringiensis, Candida succiphila, Ambrosiozyma monospora, and Candida species YB-2248 have the ability to ferment arabinose to ethanol. These yeasts can also ferment xylose. Nontraditional yeasts, Candida shehatae, Pachysolen tannophilus, and Pichia stipitis, fermented a mixture of glucose and xylose to produce ethanol. Organic nitrogen sources enhanced ethanol and xylitol production from xylose during fermentation by Candida shehatae ATCC 22984. Pachysolen tannophilus immobilized in calcium alginate beads fermented xylose to ethanol on a continuous basis. The yeast Candida peltata NRRL Y-6888 preferentially utilized glucose > xylose > arabinose from mixed substrates.

The yeast Pichia stipitis NRRL Y-7124 has the ability to ferment xylose to 76% of theoretical ethanol yield. Lignocellulose hydrolyzates containing xylose and glucose provided good substrates for ethanol production by Pachysolen tannophilus NRRL Y-2460 and S. cerevisiae NRRL Y-2235. A fermentation process produced ethanol from pretreated biomass comprising xylose and cellulose using cellulase,  $\beta$ -glucosidase, xylose isomerase, and Saccharomyces pombe ATCC No. 2476.

E. coli KO 11 was reported to be capable of fermenting xylose into ethanol. Mutants of E. coli KO 11 have been used to ferment arabinose and xylose to ethanol selectively in the presence of high concentrations of glucose. Two hyperproductive strains of the mutants retained the ability to metabolize all three sugars with faster fermentation and higher alcohol yields than KO 11. A novel ethanologenic E. coli strain FBR3 acts as biocatalyst for converting lignocellulosic hydrolysates containing mixed sugars (e.g., arabinose, xylose, and glucose) into ethanol.

Genetic engineering played a significant role in overcoming the inability of common microorganisms to ferment pentose sugars. S. cerevisiae strain 1400 (pLNH 32) has been genetically engineered to ferment xylose. This recombinant strain fermented a mixture of glucose, xylose, arabinose, and galactose into ethanol at 90% yield in 24 h. Another recombinant organism, *Klebsiella oxytoca* strain P2, has the ability to ferment mixed sugars in which sugar utilization is in the order of glucose > arabinose > xylose and ethanol production is in the order of xylose > glucose > arabinose. Recombinant strains Klebsiella oxytoca and E. coli can ferment pentose sugars to ethanol with high selectivity and at reasonable ethanol concentration and fermentation time. Hydrolyzates of corn hulls and germ meal containing mixed sugars (e.g., arabinose, glucose, and xylose)

# **Whey Fermentation**

Whey is the lactose-rich, watery liquid remaining after the curd is formed in the manufacture of fermented or acidified dairy products such as cheese. Liquid whey generally contains 4.8% lactose, 0.6% protein, 0.6% salts, 0.05% fat, and the balance being water. Spraydried whey has 3.5-5% moisture, 11-14.5% protein, 0.5-1.5% fat, 9.8-12.3% ash, and 61-75% lactose. Many dairy companies have to contend with the utilization or disposal of liquid whey as a by-product in the process. Ethanol production is one possible avenue by utilizing whey as a feedstock for fermentation.

Lactose, a disaccharide, present in whey can be hydrolyzed by lactase enzyme into galactose and glucose. Normal distillers yeast (S. cerevisiae) preferentially ferments glucose first, and then slowly ferments galactose into alcohol. Some strains of yeast, e.g., Kluyveromyces marxianus, variety marxianus (previously known as *Kluyveromyces fragilis*), can ferment lactose without prior hydrolysis. Several strains of yeast belonging to Kluyveromyces marxianus, Kluyveromyces lactic, Candida pseudotropicalis, Candida versatilis, and Trichosporon melibiosaceum have reasonable yield of ethanol during batch fermentation of lactose. Ethanol was also produced by transformed strains of S. cerevisiae acting on whey or by recombinant flocculating S. cerevisiae expressing the genes of Kluyveromyces marxianus coding for  $\beta$ -galactosidase and lactose permease acting on cheese whey permeate.

Maximum ethanol yield is achieved by batch fermentation of cheese whey or continuous anaerobic fermentation of whey by *Candida pseudotropicalis*. In a small cheese plant, whey waste fermentation with *C. pseudotropicalis* ATCC 8619 resulted in daily production of 7201 of 190 proof alcohol and 40 kg of *Candida* yeast from 25 0001 of whey.

Cofermenting food processing wastes with cheese whey, which acted as a wetting agent and provided macro- and micronutrients for the yeast, resulted in increase in alcohol yield and decreased fermentation time. These advantages were also attained in another co-fermentation process involving whey permeate from the dairy industry and starchy waste from the potato industry treated with a mixed yeast inoculum of *Kluyveromyces fragilis* and *Schizosaccharomyces pombe*. Simularly, liquid whey supplemented with corn and the saccharified mash fermented with a mixed yeast inoculum of *Kluyveromyces fragilis* and *Saccharomyces cerevisiae* improved the yield of ethanol.

**Table 7** HPLC analysis of fermentation liquor of a 60 h mash from a dry corn milling/milo/wheat starch continuous fermentation process

Component	Retention time (min)	Amount <sup>a</sup> $(g I^{-1})$	
Maltotetraose	7.9	14.4	
Maltotriose	8.6	0.9	
Maltose	9.6	3.0	
Citric acid	10.8	0.7	
Glucose	11.2	3.3	
Fructose	12.2	0.4	
Succinic acid	15.1	0.4	
Lactic acid	16.2	4.6	
Glycerol	16.8	6.8	
Acetic acid	18.9	0.6	
Methanol	23.1	2.2	
Ethanol	25.6	12.0	

<sup>&</sup>lt;sup>a</sup>Units are in g I<sup>-1</sup> except for ethanol, which is in vol.%.

# **Monitoring Progress of Fermentation**

Conventional high-performance liquid chromatography (HPLC) procedures are used to track the progress of fermentation by analyzing mash composition. A typical chromatographic result is shown in Table 7, where fermentation liquor of a 60 h mash from a dry corn milling/milo/wheat starch continuous fermentation process is assayed using a Waters HPLC Model 515 pump equipped with an autosampler and a refractive index detector. Separation of components is accomplished using Bio-Rad's Aminex HPX-87H column and 5 mM H<sub>2</sub>SO<sub>4</sub> eluent operating isocratically at 60°C. Ethanol is the major component with minor or trace amounts of methanol, glycerol, lactic acid, acetic acid, succinic acid, citric acid, maltotetraose, maltotriose, maltose, fructose, and glucose.

A fully automated system can also be used for online monitoring of ethanol in fermentation liquor. In this system, ethanol is determined on precleaned filtrate by liquid chromatography on a reversed-phase column and amperometric detection with a biosensor consisting of a carbon-paste electrode modified with co-immobilized horseradish peroxidase and alcohol oxidase. Biosensors based on amperometry and constructed using immobilized cells of either *Gluconobacter oxydans* or *Pichia methanolica* also provide accurate estimates of glucose and ethanol in mixtures.

Another type of monitoring equipment includes an automated glucose analyzer modified to form a multichannel flow-injection analysis system for the sequential determination of glucose, ethanol, and glutamate. The system is equipped with a mini-reactor column containing immobilized glucose oxidase, alcohol oxidase, or glutamate oxidase. By comparison, another flow-injection analysis setup employs a reagentless alcohol dehydrogenase working

electrode, a platinum auxiliary electrode, and an Ag/AgCl reference electrode.

# **Distillation Processes**

A simple distillation column to separate an ideal, binary mixture contains several elements. These elements consist of the feed (which contains the two components to be separated), the source of energy (primarily steam to drive the process), the overhead product (consisting primarily of the feed component with the lower boiling point), the bottoms product (containing the feed component with higher boiling point), and the condenser (which transforms and splits the vapor into a liquid (reflux) and an overhead product).

If the ethanol-water mixture is an ideal one, the distillation system described above would be able to separate the beer feed into a relatively pure ethanol overhead product and a bottoms product of stillage free of ethanol. However, the ethanol-water mixture in the beer feed is not an ideal system. Distillation of an ethanol-water mixture follows the vapor/liquid equilibrium diagram, in which a point is reached where the vapor boiling off of the liquid is of identical composition as the liquid from which it is being evolved. What is formed is a constant boiling mixture or an azeotrope with 95% ethanol content. To produce pure ethanol, the simple distillation process is modified, and the vapor/liquid information is divided into three distinct zones of process and equipment requirements namely stripping, rectifying, and dehydration.

Efficient distillation processes (see Beverages: Distilled) have been in commercial operation for many years for the production of high-grade, 190 proof ethanol. After fermentation the beer feed is preheated using recovered heat from effluent streams and vapors in the process. The preheated beer is degassed and pumped to the beer still equipped with stripping travs below and above the feed point. Condensed vapors from the top of the column are fed to the extractive-distillation column, which removed the impurities as overhead product to be condensed as a low-grade ethanol. The purified, diluted ethanol from the bottom of the column is pumped to the rectifying column, which has an integral stripping section. The high-grade ethanol product is taken as a side draw from one of the upper trays of the rectifying column. Fusel oils are drawn off below the product draw tray to avoid buildup of these impurities in the rectifying column. Fusel-oil draws and overhead "heads" cut are sent to the concentrating column to recover ethanol, which is fed back to the extractivedistillation column for re-purification and recovery of ethanol.

# **Anhydrous Ethanol Production**

To produce anhydrous ethanol, a dehydrating column and an entrainer recovery column are put in operation. The entrainer used to remove water as a ternary azeotrope may be either benzene, heptane, cyclohexane, n-pentane, or diethyl ether. The three-component azeotrope boils at a lower temperature than any of the components, and passes overhead from the column, carrying the water upward. In this process, the alcohol feed enters the dehydrating column near the top, and comes in contact with the entrainer. In this section of the column, the three-component mixture seeks to form its azeotrope, but is deficient in water and contains more ethanol than the azeotrope composition. This excess ethanol is rejected downward in the liquid, and is withdrawn as an anhydrous product from the bottom of the column. On the other hand, the water joins the entrainer, passing upward as vapor to form a mixture that is near the ternary azeotrope composition. The condensed mixture separates into two layers in the decanter, and the entrainer-rich layer is refluxed from the decanter back to the top of the dehydrating column. The aqueous layer is pumped to the entrainer-recovery column, in which the entrainer and ethanol are concentrated overhead and the stripped water emerges from the base.

Significant improvements in the above processes were implemented to achieve maximum recovery during the production of motor fuel-grade ethanol. Fermented beer is preheated in a multistage heat exchanger employing a "boot-strapping" operation which picks up heat from the dehydrating system, rectifying column, and hot stillage. The preheated beer is degassed to remove residual carbon dioxide before entering the pressurized beer column. Heat is provided to the beer column by steam through a forced-circulation reboiler. The ethanol stripped from the beer in the lower part of the column is rectified to 95% concentration, and is taken as a side draw a few trays below the top of the rectifying section. The condensed overhead vapors are refluxed to the top of the beer column, with a small draw of "heads" taken to avoid accumulation of congeners. Fusel-oil draws are also taken from the rectifying section of the beer column into the decanter. The 95% ethanol enters the dehydrating column for anhydrous ethanol production according to the process described previously.

Ternary azeotropic distillation for anhydrous ethanol production is being supplanted with molecular sieve dehydration utilizing pressure-swing adsorption technology. Molecular sieves are manufactured from potassium aluminosilicates, and are described as hard, granular substance with spherical or cylindrical shape. The typical sieve used in ethanol dehydration has an average diameter of the interstitial passageway of 3 Å. The separation of ethanol—water mixture is thus possible because the ethanol molecule has a mean diameter greater than 3 Å, and water molecule has a mean diameter less than 3 Å and can be adsorbed within the molecular sieve structure. The ethanol molecules are too large and pass out of the vessel leaving the water behind. Hot gas regeneration method was employed to displace the water from the molecular sieve beads. Physical deterioration of the beads occurred due to excessive thermal shock, especially for dehydration systems in the liquid phase. During the 1980s, vapor-phase, pressure-swing, vacuum-purge adsorption technology was applied to molecular sieve ethanol dehydration. This technology extended molecular-sieve bead life by a mild regeneration process using part of the recycled, superheated anhydrous ethanol vapor.

The adsorptive dehydration of aqueous ethanol in the gas or liquid phase has been conducted using carbon molecular sieves, which preferentially absorb water. The carbon molecular sieves have average effective pore diameter of 2.0-5.0 A. Ground corn having operational temperature ranges between 80°C and 100°C has also been used as an adsorbent to remove water from ethanol vapors. The mechanism of adsorption is believed to involve transport of water molecules into the structure of adjacent starch granules immobilized on the surface of corn grit particles. Selectivity for water in a packed bed separation process with pure starches increases with increasing ratio of amylose to amylopectin. Corn grits treated with α-amylase have enhanced desiccant or water adsorption capacity useful in dehydrating aqueous ethanol.

See also: Beverages: Distilled. Grain Production and Consumption: Overview. Maize: Dry Milling; Wet Milling.

# **Further Reading**

- Agricultural Engineering Department (1981) Alcohol and Vegetable Oil as Alternative Fuels: Proceedings of Regional Workshops. West Lafayette, IN: Purdue University.
- Alico DH (1982) Alcohol Fuels: Policies, Production, and Potential. Boulder, CO: Westview Press.
- BBI International (2001) Ethanol Plant Development Handbook. Cotopaxi, CO: BBI International.
- Ho WSW and Luo RG (1997) Preprints of Topical Conference on Separation Science and Technologies Parts I and II. San Diego, CA: The American Institute of Chemical Engineers.
- Hunt VD (1981) *The Gasohol Handbook*. New York: Industrial Press.

- Hurn RW, Marshall WF, and Allsup JR (eds.) (1991) Oxygenates in Motor Fuel Formulation. Warrendale, PA: Society of Automotive Engineers.
- Jacques KA, Lyons TP, and Kelsall DR (eds.) (1999) *The Alcohol Textbook*, 3rd edn. Nottingham: Nottingham University Press.
- Lyons TP and Jacques KA (eds.) (1999) Biotechnology in the Feed Industry: Proceedings of Alltech's Fifteenth Annual Symposium. Nottingham: Nottingham University Press.
- National Research Council (1983) Alcohol Fuels: Options for Developing Countries. Washington, DC: National Academy Press.
- National Research Council (2000) Biobased Industrial Products: Priorities for Research and Commercialization. Washington, DC: National Academy Press.
- National Corn Growers Association (2002) *The World of Corn.* St. Louis, MO: National Corn Growers Association.
- O'Connor J and Cropley J (1998) Recent ethanol developments: production, policy, and switch capacity. In: *Starch and Fermentation Analysis June 1998*. New York: LMC International Ltd.
- O'Connor J and Cropley J (2000) World ethanol trade and Brazil's role. In: *Starch and Fermentation Analysis June* 2000. New York: LMC International Ltd.
- Parsons RA (ed.) (1981) Feed and Fuel from Ethanol Production. Ithaca, NY: Cornell University.
- Paul JK (ed.) (1979) Ethyl Alcohol Production and Use as a Motor Fuel. Park Ridge, NJ: Noves Data Corporation.
- Piggott JR and Paterson A (eds.) (1989) Distilled Beverage Flavour: Recent Developments. New York: VCH Publishers.
- Renewable Fuels Association (2002) *Growing Homeland Energy Security: Ethanol Industry Outlook* 2002. Washington, DC: Renewable Fuels Association.
- Reynolds RE (2000) The Current Fuel Ethanol Industry: Transportation, Marketing, Distribution, and Technical Considerations. Bremen, IN: Downstream Alternatives.
- Renewable Fuel Association (2002) Growing Homeland Energy Security: Ethanol Industry Outlook 2002. Washington, DC: Renewable Fuels Association.
- Rousseau RW (ed.) (1987) Handbook of Separation Process Technology. New York: Wiley.
- USI Chemicals (1981) *Ethyl Alcohol Handbook*, 5th edn. New York: USI Chemicals.
- Watson SA and Ramstad PE (eds.) (1987) Corn: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

# **Relevant Websites**

http://www.ethanolrfa.org — This is the website for the Renewable Fuels Association (RFA) based in Washington, DC. RFA is the national trade association for the US ethanol industry working to secure a strong marketplace for ethanol. Its membership includes businesses, individuals, and organizations involved in the production, blending, marketing, and promotion of ethanol. RFA serves as a link between the ethanol industry and the federal government to promote increased production and use of ethanol through supportive policies, regulations, research and development iniatives.

http://www.ncga.com - The National Corn Growers Association (NCGA) with 32 000 members is the largest national nonprofit organization representing the interests of US corn growers. The national headquarter is located in St. Louis, MO with offices in Washington, DC. With the help of corn growers nationwide, NCGA defines the future of corn through research, market development, public policy, production, and education. It publishes "The World of Corn," an annual publication that reports US Department of Agriculture data on the production and consumption of corn in the US and around the world. http://www.atcc.org - The American Type Culture Collection (ATCC) is a global nonprofit bioresource center that provides biological products, technical services, and educational programs to private industry, government, and academic organizations around the world. ATCC was established in 1925 when a committee of scientists recognized a need for a central collection of microorganisms that would serve scientists all over the world. Its headquarter and laboratory facilities are in Monassas, VA.



# GENETICALLY MODIFIED GRAINS AND THE CONSUMER

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# Introduction

Consumer opinion about genetically modified (GM) grains and foods ranges from excitement to resolute fear. Somewhere in the middle lies the consumer majority. If consumers have heard of GM food, they have mostly heard contradictory snippets from Malthusian and humanitarian discourses calling for biotech solutions that could help feed the world to the apocalyptic sounding "Frankenfood," which like Mary Shelley's 1818 novel evokes the dangers associated in messing with nature's order. In the end, the ultimate success or failure of GM grains will not depend on laboratory or farm research successes, but on consumer acceptance. This article covers the following: (1) the range of world consumer opinion concerning GM grains and foods and (2) an assessment of the factors involved in forming those opinions.

# **Consumer Perception of GM Grains and Foods**

Much of the world has never heard of GM foods or grains. Even in the best cases (developed nations that have active campaigns and education movements on both sides of the spectrum concerning GM foods), polls only show that 70% of consumers are familiar with GM foods. In many cases, the awareness and actual knowledge or willingness to learn more is much smaller (as low as 25% in some surveys). Table 1 gives some comparative data for some countries in Europe.

When consumers in the world hear of GM foods, their perception could be grossly summarized as wary. Of course, the spectrum of opinion varies drastically across geographical, economic, political, and social lines. Interestingly, both in the USA and

Europe, consumers give lower acceptance ratings when asked about the genetic modification of foods than when asked the same question using the term "biotechnology."

The greatest advocates for GM foods generally come from the USA and a few other select countries. The scientific and governmental leaders in the USA believe that GM foods, when appropriately tested and used, could be a boon for the world in terms of food supply, environmental protection, and economic development. Consumer acceptance was slowly increasing until the Starlink debacle occurred. Starlink corn was approved for use in animal food. It was not approved for use in human food because regulators wanted further testing to ensure there were no allergy risks. Most consumers failed to understand this and assumed that the recall of the product meant it was unsafe rather than merely not receiving final approval. Now that the brouhaha has died down, there is again slowly growing acceptance.

The voices of Europe, led by France, take a more skeptical approach and possibly trade protectionist approach. Scientific and governmental leaders in Europe do not completely reject GM foods, but instead believe they have not been proven safe and therefore invoke "the precautionary principle." This helps protect their markets. Interestingly some countries are more accepting of GM crops than they are of the food. This seems to be somewhat contradictory (Table 2).

The opinions of the governments in the rest of the world fall somewhere in between those of USA and Europe, with data for developing nations still quite scarce. It is not surprising that consumer perception generally follows the advice of their scientific and political leaders. Thus, consumers in the USA are far more accepting of the idea of GM foods than the rest of the world.

#### **Developing Nations**

A 2000 study published in *Scientific American* shows that globally developing nations account for about

Table 1 Awareness and knowledge of biotechnology in selected countries in Europe

	% Who talk about it at least some	% Who would read or view to learn more	Mean number of known applications	Knowledge (mean correct score out of 10)	% Engaged in the process
Denmark	50	77	2.23	6.60	47
Finland	43	68	1.71	6.21	31
France	37	80	1.76	5.70	29
UK	23	67	2.00	5.90	26
Germany	40	72	1.78	5.30	25
Ireland	18	64	1.50	4.86	18
Spain	24	41	1.70	5.02	15
Europe, overall	32	68	1.79	5.46	25

Adapted from George et al. (2002) Eurobarometer 58.0. Europeans and Biotechnology in 2002. Luxembourg, Office for Official Publications of the European Communities. Brussels, European Commission, Research DG, 2002 (http://europe.eu.int/comm/public\_opinion/archives/eb/ebs\_177\_en/pdf).

**Table 2** Support for GM crops and food in selected European countries

Countries which support both GM crops and food

Spain (strong support)

Portugal

Ireland

Finland

Countries which support GM crops but not GM food

Belgium

Germany

Netherlands

Uk

Countries which support neither GM crops nor food

Sweden

Denmark

Germany

France (strong opposition)

Greece (strong opposition)

Adapted from George *et al.* (2002) Eurobarometer 58.0. Europeans and Biotechnology in 2002. Luxembourg, Office for Official Publications of the European Communities. Brussels, European Commission, Research DG, 2002 (http://europe.eu.int/comm/public\_opinion/archives/eb/ebs\_177\_en/pdf).

one-quarter of the area employed for GM crops. Proponents of GM foods usually cite the developing world as the greatest potential benefactor of GM foods, yet studies of what the people in developing countries think of GM foods are few are far between.

One of the great worries among GM food opponents is the potential to create further economic imbalance between the impoverished and wealthy countries. So while many developing nations look at GM food, especially grains, with a keen eye towards solving many health and hunger issues, many leaders in developing nations fear that GM food would not move their countries towards economic self-sufficiency.

In fact, in 2002, the African nation of Zambia rejected a deal proposed by the USA in which they would accept GM grain from the International Federation of the Red Cross. In a statement, the Zambian government said it would prefer its poor

to die than to make the entire national food supply unhealthy. This shows the extent of pervading fear about this technology.

Leaders and scientists in South America seem a little more welcoming of GM food. Argentina, for example, is the second largest adapter by acreage of GM food crops in the world. In Southern Voices: An Online Debate on Biotechnology and Food conducted in 2001 by the Network University in The Netherlands, scientists and leaders from developing nations gave their opinions on biotechnology. Many Latin American nations (as well as other developing nations) were excited about the prospects of GM food, but favored a type of "open source" biotechnology, in which patent protection would not create a dependency on developed nations.

**India** To date, there has been no comprehensive study of how Indians feel about GM foods, but scientific and government officials are trying to jump start biotechnology in India. At the Bangalore Bio 2003 conference, a majority of Indian scientists came out in favor of GM foods, arguing that the number of infant deaths (61 out of every 1000 die from disease and hunger) could be reduced with GM foods. However, the scientists argued that GM foods should be researched, produced, and approved in India so they would not have to rely on the West for such technology. They argued that rich European nations have the luxury of choosing not to accept GM foods, but in countries like India the acceptance of biotechnology ends up as a choice between feeding and not feeding people.

Africa Similar to the resistance to GM food in Zambia, the governments of Zimbabwe, Mozambique and Botswana all initially protested the introduction of GM food crops into their countries, but eventually relented. However, African nations worry about GM foods. An international campaign is currently

underway in Malawi to reject 250 000 metric tons (t) of GM maize. The latest reports, however, indicate that most of the Malawian farmers have decided to ignore the warnings and plant the maize.

In a different development, in March 2003 South African consumers started a campaign for GM food labeling, indicating that consumers in South Africa are aware of GM foods and want the right to choose between eating them or not.

# **Pacific Nations**

It may be stated that major nations on the Pacific, including Australia, New Zealand, Japan, China, and India provide a glimpse of the middle ground in this debate. Though varied in their opinions, these nations seem to be the moderates in the GM food debate — cautious, yet accepting. In countries like Australia and New Zealand, consumers seem evenly split in the debate over GM foods. And while no one is yet allowed to produce GM foods for public consumption in Australia and New Zealand, both countries are actively researching the production of biotech food crops.

Like in Europe, most governments in Asia have not given the go-ahead for GM food crop production, but according to a 21 February 2003 article in the *New York Times*, other major Asian countries, including Japan, Thailand, The Philippines, and Malaysia, have set aside billions of dollars for research on biotech crops. The same article reports that China is so far ahead in its research on GM food crops that it stands to dominate the region in agricultural production if GM food gains acceptance.

Australia A 2001 survey of Australian consumers conducted by the government agency, Biotechnology Australia, showed a growing acceptance of biotechnology, from 28% in 1999 to 35% in 2000 to 49% in 2001. The strongest acceptance among consumers was for GM foods that might induce health benefits (lower cholesterol, reduction in allergens etc.). The survey showed lowest consumer acceptance of foods engineered to ward off pests or improve taste.

Australian agencies are not totally convinced, however. In May 2003, despite the overall rise in acceptance for GM foods, Victoria, a southern Australian state, joined the rest of Australian canola producing states by halting the release of GM canola for at least another year. GM canola was to be Australia's first GM food crop.

New Zealand A 1999 study, conducted by Hort-Research in collaboration with AgResearch, Forest Research Institute, Auckland University, and Massey

University on New Zealanders' attitudes towards GM foods, found the country split in half between those that favored GM foods and those that opposed them. New Zealand also seems to show indications of a very polarized country, with 25% of the 908 responders having extreme negative feelings towards GM foods and 18% having very positive feelings towards them.

The study also showed some interesting demographic differences. Young (18–24 years) and male groups were the most likely to support GM foods, whereas older (35+ years) and female groups were more likely to oppose them. The study also indicated stronger approval for GM foods in urban over rural areas.

New Zealand's scientific, governmental, and agricultural communities are still very cautious over the use of GM grains or oilseeds. However, in May 2003, New Zealand joined a World Trade Organization (WTO) disputes case against the European Union (EU) because of its refusal to open markets to GM foods. This does not imply that New Zealand supports the production or trade of GM foods, but that the government believes in a trade system that sets health and environmental standards based on scientific evidence and risk analysis.

China In 2001, China implemented a strict labeling policy on GM soybeans. The reason given for the move was public outcry and safety. Some think the labeling requirement may have been less about public perception or fears, and more about economic protectionism.

China has halted the commercial production of biotech food crops that had already been approved due to opposition from major trade partners. Nevertheless, despite the labeling and export concerns, China remains the world's second largest spender on GM food research (US expenditure stands at \$1.5 billion).

Japan Surveys in 2001 showed that Japanese consumers appear less ready to embrace GM foods than other Pacific nations. The Biotechnology Strategy Council, a government agency erected in July 2002, was partially conceived to fight this perceived problem. One of its primary goals is to educate the public about the issues surrounding biotechnology.

# **European Nations**

A study conducted by the American Association for the Advancement of Science in 1999 looked at the difference between European and US perceptions over GM foods. A similar study, the Eurobarometer, conducted every two to three years (most recently in 2002 – "Europeans and biotechnology in 2002") by The European Commission breaks down the various European opinions on biotechnology issues. Both studies show a wide gulf between US and European acceptance of GM foods.

Seventy-one percent of the  $16\,500$  consumers interviewed in EU countries for the Eurobarometer responded that they did not want to consume GM foods. This contrasts with a commonly quoted rate of  $\sim 70\%$  acceptance rate by US consumers. While still in the minority, the Eurobarometer showed that European students, youth, higher income people, and men had a higher acceptance rate of GM foods, whereas, lower income people, the elderly, and women rejected GM foods at a much higher rate than the survey average.

EU candidate countries According to the Eurobarometer, candidate countries (Slovenia, Czech Republic, Slovakia, Turkey, Malta, Romania, Lithuania, Estonia, Hungary, and Cyprus) survey responses about attitudes to GM food reflect the opinions in the EU. Fifty-two percent of those queried in the candidate countries (compared to 56% in EU countries) viewed GM foods as dangerous.

Southern Europe (Italy, Greece, Portugal, and Spain) A study of 252 food shoppers in Modena, Italy during the Fall of 2001 conducted by Cal Poly State University in California showed that only 28% of the consumers queried in Italy knew what GM foods were. Only 43% of the shoppers queried said they would even consider purchasing GM foods.

The 2002 Eurobarometer showed that Greece was one of the four countries (France, Austria, and Luxembourg) that was particularly hostile to GM foods. In contrast, the 2002 Eurobarometer showed that Spain and Portugal were two of only four countries in Europe (joining Ireland and Finland) that showed overall support for GM foods.

British Isles England and Scotland share an overall disapproval for GM foods. In separate studies for the two countries in 2003 (Mori Environmental Research Bulletin and Public Perceptions of Food and Farming in Scotland), about 56% of consumers in each country were against GM foods, just slightly less than the rest of Europe. Only 14% of the English surveyed would themselves buy GM food products. Consumers in the Scottish survey stated they would neither buy food labeled as "GM," nor actively seek out additional information.

Ireland was more supportive of GM food. A majority of the consumers supported GM foods according to the 2002 Eurobarometer. However,

a 2000 study by Cal Poly State University, showed a slight majority opposed GM foods. This either falls in the bounds of survey error or suggests a growing acceptance of GM foods.

Scandinavia (Finland, Denmark, Norway, Sweden, and The Netherlands) Among the EU countries, the Scandinavian countries are the most engaged in GM food research. While most Scandinavian countries still have a majority of consumers opposed to GM foods, consumers in the Scandinavian countries are the most supportive (as a group) of GM foods. The percentages are closer to an even split among the countries, compared to the 71% EU rejection average.

Western Europe (Germany and France) In France, a population particularly hostile towards GM foods, much of the public perception can be traced to two factors: first, France's age old traditions celebrating food as an important cultural placard; and second, a strong grassroots campaign against GM foods conducted by a series of well-received environmental, consumer, economic, and political organizations. Consumers in Germany do not go as far as the French, but a large majority of Germans align with the French on this issue.

#### **North American Nations**

North Americans are the greatest proponents for GM foods in the world, and consumers reflect the opinions' of scientists and leaders. Multiple surveys between 2001 and 2003 show a varying degree of public acceptance, from a low of 32% to a high of 71%. Even with the varying degree of sample results, most surveys do show a majority of Americans and Canadians support and accept GM foods. Caribbean consumers according to a 2001 survey were less accepting of GM foods and grains than their North American counterparts.

April 2003 studies conducted by the Universities of Calgary and Alberta show Canadian consumers support GM food (52%), but approval has declined over the past 6 years. The study also shows that more than half of consumers surveyed would buy GM foods if they were cheaper than nonmodified foods. Seventy-five percent of Canadians want all GM food to be clearly labeled.

According to surveys conducted in the USA, 70% have heard or read about biotechnology but there is a range of opinions about GM foods and grains. Like Canadians, over 85% of US consumers want these products labeled. US consumers respond with the greatest acceptance when queried about specific applications. For example, 75% of US consumers believe that GM grains can help solve world hunger

and world malnutrition. US consumers respond with more resistance when they are asked about their personal choices or beliefs. In a 2001 survey conducted by BIGresearch only 23% of respondents answered in the affirmative to questions of "Would you eat GM food products?" and "Is GM food safe to eat?" About half were undecided and about 30% answered in the negative. Furthermore, in a 2001 ABC News poll, 57% of US consumers said they would be less likely to purchase a product labeled GM.

# **Factors Influencing Consumer Perception**

Social, economic, and political factors contribute to consumer opinions around GM grains. Understanding of each of these contributes to the success or failure of GM foods. Nine key factors contribute to consumer opinion about GM grains: use; scientific literacy; trust in regulatory organizations; labeling; safety (environmental and health); perception of benefit; price; religion; and global and socio-economic issues.

#### Use

The biggest factor when it comes to consumer perception is the understood use of biotechnology. Among uses for biotechnology, genetic modification for food and food grains usually falls somewhere in the middle of the spectrum in terms of acceptability, far below the acceptance of medical uses. Furthermore, consumers differentiate uses within the realm of GM grains. Consumers find GM grains that reduce allergens or pesticides far more appealing than GM foods providing better taste or nutrition. This also suggests that many surveys may have flaws if potential use remains unspecified.

# **Scientific Literacy**

Another major factor in consumer acceptance of GM grains may be scientific literacy. Some surveys have shown that people who understand the concepts of DNA and gene splicing are more accepting of GM foods. However, a major 1999 study conducted by the American Association for the Advancement of Science, compared scientific and genome literacy in Europe and the USA. While the European consumer scored significantly higher compared to their US counterpart in terms of genome knowledge, they showed much greater mistrust of GM grains and foods.

# **Trust in Regulatory Organizations**

European consumers also have greater mistrust for a large number of scientific and regulatory bodies that come out in favor of the safety of GM food. In a comparison of US and UK consumers, 76% of US consumers trusted the government's role "in securing the safety of general food supply." Only 48% of UK consumers felt that same way. The Eurobarometer showed similar feelings - only 32% of respondents felt that "public authorities can be trusted to make good decisions on GMOs."

# Labeling

One of the major battlegrounds in terms of public trust is the issue of labeling. In almost every survey upwards of 85% (and sometimes up to 98%) of people want GM foods labeled clearly. Most consumers believe they have the right to know what they are eating and have the right to choose not to eat GM foods if they are uncertain about them. The acceptance rate for GM food rises significantly in most surveys if all GM foods were to be clearly labeled.

# Safety

Underlying ideas of trust, labeling, literacy, and use is the consumer's uncertainty about the environmental and consumptive safety of GM food. According to several surveys, if a consumer has heard of a setback or problem with GM food production or consumption (regardless of whether the concerns have validity or not), they are far more likely to reject GM food.

It would seem that the media would thus play a huge role in the reception of GM food. Surprisingly, a study of media coverage showed that the GM foods are portrayed in a more positive light in Europe than in the USA. However, pro- and anti-GM food campaigns continue to be argued primarily through the media.

Media coverage of the Starlink corn issue certainly polarized public opinion in the USA at the time of the recall. In this case the media did little to explain that the product was recalled not because it was unsafe, but rather because it had only received approval for animal, not human food.

# **Perception of Benefit**

For many there is a perception that only agribusiness benefits. Farmers benefit by being able to grow more grain on less space, to improve resistance to weather and disease, and to reduce or eliminate the use of herbicides and pesticides. While these all reduce the farmer's cost and increase profit, consumers fail to realize that they also benefit by having less land devoted to food and grain production. Many consumers fail to consider environmental benefits from less pesticide and herbicide spraying and water quality benefits with decreased pesticide runoff. Until consumers actually believe and see that rice can have more vitamin A, sorghum with more available

minerals and fewer toxic factors, protein, wheat without the protein sequence that causes gluten intolerance, and grains that are less allergenic, there will still be large pockets of consumer resistance.

Golden rice is perceived by many as an example of biotechnology's promise. Every year, over half a million children in developing countries suffer from preventable blindness. Rice has been genetically engineered to contain the carotenoid precursor of vitamin A. This genetically engineered rice is referred to as "golden rice" because of its yellowish color. In 2001 two biotechnology companies, Greenovation and Syngenta offered the GM rice to small-scale farmers in developing countries free of charge and without limitations for humanitarian purposes. Because rice is already a staple crop in developing countries, the vitamin A containing rice would allow people in such areas to obtain more dietary vitamin A and potentially reduce blindness and other effects of vitamin A deficiency.

Interestingly, golden rice set off a major debate. Activist groups such as Greenpeace have tried to discredit the gesture by stating that the rice will not provide enough vitamin A and that it will fail to address blindness because of other malnourishment. The Rockefeller Foundation, a funder of the development of golden rice, says that the calculations of the activist groups about the amount of rice that needs to be eaten to address the vitamin A problems are based on some of the first versions of the rice and are not accurate for more currently available rice with greater vitamin A levels. The issue of golden rice has even split members of Greenpeace into those who oppose the technology under any circumstances and those who believe that this rice has a useful purpose and do not want to oppose the humanitarian effort.

#### **Price**

Two contradictory studies show that product price may or may not be important to consumers. A Canadian study asked whether consumers would buy GM food if it were significantly cheaper and tasted the same. A majority of respondents said that they would. In a separate study conducted by the University of Southern Illinois 56% of UK consumers and 37% of US consumers said that they would be willing to pay a premium for non-GM breakfast cereals. Only 22% in each country said that they would not be willing to do so.

#### Religion/Morality

There has not been much research on religion and GM food acceptance, but it remains a key factor

among consumers according to a Pew Initiative on Food and Biotechnology study conducted in 2001. The study, conducted among people of faith in the USA, showed that in the USA people of faith supported biotechnology less than the rest of the public. It showed that the highest approval rating for biotech was among Jews (55%), followed by Catholics (42%), Protestants (37%), and Muslims (32%) approved). The number one reason respondents said they disapproved of biotechnology is that man should not be playing God. (These statistics exist despite approval for GM food by many expert ethicists, including the Nuffield Foundation, the Church of England and even the Vatican.) Unlike groups of consumers that may be swayed by more scientific proof to the safety of GM food or a system of food labeling, those with moral objections to GM food may never be swayed to accept GM food.

#### **Economics/Concentration of Power/SES**

Socio-economic status (SES) is also a major factor in determining what consumers think of GM food and grains. Regardless of the overall sentiment in a particular country, most surveys show that consumers in higher SES brackets accept GM food with far more regularity than those in lower SES brackets.

Many consumer groups and policy makers are concerned that GM food will create an even greater inequity between developed and developing nations because developing nations will have to purchase expensive GM seeds from Western companies. Furthermore, farmers in the US and elsewhere have expressed concern over using GM seeds even if it will save them money in production because many countries either have strict labeling requirements or outright bans on GM food. Public perception is waiting to see how the economics of GM food bears out. If the industry fails to convince farmers in developed nations that they can sell the product or in developing nations that the industry can service them without disrupting the local economies, they will further impair their image among the public.

GM grains offer potential for improved yield and nutritional advantages. With careful balancing of environmental and ethical issues, vigilance about allergies and other safety issues, clear consumer education and awareness of sharp cultural differences surrounding GM grains, the potential benefits of this technology can be harvested.

See also: Consumer Trends in Consumption. Food Safety through the Production Chain. Fortification of Grain-Based Foods. Genomics. Labeling of Grain-Based Foods. Nutraceuticals from Grains. Nutrition: Beriberi, A Deficiency Related to Grains.

# **Further Reading**

- Bauer MW, Durant J, and Allum NC (1999) Worlds Apart? The Reception of Genetically Modified Foods in Europe and the US. Washington, DC: American Association for the Advancement of Science.
- Brown K (2001) Genetically modified foods: are they safe? Scientific American 284(4): 51-65.
- Bruhn C (1999) Consumer perceptions and concerns about food contaminants. Advances in Experimental Biology and Medicine 459: 1-7.
- Bucchini L and Goldman LR (2002) Starlink corn: a risk analysis. *Environmental Health Perspectives* 110: 5–13.
- Fischhoff B and Fischhoff I (2000) Publics' opinions about biotechnology. AgBio Forum vol. 4 (3&4), Col. 2, http://www.agbioforum.org.
- Gaskell G (2000) Agricultural biotechnology and public attitudes in the European Union. *AgBioForum* 3: 87–96.
- George G, et al. (2002) Eurobarometer 58.0. Europeans and Biotechnology in 2002. Luxembourg, Office for Official Publications of the European Communities. Brussels, European Commission, Research DG, 2002. (http://europe.eu.int/comm/public\_opinion/archives/eb/ ebs 177 en/pdf).
- IFIC (International Food Information Council Federation) (2002) US Consumer Attitudes Towards Food Biotechnology. Washington: IFIC (online).
- King J (2002) Biotechnology: a solution for improving nutrient bioavailability. International Journal of Vitamin and Nutrition Research 72: 7-12.
- Lonnerdal B (2003) Genetically modified plants for improved trace element nutrition. Journal of Nutrition 133(5 suppl. 1): 1490S-3S.
- Mazur BJ (2001) Developing transgenic grains with improved oils, proteins and carbohydrates. Novartis Foundation Symposium 236: 233-239 (discussion 240-1).

- McHugen A (2000) Pandora's Picnic Basket: The Potential and Hazards of Genetically Modified Foods. Oxford: Oxford University Press.
- Moon W and Balasubramanian SK (2001) Public Perception and Willingness-To-Pay a Premium for Non-GM Foods in the US and UK. Carbondale, IL: Southern Illinois University.
- PEW (Pew Initiative on Food and Biotechnology) (2002) Results of several surveys of Americans on questions pertaining to agricultural biotechnology (www.pewagbiotech.org).
- Taylor SL and Hefle SL (2002) Genetically engineered foods: implications for food allergy. Current Opinions in Allergy and Clinical Immunology 2: 249-252.
- Trewavas A (2002) Malthus foiled again and again. Nature 418: 668-670.
- Thomson JA (2002) Research needs to improve agricultural productivity and food quality, with emphasis on biotechnology. Journal of Nutrition 132: 3441S-2S.
- Zimmermann MB and Hurrell RF (2002) Improving iron, zinc and vitamin A nutrition through plant biotechnology. Current Opinions in Biotechnology 13: 142-145.

# **Relevant Websites**

http://www.agbioforum.org. http://europa.eu.int. http://www.ncbi.nlm.nih.gov. http://www.agbiotechnet.com - AgBiotechNet. http://www.genengnews.com/top100.asp. http://www.whybiotech.com. http://biotech.icmb.utexas.edu. http://pewagbiotech.org. http://www.ers.usda.gov/amberwaves.

# **GENOME MAPPING**

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Published by Elsevier Ltd.

# Introduction

Genome maps are very similar to road maps except that, instead of traversing across land, they traverse across the chromosomes of an organism. Genetic markers serve as "landmarks" along the chromosome and provide researchers information as to how close they may be to a gene or region of interest. There are two types of genome mapping - physical mapping and genetic linkage mapping - in which distances are measured in terms of base pairs and recombination frequency, respectively.

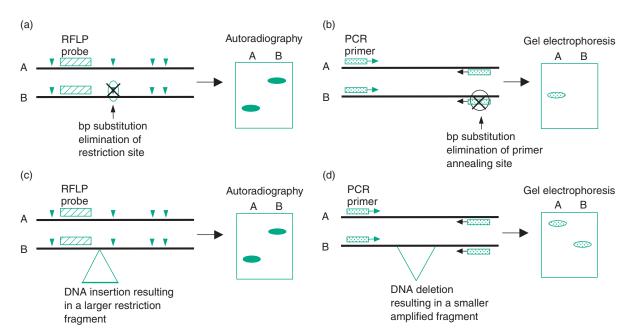
Genetic markers are differences in the DNA sequence of chromosomes derived from two different parents, and these polymorphisms can be visualized in several different ways. Morphological markers are visible markers, which can be identified by simply observing the phenotypes of individuals. Isozymes are protein variants which, after separation by

electrophoresis, can be visualized by a colorimetric activity assay for the relevant enzyme. DNA markers are visualized by chemical staining of the DNA itself or by fluorescence; they can also be labeled with a radioisotope and then visualized by autoradiography. Genetic polymorphisms can also be monitored by direct sequencing of the DNA itself. Currently, markers of choice are DNA-based markers because of their universal applications, information content, and ease of automation, and these will be discussed in detail.

# **Molecular Basis of DNA Markers**

DNA markers reflect differences in the DNA sequences of two parents of a mapping population. These differences, or polymorphisms, can arise through mutations resulting in a single nucleotide difference, errors in DNA replication, or insertions and deletions of larger tracts of DNA. Restriction enzymes are used routinely in molecular biology and genomics. Restriction enzymes recognize specific DNA sequence palindromes, usually 4, 6, or 8 bp in length, and

cleave the DNA at that site. In restriction fragment assays, a single nucleotide differing between two genotypes can result in a genetic marker when the base substitution results in the abolishment of a particular restriction site and, thus, a larger fragment in one genotype compared to the other (Figure 1a). This results in a discrete marker representing an individual's genotype that follows the laws of heredity and can be screened in the progeny. In polymerase chain reaction (PCR)-based assays (see below), a single base substitution within a primer annealing site can render the site noncomplementary and prohibit the fragment from being amplified (Figure 1b). Genotypes with or without the particular base substitution can be distinguished by the absence or presence of an amplicon in PCR-based assays. Thus, single base substitutions are specific to particular restriction enzymes or PCR primers. Rearrangements of the DNA between two restriction sites, or two primer annealing sites, can also generate DNA markers (Figures 1c and 1d). Such rearrangements can usually be detected using several different restriction enzymes or PCR primer sets that flank the rearranged region.



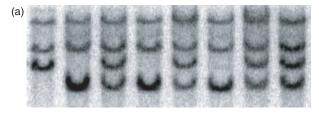
**Figure 1** The molecular basis of DNA markers: (a) The DNA of two genotypes (A and B) is digested with a restriction enzyme, which recognizes specific sequences (arrowheads). An RFLP probe, which is a short fragment of DNA, hybridizes to complementary sequences in the A and B genotypes. The fragment detected by the probe in genotype B is larger than that detected in genotype A, because a single base-pair substitution has eliminated one of the flanking restriction sites. The size differences of the hybridizing fragments are visualized as "bands" by autoradiography. (b) PCR analysis of a target sequence in genotypes A and B, where a single base-pair substitution has occurred within the annealing site of the right primer in genotype B resulting in the lack of annealing and, therefore, the absence of an amplicon which is viewed after gel electrophoresis. (c) RFLP analysis of genotypes A and B, where the detected fragment in B has undergone an insertion of a DNA fragment creating a larger restriction fragment than that in A. (d) PCR analysis of genotypes A and B, where genotype B has undergone a deletion of a fragment of DNA between the two priming sites creating a smaller amplicon than that in A.

# **Methods of Visualizing DNA Markers**

DNA markers can be detected in several different ways, some of the most common being restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), sequence tagged sites (STSs, also known as cleaved amplified polymorphic sequences (CAPS)), microsatellites (also known as simple sequence repeats (SSRs)), and single nucleotide polymorphisms (SNPs).

RFLPs are detected by hybridizing a labeled DNA probe, a short piece of DNA usually 0.2–2.0 kbp in length, to genomic DNA that has been digested with a restriction enzyme, separated through an agarose gel, and transferred to a nylon membrane. Hybridized fragments are then observed as "bands" by autoradiography in cases where probes are radioactively labeled (Figure 2a). RFLPs were the first type of DNA markers used that could be applied to essentially any organism, and they are still used routinely in applications such as comparative mapping, mapbased cloning, etc. Nonradioactive labeling protocols are now available and used to some extent, which have rendered RFLPs more user friendly. However, due to their relatively low-throughput capabilities, the more user-friendly PCR-based markers are now preferred for generating whole genome maps.

RAPDs, AFLPs, STSs, microsatellites, and SNPs are all PCR-based markers. PCR markers rely on the development of a specific oligonucleotide, or primer, to serve as a start site for amplification. The template



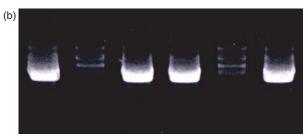


Figure 2 (a) RFLP fragments detected by autoradiography. (b) PCR amplicons detected by agarose gel electrophoresis followed by staining with ethidium bromide and visualization under UV light. The two lanes missing the amplicon are genotypes possessing deletions of the target sequence.

DNA from which a target fragment is to be amplified is mixed together with the primers, nucleotides, and an enzyme that polymerizes DNA (usually Taq polymerase). In the PCR process, the samples are first heated to 94-96°C to denature the double stranded target DNA. For the second step, the temperature is lowered to 50-65°C for one to several minutes allowing the left and right primers to anneal to complementary sequences on the target DNA. For the third step, the temperature is raised to 72°C for one to several minutes allowing the *Taq* polymerase to attach at each priming site and extend (synthesize) a new DNA strand. These three steps constitute one cycle. When 30 cycles are performed in succession, the exponential amplification of fragments will result in over one billion copies of the target fragment. Once the PCR is complete, samples are electrophoresed through an agarose or polyacrylamide gel, stained with a chemical, and visualized by UV light or other means (Figure 2b).

RAPD markers are detected using short (10 mer) random oligonucleotides as primers to amplify genomic DNA sequences. RAPDs have not been widely used for genomic mapping because of the unpredictable behavior of the short primers in PCR reactions, which leads to low repeatability.

The AFLP technique combines the use of restriction enzymes with PCR. To detect AFLPs, genomic DNA is digested with two restriction enzymes simultaneously and oligonucleotide adapters are ligated to the ends of the fragments to serve as priming sites. Oligonucleotide primers having from one to three selective nucleotides in addition to the adapter sequence are used in PCR amplification, and the fragments are separated on a polyacrylamide gel. To visualize AFLPs, one primer is end-labeled with radioactivity or fluorescence, or the DNA fragments are directly stained with chemicals. AFLPs provide a very high throughput method for identifying DNA markers as multiple markers can be identified with each restriction enzyme/primer combination. In theory, the number of markers potentially identifiable by AFLP is virtually unlimited, because they are limited only by the number of restriction enzyme/primer combinations surveyed.

Microsatellites consist of di-, tri-, or tetra-nucleotide repeats, and DNA sequences flanking the repeats are used as priming sites in PCR reactions. These short tandemly repeated sequences tend to be imprecisely replicated during DNA synthesis. As a result, the number of repeats within a microsatellite tends to be highly variable even among members of the same species. Therefore, microsatellites tend to produce a high degree of polymorphism. Although microsatellites are costly to identify because of the

amount of sequencing required, once specific primers are constructed they are very user friendly and efficient.

STS markers are usually detected using PCR primers designed on the basis of sequenced RFLP clones that have previously been mapped. Genomic sequences amplified using STS primers are usually digested with a four-base cutter enzyme to reveal polymorphisms. STS markers are also very user friendly and tend to be highly reproducible compared to methods based on arbitrary primers due to the larger primers and increased specificity. Also, STSs can be selected based on prior knowledge of the map position of the corresponding RFLP probe.

SNPs are the most common type of genetic difference between members of the same species. An SNP is a single base-pair difference at a specific site in the DNA. There are many ways of identifying SNPs. Currently, most procedures involve target sequence PCR amplification followed by electrophoresis, sequence detection, or mass spectrometry.

# **Genetic Linkage Mapping**

The high-density genetic linkage maps facilitate map-based cloning experiments, quantitative trait mapping, marker-assisted breeding, and evolutionary studies (for further related readings, see Genomics). Genetic mapping relies on the fact that nuclear genomes are made up of chromosomes, which contain both genes and noncoding DNA. When homologous chromosomes pair at meiosis, they recombine at various positions along the chromosomes. Thus, recombination is the basis for genetic linkage mapping and determining the order of markers along the chromosome, i.e., markers are separated by genetic distances calculated based on the amount of meiotic recombination that occurs between them.

An example of genetic linkage mapping of three linked markers in 20 F2 progeny is presented in Figure 3. The markers include two DNA markers (A and B) and one morphological marker (disease resistance gene "R"). The DNA markers are codominant and, therefore, all possible genotypes can be determined in the F<sub>2</sub> progeny (homozygous for parent A, homozygous for parent B, and heterozygous). For the morphological marker, disease resistance is dominant and, therefore, the genotypic classes of heterozygous and homozygous for the resistant parent (parent A) cannot be distinguished (resistant plants can have allelic compositions of "RR" or "Rr," and susceptible plants have "rr"). Inspection

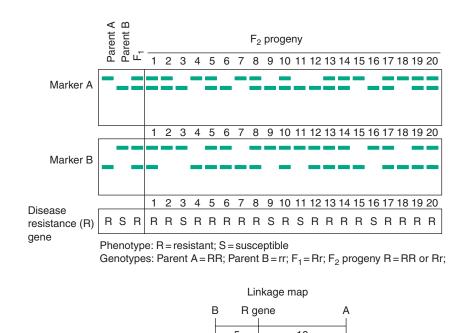


Figure 3 Genotypic data of two DNA markers (A and B) and phenotypic data of one morphological marker (disease resistance gene "R") for two parents, the F<sub>1</sub> plant derived from crossing the two parents, and 20 F<sub>2</sub> individuals. The DNA markers are co-dominant; thus, all possible genotypes can be distinguished (homozygous for parent A, heterozygous, and homozygous for parent B). The morphological marker "R" is dominant and therefore the genotypes of resistant F2 individuals cannot be distinguished (resistant plants can be either homozygous for parent A (RR) or heterozygous (rr)). The resulting genetic linkage map of the three loci and genetic distances separating them is shown at the bottom.

of Figure 3 indicates that there are three individuals (2, 6, 12) with genotypes that differ between markers A and B. Between A and R, there are two individuals (6 and 12) with differing genotypes, and one individual (2) has differing genotypes between markers B and R. This suggests that marker R (disease resistance gene) lies between markers A and B. The two recombination events between markers A and R translate into 10 map units  $(2/20 \times 100 = 10)$ , and there are 5 map units between markers B and R  $(1/20 \times 100 = 5)$ .

This type of analysis can be applied to hundreds, or even thousands of markers to construct complete genetic linkage maps of chromosomes. Fortunately, there are various computer software programs available to handle such large datasets and to determine the most likely marker orders and intermarker distances.

The number of individuals surveyed in a mapping population determines the precision of the genetic distance measured. In the example, only 20 individuals were surveyed, and if no recombinants were identified between two markers, this would translate to a genetic distance of 0 map units between the markers. If 100 individuals were surveyed, then one or more recombinants may be identified leading to a genetic distance of 1 or more map units. Generally, initial genetic maps of plant species are generated using 80-120 individuals, which allows for the detection of recombination between markers 1-3 map units apart. This level of precision is considered acceptable and, at the same time, the amount of labor and cost is considered manageable. However, certain mapping experiments such as map-based cloning of genes by chromosome walking require much higher resolution in order to separate markers extremely close to the target gene. In these experiments, it is not uncommon to survey 3000-5000 individuals to obtain the necessary level of precision.

In plants, most populations are derived from crossing two highly homozygous parents. The population shown in the example in Figure 3 is an F<sub>2</sub> population. While F<sub>2</sub> populations are commonly used and generally a good choice for chromosome mapping, other types of populations - such as backcross (BC), doubled haploid (DH), and recombinant inbred (RI) – are also commonly used. However, DH technology is not easily accomplished in some crops, and it is currently impossible in others. Each type of population has its advantages and disadvantages. F2, BC, and DH populations can be developed very rapidly, while RI populations are developed by advancing each line by single-seed descent for many generations with the goal of selfing to homozygosity. F<sub>2</sub> and BC populations are short lived and provide limited opportunity to obtain DNA and phenotypic data, while DH and RI populations provide essentially pure lines that enable them to be tested for traits in replicated experiments over several environments if desired. Thus, RI and DH populations are preferred for mapping of quantitative traits that may be affected by environmental influences. F<sub>2</sub>, BC, and DH populations have undergone only one cycle of meiosis, but an F<sub>2</sub> population has undergone recombination along two homologous chromosomes, and, therefore, provides twice the recombination information as BC or DH. RI populations have undergone several cycles of meiosis but contain two identical homologues and, therefore, provide about the same amount of information as an F<sub>2</sub>.

The development and analysis of genetic linkage maps lead to an abundance of information regarding genome structure. From a more applied perspective, they provide knowledge regarding the locations of genes and DNA markers associated with them. In a segregating population, morphological markers can be scored and analyzed in the same manner as DNA markers. The difference in scoring for morphological makers compared to DNA markers lies in the fact that, for morphological markers, the genotype is determined based on visualization of the plant's phenotype, whereas DNA markers are scored at the DNA level. For example, a population segregating for resistance to a particular disease would be scored based on the reaction of each individual to the disease as being one of either parental type. Inclusion of this phenotypic data with genotypic DNA marker data for map generation might reveal that the disease resistance gene is flanked by closely linked DNA markers. Such markers are valuable tools that can be employed by plant breeders, who wish to move the disease resistance gene into elite lines for the development of new and improved varieties. Using the markers to make selections is known as marker-assisted selection (MAS). MAS has advantages over selecting for the trait itself in that markers are not affected by environmental factors as phenotypic traits sometimes are. In addition, MAS allows breeders to make selections in early generations and growth stages, allowing them to eliminate undesirable material early on.

# **Physical Mapping**

In contrast to genetic mapping where distances between landmarks are calculated based on the percent recombination that occurs between them, physical mapping determines actual physical distance. Physical mapping can be done cytologically by chemically staining and viewing whole chromosomes using

techniques such as in situ hybridization and C-banding. Such techniques have very low resolution in terms of physical mapping, because chromosomes are viewed at the cellular level usually at metaphase. However, recent techniques such as fiber fluorescence in situ hybridization (FISH) where nuclear DNA is lysed on a glass slide and used for *in situ* mapping can provide a much higher resolution (see below). The highest-resolution physical mapping is obtained by sequencing the DNA itself. It is usually preceded by constructing local contiguous sequences (contigs) of large-insert DNA clones and anchoring the contig to a genetic map.

# In Situ Hybridization

The *in situ* hybridization (ISH) technique was developed in the 1970s and allows the localization of genes or DNA sequences directly on chromosomes in cytological preparations. The ISH technique uses probe DNA that is labeled with biotinylated dUTP or digoxygenin-dUTP, and the hybridization sites are detected by enzymatic reporter molecules such as horseradish peroxidase or alkaline phosphatase conjugated avidin/streptavidin. ISH has been used successfully to determine the physical location and distribution of dispersed or tandemly repetitive DNA sequences on individual chromosomes. For example, it has been used to determine the physical location of multicopy gene families such as the 5S and 18S-26S ribosomal genes.

FISH uses fluorochromes for signal detection. The FISH technique allows different DNA probes to be labeled with different fluorochromes that emit different colors (multicolor FISH). Thus, the physical order of two or more probes on a chromosome can be determined simultaneously. Also, FISH can allow more precise mapping of probes, because the fluorescent signals can be analyzed with special cameras and digital imaging tools.

In humans, the order of two DNA probes can be determined by ISH on metaphase chromosomes only if the two sequences are separated by at least 1 Mb. However, when ISH is done using interphase nuclei, DNA sequences separated by as little as 50 kb can be resolved. Plant metaphase chromosomes are more condensed than human metaphase chromosomes, and this may be one reason why ISH using lowcopy probes is more difficult in some plant species. Thus, it has been suggested that interphase nuclei be exploited for ISH mapping in plants. Subsequently, experiments where DNA probes were hybridized to maize interphase nuclei suggested that the resolving power of interphase FISH mapping can be as little as 100 kb.

More recently, the FISH technique has been used successfully to determine the physical location of bacterial artificial chromosome (BAC) clones on interphase and metaphase chromosomes. Rice BAC clones have been hybridized to rice (Oryza sativa L.) chromosomes revealing that the repetitive DNA sequences in the BAC clones could be efficiently suppressed by using rice genomic DNA as a competitor in the hybridization mixture. The successful application of this technique to plants with very large genomes may depend on the size of the genomic clones analyzed and the amount of repetitive sequences in the genome.

# Fiber FISH

Many plant species have small chromosomes that are not suitable for classical genetic studies and ISH analysis of metaphase chromosomes. A new FISH technique that uses extended DNA fibers has been developed. In the fiber FISH technique, chromatin fibers are extended across a glass slide and a probe is labeled as with standard FISH and hybridized to the extended fibers. In humans, fiber FISH has been used to analyze overlapping clones, detect chromosomal rearrangements, determine the physical distances between genes, measure the sizes of long DNA loci, and aid in the positional cloning of specific genes. Fiber FISH was used in Arabidopsis thaliana to measure clusters of DNA repeats as long as 1.71 Mb, which is more than 1% of the Arabidopsis genome. However, it was found that fiber FISH signals derived from small DNA fragments (<3 kb) were often observed as single spots on extended DNA fibers, and it was concluded that these signals were difficult to distinguish from background noise.

# **Aneuploid Mapping**

Wheat is unique in that it can tolerate a high degree of aneuploidy (abnormal chromosome number, compliment, or constitution) because of its polyploid buffering capacity. Some of the most important and useful genetics stocks ever developed in wheat are the nullisomic-tetrasomic (NT) lines and the ditelosomic (dt) lines. NT lines are lacking one pair of chromosomes and the absence of the pair is compensated for by an extra pair of homoeologous chromosomes. Ditelosomic lines are lacking one pair of chromosome arms. For example, the line dt1AS has the complete normal chromosome constitution except that the long arms of the pair of 1A chromosomes are missing. For more information on the aneuploids of wheat, see Wheat: Genetics.

With today's molecular technology, the power and utility of the wheat aneuploids is even more greatly realized. DNA markers can be quickly located to a specific chromosome or chromosome arm using a single hybridization or amplification reaction without the need for polymorphism. Chromosomal arm maps have been developed that locate DNA clones to specific chromosome arms. These maps are useful in that they can be applied to gene tagging, linkage and mapping of QTLs, cytogenetic manipulations, estimation of genetic distance, and evolutionary studies.

Due to their polyploid buffering capacity, wheat and other polyploids are more amenable to cytogenetic manipulations than diploid plant species. However, cytogenetic stocks such as primary trisomics have been developed in plant crops such as tomato, rice, and soybean (see Rice: Genetics and Soybean: Germplasm, Breeding, and Genetics). A primary trisomic individual contains a normal chromosome complement plus an extra complete chromosome (2n = 2x + 1), and it can be used as a tool to locate genes and specific genetic linkage groups (maps) to specific chromosomes. Trisomics cause segregation distortion in critical F<sub>2</sub> populations for phenotypic traits and DNA-based markers. For example, a DNA marker is selected from a previously developed genetic linkage map and used to screen a complete set of primary trisomic populations. The marker will segregate in a Mendelian fashion in all populations except for the critical one, i.e., the population derived from the trisomic that harbors the DNA sequence detected by the DNA marker. Using this method, genetic linkage groups can be assigned to their corresponding chromosomes.

#### **Chromosome Deletion Mapping**

Another unique system in wheat is the use of gametocidal (Gc) factors to construct chromosome deletion lines. Gc chromosomes are introduced into wheat by interspecific hybridization with the related Aegilops species and backcrossing. Plants monosomic for the Gc chromosome produce two types of gametes. Only those gametes possessing the Gc chromosome are normal. Gametes lacking the Gc chromosome undergo structural chromosome aberrations and, in most cases, are nonfunctional. However, if the damage caused by the chromosome breakage is not sufficient to kill the gamete, it may still function and be transmitted to the offspring.

The *Gc* system has been used to develop wheat lines with terminal chromosome deletions. These stocks have proven very useful for the physical mapping of genes and DNA markers to subarm locations and for the development of physical maps, which have been constructed for all seven homoeologous

chromosome groups of wheat. In addition, chromosome bin maps of most of the expressed genes in the wheat plant have been constructed using a set of wheat aneuploid and deletion lines (http://wheat.pw.usda.gov/wEST/binmaps/).

Terminal chromosome deletions have also been used to construct physical maps in maize. The r-XI system, a small intercalary submicroscopic deletion located on the long arm of chromosome 10 in maize, has been shown to induce terminal deletions and monosomy. Terminal chromosome deficiencies have been developed using r-XI and identified with recessive morphological markers. RFLP markers were physically mapped in relation to terminal deletions occurring on various chromosome arms. Some insights were gained regarding physical distances between markers, but discrepancies in marker order along the physical maps compared to the genetic maps were observed suggesting that multiple rearrangements may have occurred in the deletion lines. A major disadvantage to this system is that utilization of the terminal deletion lines in maize for long-term physical mapping is impossible. The lines cannot be propagated because maize is diploid and many of the genes are unique in the maize genome. For more information on maize, see Maize: Genetics.

#### **Large-Insert Clone Contigs**

The construction of physical contig maps is important for facilitating positional cloning of genes, sequencing of genomic DNA, and detailed analysis of chromosome and genome structure. Physical contig mapping is the arrangement of large-insert clones (yeast artificial chromosomes (YACs), BACs, cosmids) in a linear array that represents the DNA sequence along the chromosome.

Clones are selected by screening a library with DNA probes used to detect genetic markers on a genetic linkage map of the organism. Several DNA probes that detect closely linked genetic loci will hybridize to corresponding large-insert clones, and these clones can then be arranged into a contig based on overlapping segments and fingerprinting. YAC and BAC contigs are currently being developed in many crop species.

Once a physical contig map is complete, the structure and organization of the genome, such as the distribution of repetitive and single-copy sequences, can be discerned. Large-insert clone libraries have been developed for many plant species, but in most cases it is not yet a priority to develop contigs that cover entire genomes. Instead, the large-insert libraries are being used to construct small localized contigs of genomic regions that possess genes of interest.

#### Comparing Physical Distance to Genetic Distance

Physical maps have led to a wealth of information regarding the physical locations of morphological traits and evolutionary translocation breakpoints, and genome-wide structure and organization. Comparisons of the physical maps with genetic linkage maps can reveal the physical distribution of genes and recombination along the chromosome. For example, RFLP probes derived from mRNA (called cDNA probes) represent expressed genes and, thus, the physical mapping of cDNA probes will reveal the physical locations of expressed genes. Therefore, when sets of cDNA probes are mapped genetically as well as physically, one can infer the relationship between physical distances and genetic distances among the common markers. In wheat, physical maps constructed using the chromosome deletion lines have been compared extensively to corresponding genetic maps of the same chromosomes. This work has revealed that genes and DNA markers tend to be clustered in small physical segments that undergo a high degree of recombination (Figure 4). The gene-rich regions are separated by large gene-poor segments that undergo very little recombination. This work has facilitated BAC contig construction of regions containing genes of interest for the purpose of positional cloning.

In barley, physical maps generated based on translocation breakpoints were compared to corresponding genetic linkage maps. The results agreed with those found in wheat by deletion mapping and showed that the barley genome consists of relatively small gene-rich regions that are hot spots for recombination interspersed among large segments that are gene poor and undergo very little recombination. The information obtained by physical mapping of translocation breakpoints has facilitated the construction of BAC contigs and positional cloning of important genes by allowing researchers to focus on the generich regions of the genome (for further reading, *see* Barley: Genetics and Breeding and Genomics).

More intricate comparisons of physical and genetic relationships can be obtained by comparing local BAC contigs to genetic maps. The primary goal of such experiments is to identify a large-insert clone containing the gene of interest, but additional important information is obtained. For example, once a physical contig map of the region is developed, it can be compared to the genetic linkage map of the corresponding region to calculate physical to genetic distance ratios. This is important information because recombination is known to be distributed nonrandomly throughout the genomes of many plant species causing the

physical to genetic distance ratios to be highly variable depending on the characteristics of the region. Such information allows the investigator to determine the physical distance between the gene of interest and the closest DNA markers on the genetic map.

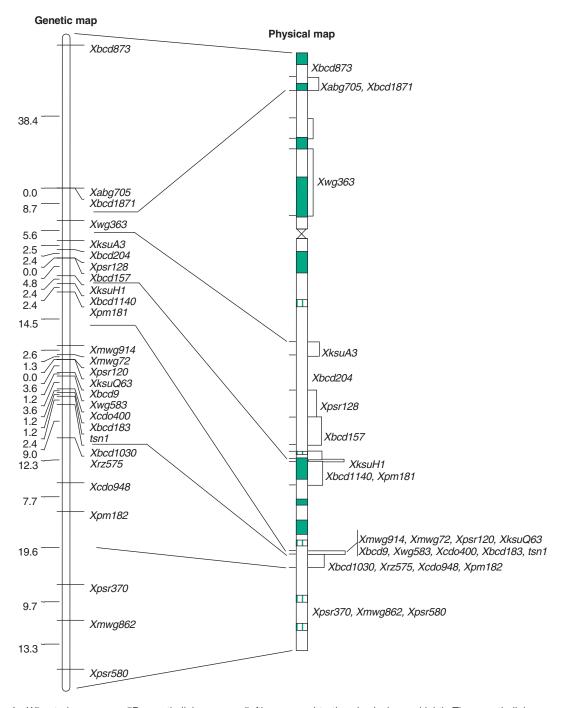
#### **Comparative Mapping**

Much effort has been put forth in comparing the genomic relationships among grasses and among members of other plant families. For example, comparative mapping experiments among members of the Poaceae such as wheat, rice, barley, rye, oat, and maize have revealed remarkable similarities in gene content and marker synteny at the chromosome level. It is well established that DNA probes cloned from these related species commonly identify sets of orthologous loci that lie at approximately the same positions relative to each other and to the centromeres. Consensus maps of several chromosomes uniting loci from homoeologous wheat genomes and the corresponding chromosomes of barley, Ae. tauschii, T. monococ*cum*, and rice have been presented. These experiments have shown that the genomes of barley, Ae. tauschii, and T. monococcum are essentially collinear with wheat genomes. The genomes of more distantly related cereals such as oat, rice, and maize can be divided into linkage blocks that have homology to corresponding segments of the wheat genome.

The degree of genomic similarities observed at the chromosome level among grass genomes led to the notion that information from the small genome of rice could be directly applied to the much larger genome of wheat. However, even though a substantial degree of synteny is observed at the chromosome level, studies of the degree of microcollinearity between rice and wheat show less promise for gene discovery in wheat.

#### **Future Mapping Prospects**

The ultimate goal in map construction is the deciphering of the linear DNA sequences of the full complement of chromosomes of an organism. Breakthroughs in high-throughput DNA sequencing have led to the complete sequencing of the five pairs of chromosomes of *Arabidopsis* (genome size 126 Mb, see http://mips.gsf.de/proj/thal/) and the target is within reach for the 12 pairs of rice chromosomes (genome size 426 Mb, see http://www.usricegenome.org/) (see Rice: Genetics). In large genome crops such as barley ( $\sim 5.3 \times 10^6$  bp) and wheat ( $\sim 16 \times 10^6$  bp), more than 80% of DNA consists of repetitive elements making it difficult to sequence



**Figure 4** Wheat chromosome 5B genetic linkage map (left) compared to the physical map (right). The genetic linkage map was constructed using a BC population and the physical map was constructed using the chromosome deletion lines of wheat. On the genetic linkage map, units separating markers are shown on the left, and markers are indicated on the right. On the physical map, tick marks on the left of the chromosome indicate deletion breakpoints, black and hatched regions on the chromosome represent dark and light C-bands, respectively, and DNA markers and their bin locations are shown to the right. Lines drawn between the maps indicate where deletion breakpoints occur relative to the genetic map. Notice that the centromeric region is nearly void of DNA markers and recombination, while more distal regions possess most of the DNA markers and recombination.

the entire genome. However, gene-rich regions can be targeted for sequencing as is already being done for maize (http://www.zmdb.iastate.edu/), and plans are underway for such a project in wheat. The goal will be

to determine the genomic as well as full-length cDNA sequences of the entire set of genes in all organisms of economic significance. Once genomic and DNA sequence of most genes is known, the techniques

such as SNPs will become very important for analyzing allelic variation of useful genes (gene mining) in diverse germplasm for novel gene discovery and manipulation for crop improvement.

See also: Barley: Genetics and Breeding. Canola: Genetics and Breeding. Genomics. Maize: Genetics. Rice: Genetics. Soybean: Germplasm, Breeding, and Genetics. Wheat: Genetics.

#### **Further Reading**

- Appels R, Morris R, Gill B, and May C (1998) *Chromosome Biology*, p. 401. Boston: Kluwer Academic.
- Devos KM and Gale MD (2000) Genome relationships: the grass model in current research. *Plant Cell* 12: 637–646.
- Faris JD, Friebe B, and Gill BS (2002) Wheat genomics: exploring the polyploid model. *Current Genomics* 3: 577–591.
- Feuillet C and Keller B (1999) High gene density is conserved at syntenic loci of small and large grass genomes. *Proceedings of the National Academy of Sciences USA* 96: 8265–8270.
- Jiang J and Gill BS (1994) Nonisotopic *in situ* hybridization and plant genome mapping: the first 10 years. *Genome* 37: 717–725.

- Lander ES and Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121: 185–199.
- Liu BH (1997) Statistical Genomics: Linkage, Mapping and QTL Analysis. Boca Raton, FL: CRC Press.
- Paterson AH (1996) Making genetic maps. In: Paterson AH (ed.) *Genome Mapping in Plants*, pp. 23–39. Austin, TX: R G Landes Company.
- Redei GP (1999) Genetics Manual, pp. 1141. Singapore: World Scientific.
- Tanksley SD, Young ND, Paterson AH, and Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. *Biotechnology* 7: 257–263.
- Tanksley SD, Ganal MW, and Martin GB (1995) Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. *Trends in Genetics* 11: 63–68.

#### **Relevant Websites**

http://wheat.pw.usda.gov. http://www.gramene.org. http://www.tigr.org. http://www.hgmp.mrc.ac.uk. http://www.maizegdb.org. http://www.arabidopsis.org. http://www.plantgdb.org.

## **GENOMICS**

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#### Introduction

Emerging techniques of functional genomics are being enthusiastically embraced in both the private and public sectors of scientific endeavor. Although the techniques have been developed relatively recently, they have quickly evolved and expanded into an exciting set of technologies that is attracting massive research investment around the world. It remains difficult to come up with a succinct, yet informative definition of functional genomics and its related technologies, but the ultimate objectives of the technologies are clear. The following would together constitute an ideal end point for

genomics studies:

- the determination of the complete nucleotide sequence of a genome,
- the identification of all the structural genes in that sequence,
- well-defined functions for all the genes,
- a detailed knowledge of the regulatory control of the genes during normal growth and development, and in response to environmental stresses, and
- an understanding of the complex interactions that occur in genetic and cellular networks.

Thus, in functional genomics one is endeavoring to determine the function of large sets of genes or, ideally, the entire genetic complement of an organism. Functional genomics then draws the profiling of proteins and metabolites into the analysis. If the ambitious goals outlined above are to be achieved

in a reasonable time frame, given that plants might have between 25 000 and 40 000 genes, the attendant technologies must allow high throughput collection of data and high throughput analyses of gene structure and function. It is also becoming clear that the noncoding regions of the genome play a critical role in various aspects of genome behavior and gene expression. Analysis of these regions poses special problems that have not yet been addressed in plant genomics.

The key feature of genomics and associated technologies is that they represent a fundamental change in our approach to biological science. In the past, we usually investigated a biological phenomenon with a particular hypothesis in mind, and with one or a small number of genes or gene products in our sights. For example, a starting hypothesis that a plant's response to salt stress might be linked with the capacity of Na<sup>+</sup> transporters to move Na<sup>+</sup> ions across various cellular membranes would have focused experiments on measuring changes in these transporters at the gene expression or biochemical levels. In contrast, the genomics approach involves no preconceived ideas as to what genes or proteins might be involved, in a particular phenomenon, but instead involves the high throughput collection and analysis of data in a broader, nonbiased manner. In the salt stress example, this would entail application of the stress to the plant and the subsequent collection of expression data for as many genes as possible. Identification of all up- and down-regulated genes would follow, together with attempts to rationalize the shifts in gene expression with the plant's response to salt stress on a much broader scale. This broad approach can easily be justified, not only because genes seldom act alone, but also because it acknowledges the high level of complexity of cellular networks and the participation of multiple genes or proteins in plant processes. Although genomics technologies adopt this broad approach, this is not to say that the technologies cannot be focused on more specific components of an overall phenomenon. In the salt stress example it might be desirable to limit the study to effects on ion carriers or pumps. Conversely, genomics methods can generate an overwhelming amount of data that present major difficulties for subsequent analysis of the process. Salt stress is an example of the latter, because up to 25% of genes in plant systems can be up- or downregulated in response to the stress.

Genomics technologies were initially developed on model organisms such as the yeast Saccharomyces cerevisiae, the nematode Caenorhabditis elegans, the fruit fly Drosophila melanogaster, mice, and humans. An early decision to use Arabidopsis thaliana as the model for genomics studies in plants was based largely on the relatively small genome of this plant and its short life cycle. Attention soon turned to the commercially important cereals – rice, wheat, maize, and barley. These species are members of the Poaceae family, and together provide the vast majority of carbohydrate requirements in human diets. At the same time, the technology was becoming broader in its scope. Early transcript analyses showed that mRNA levels are not necessarily related to cellular levels of the protein products of the mRNA; this led to an interest in defining the complement of proteins in plant tissues grown under various conditions, and became known as proteomics. Moreover, protein levels themselves were often difficult to relate to a cellular response; this led, in turn, to an interest in metabolite profiles in plants as the ultimate reflection of gene expression in cellular processes. Metabolomics had evolved.

In this article, we will outline the essential features of the genomics, proteomics, and metabolomics technologies as they apply to the common cereals, and how they are used in a complementary manner to generate a list of candidate genes that jointly control the phenomenon of interest. Once the candidate genes are identified, their participation in a particular cellular function must be confirmed. The major strategies for functional analysis currently in use for this purpose will be summarized. As mentioned above, coupling genomics, proteomics, and metabolomics technologies with functional analyses of genes is referred to here simply as functional genomics. The high throughput imperatives of functional genomics programs require the development of specialized equipment, such as robots, automated mass spectrometers, and high capacity nucleotide sequencers. It should also be emphasized that functional genomics would not be possible without the spectacular evolution of computing power that has occurred since the early 1990s. These points will be illustrated by reference to our functional genomics programs on early grain development and cell wall biosynthesis in wheat and barley. Other specialized resources, including mutant libraries and high-density genetic maps, represent critical support technology for functional genomics programs. Finally, the potential economic, social, and environmental benefits that might flow to cereal producers and consumers as a result of functional genomics programs will be explored.

#### **Genome Analysis**

The traditional use by cereal breeders of phenotypic markers in selection of superior quality and productivity traits was significantly enhanced through the

development of DNA molecular markers in the 1980s. In particular, restriction fragment length polymorphisms (RFLPs) allowed the first molecular analysis of cereal genomes. Subsequently, amplified fragment length polymorphisms (AFLPs), randomly amplified polymorphic DNAs (RAPDs), simple sequence repeats (SSRs or microsatellites), and single nucleotide polymorphisms (SNPs) have been developed as additional methods for the generation of high-density genetic maps of cereals. Furthermore, quantitative trait loci (QTLs) controlling complex, polygenic traits, can be mapped, so that major genetic loci that influence traits such as components of quality, tolerance to stresses such as drought, kernel weight, seed dormancy, and susceptibility to mineral toxicity in soils can be incorporated into the genetic maps. Similarly, cloned genes can be readily mapped, provided polymorphisms can be detected in or around the genes of parental lines used to generate the mapping populations. High-density genetic maps are now available for wheat, barley, maize, sorghum, and rice. The extremely large size of the wheat hexaploid genome and its inherently low level of polymorphism initially hampered the development of genetic maps for this cereal, although better progress is now being made with AFLP and microsatellite markers.

Distances between markers or genes on genetic maps of the type described above are based on recombination frequency in segregating populations, and this frequency will vary in different regions of the genome. In contrast, physical distances represent the actual DNA sequence distances between markers or genes, expressed precisely in terms of the number of nucleotide pairs in the DNA. Increasing attention is being focused on the generation of physical maps, at least in gene-rich regions of the genome. The availability of bacterial artificial chromosome (BAC) libraries for the common cereals (Table 1), in which

Table 1 Genomic resources for the major cereals

Cereal species	Genome size (bp)	BAC libraries	Public ESTS (June 2003)	Genetic maps	Genome sequence
Rice	$4.3\times10^{8}$ (diploid)	Yes	202 000	Yes	Available in draft form (2002)
Wheat	$1.6 \times 10^{10}$ (hexaploid)	Yes	420 000	Yes	No
Barley	5 × 10 <sup>9</sup> (diploid)	Yes	346 000	Yes	No
Maize	2.5 × 10 <sup>9</sup> (diploid)	Yes	229 000	Yes	Perhaps by 2006?

large fragments of genomic DNA are cloned, has greatly assisted in the generation of the physical maps of selected regions of cereal genomes. Physical maps of the genome can be constructed by fingerprinting large collections of BAC clones and using the fingerprints to link BAC clones. Such physical maps have been constructed for the rice and maize genomes, and are well advanced for Aegilops tauschii, the D-genome progenitor of bread wheat. At a smaller scale, BAC clones can be used to rapidly sequence and characterize the regions surrounding particular genes. If, for example, a cellulose synthase (CesA) gene or genes were detected on a particular BAC clone, high throughput sequencing and restriction mapping protocols could be used to develop a physical map of the relevant regions of the BAC clone or series of overlapping BAC clones.

The logical extension to the sequencing and physical mapping of local regions of cereal genomes is to sequence the entire genome. Rice has a genome size of  $\sim 4.3 \times 10^8$  bp, which is considerably smaller than the genome sizes of the other major cereals (Table 1). In addition, an abundance of rice BAC libraries and other genetic resources meant that rice was seen as the cereal genome of choice for complete sequencing. Draft versions of the rice genome sequence have recently been released into the public databases. The genomes of other cereals are at least tenfold larger than those of rice (Table 1) and it will be some time before their complete sequences are determined. Nevertheless, targeted genome sequencing is another route to obtaining valuable physical map information for the larger cereal genomes. Irrespective of their overall size, most cereal genomes contain about the same number of genes. The number of genes has been variously estimated in the range 25 000-40 000. In cereals, the species differences in genome size lie not so much in differences in gene number, but rather in differences in the amount of tandem and dispersed repetitive DNA elements that constitute a large proportion of these genomes. It is becoming clear that active genes are concentrated in "gene-rich" regions, and there are several procedures that enable these gene-rich islands to be located and sequenced. Thus, a major effort can be directed towards sequencing and physical mapping of the gene-rich regions of the larger cereal genomes. However, the plant genome sequencing programs indicate that the analysis of the noncoding regions will also provide many important clues about genome structure and function.

Finally, the observation that cereal genomes are syntenous has greatly assisted the cross-referencing of genome sequences and genetic maps between different cereal species. Synteny is the phenomenon in which the order of individual genes along a segment

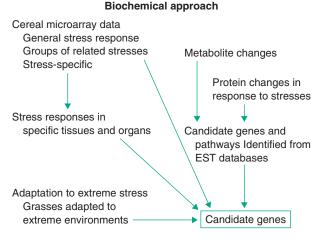
of the genomes of different cereals is conserved, despite large variations in genome size. Thus, if the order of genes can be determined along a region of the rice genome during sequencing, it is highly likely that the same genes will be arranged in the same order along the homologous region of the wheat or barley genomes. The syntenous regions might be discontinuous, through translocation events that rearrange chromosome segments, but once the homologous region is identified the order of genes therein is usually conserved. Synteny is attributable to the common ancestry of extant cereal species and, although not always perfect and not applicable to all regions of the genome, it does add enormous value to the rice genome-sequencing project, because relative gene and molecular marker positions can be extrapolated across species. Thus, synteny across the cereals and the availability of the rice genome sequence has become a critically important tool in cereal genomics (Figure 1). For the important cultivated cereals such as wheat, barley, and maize, there is extensive information on the genetic location of major loci affecting a wide range of traits, including quality, disease resistance, abiotic stress tolerance, and various physiological and developmental features. Through the rice genome sequence, the genetic data in these cereals can be linked to the physical location on the rice genome and used to identify candidate regions in the target cereal (Figure 1).

# Genetic approach Known genetic location of key loci Wheat, barley, rye, maize, rice, and sorghum Common genetic locations Gene prediction in target regions of rice Wheat and barley paralogs from EST databases Candidate genes

**Figure 1** Genetic approach and the use of synteny to identify candidate genes in cereals. The genetic loci that are linked to a specific trait can be cross-referenced to the syntenous region of the rice genetic map. This region of the rice map can be located in the rice genome sequence, and active rice genes in this region can be identified. The corresponding genes from the other cereals are thereby identified as potential candidate genes that might control the trait of interest.

#### **Transcript Analysis**

A common strategy for identifying unknown genes that might be related to a particular biological problem is to correlate expression patterns of genes. This is usually done based on the relative abundance of mRNA species and, through existing knowledge on the tissue and time frame in which the process of interest is occurring, to identify candidate genes (Figure 2). For example, it is known that large amounts of a particular polysaccharide, heteroxylan, are deposited in the cell wall at a defined time in elongating barley coleoptiles. Therefore, candidate genes might be identified from abundant mRNAs that appear in the coleoptiles just preceding the increased rate of heteroxylan deposition. The sequence of a cDNA corresponding to the mRNA might be recognized as encoding a protein or enzyme connected with wall synthesis. In this case, cDNAs derived from the CesA and cellulose synthase-like (Csl) gene families would be of particular interest, given the similarity between xylan and cellulose structure. Northern hybridization analyses are often used to monitor such expression patterns, but they can be relatively insensitive and require probes that presuppose the presence of a particular transcript; unknown genes or genes not previously suspected of having a role in wall synthesis will not be found. It can therefore be argued that it is essential not only to define the time, tissue location, and level of expression



**Figure 2** The biochemical approach to the identification of candidate genes. In this example a stress is imposed on the cereal plant, and the responses are monitored at the transcriptional level through microarray data, at the protein and metabolite levels in cell extracts, and through known information on genes that are expressed in well-characterized species that are adapted to the stress of interest. The multi-pronged approach enables the number of genes to be focused to a manageable number for functional analysis.

of a particular gene, but that it is also critical to define other genes that are coordinately expressed during the process under examination. This can be achieved through monitoring the entire transcriptome, i.e., all the transcribed mRNAs that are present in the tissue of interest.

Many early plant genomics projects were therefore focused on the high throughput definition of gene expression, as measured by profiling mRNA transcripts, with the straightforward objective of identifying all genes that were transcribed in a particular tissue at a particular time. This branch of the overall functional genomics technology is now referred to as transcriptomics. Transcript profiling can take several forms. First, mass sequencing of short segments of clones in cDNA libraries can be used to identify genes that are being transcribed. These sequences are known as "expressed sequence tags" (ESTs), and the number of cereal EST sequences in the public databases has increased in spectacular fashion over the last few years, to the point where there are now well over a million ESTs available for the major cereal species (Tables 1 and 2). It must also be remembered that EST sequence data make a major contribution to genome sequencing and physical mapping programs.

In the xylan synthase example, the databases would be searched for all CesA and Csl gene transcripts, and the ESTs could subsequently be used to isolate and characterize full-length cDNAs for all the genes being transcribed in the tissue of interest. As EST databases increase in size, searches can become semi-quantitative, such that the relative abundance of ESTs in a particular library can be correlated with the abundance of corresponding mRNAs in the tissues from which the libraries were prepared. Nevertheless, there are a number of inherent problems associated with EST analyses, including the unreliable quality of some sequences in the databases, difficulties in detecting cDNAs from genes that are transcribed at low levels, the incomplete nature of the sequences, and the fact that the sequences might correspond to nonconserved 5' or 3' untranslated regions and therefore be difficult to identify. These problems can be especially frustrating when the EST of interest is a member of a multigene family, as is frequently the case in cereals. Computer programs, through which ESTs that represent fragments of the same gene can be clustered and assembled into a single sequence (referred to as a contig), have overcome some of these difficulties.

Alternative but often less precise transcript profiling methods have now been developed. Microarraying technology is one such method that has been steadily improving over recent years. In this technique, large numbers of different gene fragments are immobilized in an ordered array on a solid support, which is variously known as a macroarray, a microarray or a chip, depending on the density of immobilized DNAs. The two main types of array currently in use are the DNA fragment array, where DNA clones, usually cDNAs or oligonucleotides of 40-80 bases, are synthesized and arrayed with a robot at densities of up to 5000 per square centimeter. The second type of array uses shorter oligonucleotides, of 10-12 residues, that are synthesized in situ on the surface of the chip at very high densities. The latter array is designed so that a single known gene is represented by several of the short oligonucleotides.

By the end of 2003 it is anticipated that chips comprising over 20 000 genes each will be commercially available for the interrogation of wheat, rice, and barley transcriptomes. To do this, the array is exposed to labeled pools of RNA (or cDNA derived from the RNA pool), from which individuals will

Table 2 Websites of interest in cereal functional genomics

Resource	Internet address		
Grain Genes	http://wheat.pw.usda.gov/index.shtml		
Rice Genome Project	http://rgp.dna.affrc.go.jp/		
Project 2010	http://www.arabidopsis.org.workshop1.html		
The Arabidopsis Information Resource	http://www.arabidopsis.org/agi.html		
Rice Transcriptional Database	http://microarray.rice.dna.affrc.go.jp		
Affymetrix	http://www.affymetrix.com/products/arabidopsis		
TIGR Arabidopsis Arrays	http://atarrays.tigr.org		
Comparative maps of wheat and rice genomes	http://wheat.pw.usda.gov/pubs/2003/Sorrells/		
Triticeae repeat sequence database	http://wheat.pw.usda.gov/ggpages/ITMI/Repeats/index.shtml		
NSF wheat genomic resources	http://wheat.pw.usda.gov/ggpages/NSF_Wheat_Resources.html		
HarvEST wheat and barley EST resources	http://harvest.ucr.edu		
Triticeae genetic linkage maps	http://wheat.pw.usda.gov/ggpages/map_summary.html		
Maize database	http://www.agron.missouri.edu/		

hybridize with the homologous DNA sequences on the array. In this way, an entire population of mRNAs from a tissue can be simultaneously hybridized, in a single experiment, with tens of thousands of known DNA probes that are immobilized at precisely known positions on the array. After the hybridization, the expression levels of each gene can be determined with a high-resolution laser scanner, and computer programs can group genes that are up- or down-regulated in increasingly sophisticated ways.

Although microarray technology is now widely used for transcript profiling, there have been technical and interpretative difficulties associated with accurate arraying of the DNA on the solid support and precise reading of the arrays. In particular, microarrays are not highly accurate and provide only approximations of mRNA levels. Several other, newer transcript profiling techniques have also been developed to help address these limitations, including serial analysis of gene expression (SAGE), restriction fragment differential display (RFDD), and massively parallel signature sequencing (MPSS). As with other genomics technologies, there is a danger that microarray analyses can overwhelm us with data. For example, in looking at changes in mRNA transcipt profiles during the early development of starchy endosperm of cereal grain, large numbers of transcripts are detected and many will follow similar developmental patterns. Faced with hundreds or even thousands of candidate genes of interest, what is the next step in terms of identifying key genes in the process? This could necessitate a refocusing of the experiment, for example, on genes already known to be involved in processes such as cell wall synthesis, and the attendant re-instatement, in part at least, of the hypothesisdriven approach!

In more general terms, profiling of mRNA transcripts is undertaken in the knowledge that mRNA levels and levels of the encoded proteins are not always correlated, for a number of reasons associated with transport, processing, and turnover of macromolecules in the cellular context. This has led to the development of proteomics as another subset of methods within overall functional genomics technologies. Results from proteome analyses can also be used to help narrow down the number of candidate genes identified in microarray experiments (Figure 2).

#### **Proteome Analysis**

The proteome may be defined as the complete complement of proteins that is present in a particular tissue under particular conditions. Again there are a number of approaches and components of proteomics technology, which are outlined in more detail in Proteomics. Traditionally, protein extracts from tissues of interest have been separated by two-dimensional (2D) gel electrophoresis that involves sequential separations based on size and isoelectric points. Between 1000 and 2000 proteins can be routinely separated on 2D gels, although as many as 10 000 have been resolved using this system. Many of the proteins will represent different isoforms from a single group of proteins, or multiple forms of a single protein with varying levels of post-translational modification. The identification of individual protein spots on the 2D gel can be based upon a combination of amino acid composition, peptide mass spectrometry fingerprinting, NH2-terminal sequence, molecular mass, and pI data, which are subsequently analyzed through appropriate protein databases (e.g., SWISS-PROT and OWL) and nucleotide databases. These procedures place heavy demands on highly sophisticated equipment, in particular expensive mass spectrometers and powerful computers. A suite of five mass spectrometers in a well-equipped proteomics laboratory might generate  $5-10\,\mathrm{GB}$  of data everyday. In the example of searching for proteins that might be involved in cell wall biosynthesis, it is advantageous in terms of data management and interpretation to enrich proteins of interest in the tissue extracts prior to 2D gel electrophoresis. Thus, the starting point for the isolation of proteins might be membrane preparations enriched in Golgi, where noncellulosic wall polysaccharide synthases would be expected to be located, or in plasma membranes, where cellulose synthases might be located. The example of cell wall polysaccharide synthases also points to some of the technical problems associated with proteomics. The enzymes are of high molecular mass and are normally membranebound. These types of proteins are typically underrepresented in 2D gel profiles, and their reliable detection requires more recently developed liquid chromatography procedures (see Proteomics for more details).

Another key activity in proteomics programs is the investigation of protein—protein interactions that are increasingly recognized as central to cellular function. In our work on the early developing wheat grain, the yeast two-hybrid system is used to progressively build up a picture of multiple interacting proteins that form transcription factor complexes and control the expression of genes that are critical for early endosperm and embryo differentiation and development. It can be confidently predicted that this component of proteomics will be increasingly important in the future.

#### **Metabolite Analysis**

Metabolomics is the high throughput study of the complete complement of metabolites in a particular tissue under defined conditions. Its evolution is based on the argument that metabolite profiles are the ultimate reflection of gene expression at the biochemical level, and that metabolites are closer to cellular function than either mRNA transcripts or proteins. Examination of a cellular proteome might lead to the identification of several key metabolic enzymes, some of which would catalyze reactions at the branch points of biochemical pathways. However, flux down a particular pathway cannot easily be predicted from the relative abundance of the enzymes in the cell, because if one enzyme has a higher catalytic efficiency than another, it could preferentially direct metabolic traffic down one of the pathways. Metabolite profiles theoretically provide a more objective measure of the final metabolic activities of the cell. In the example of cereal cell wall biosynthesis, enzymes that catalyze sugar-nucleotide interconversions could control the biosynthesis of specific wall polysaccharides. An abundance of UDP-glucose dehydrogenase and UDP-glucuronic acid decarboxylase mRNAs or proteins might suggest that the cell is actively synthesizing UDP xylose and UDP arabinose for heteroxylan biosynthesis. However, the actual detection of high levels of the sugar nucleotides themselves in the cell would provide much stronger evidence that this was in fact the case.

Metabolite profiles are determined by extraction of the tissue with aqueous or organic solvents, components are separated by gas chromatography or liquid chromatography, and individual metabolites are identified through on-line mass spectrometric analysis and database searching. One expects to find at least 500 metabolites in the extracts, of which about half would be identified against libraries of mass spectra. Standardization of the extraction and separation procedures is critically important if meaningful comparisons are to be made between different tissues or different conditions. Furthermore, the technique generates large volumes of data, which are subjected to computer-assisted simplification through hierarchical cluster analysis and principal component analysis. There is accumulating evidence that plant cells possess a surprising level of plasticity that enables them to quickly compensate for changes in gene expression. Metabolomics technologies have the potential to define in detail the regulation of biochemical networks in response to changing environmental conditions.

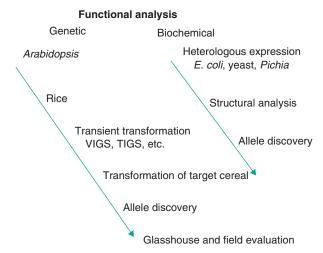
#### Phenotype Analysis

The final element of functional genomics technologies to be briefly discussed here is phenomics, which refers to the high throughput measurement of defined phenotypic characteristics. This expensive process is being applied to the analysis of very large mutant populations of cereals, in particular rice. As the mutant plants grow, phenotypic properties – such as coleoptile growth rate, root mass and length, flowering time, nutrient uptake, leaf shape, and size - are recorded, often through automated procedures, and compared with phenotypes of wild-type plants grown concurrently. Mutants with interesting or anticipated phenotypes are subsequently subjected to other genomics technologies, such as transcript, proteome, and metabolite profiling, in an attempt to fully describe the function of the gene that has been mutated.

#### **Functional Analysis Systems**

An important requirement in any cereal functional genomics project is to develop a range of systems for functional analysis of genes. Given that putative functions can be assigned to fewer than half the genes discovered in plant genome sequencing projects, there is a good chance that many genes of interest in a cellular process will require detailed functional analysis. Even if the candidate genes can be tentatively identified through database searches, it is usually necessary to provide independent corroborating evidence for their function.

There are several approaches to functional analysis of genes discovered in genomics programs (Figure 3), although none are especially amenable to high throughput analyses. Perhaps the most direct method for defining gene function is through heterologous expression of cDNAs in bacterial, yeast, Pichia pastorius, and baculovirus systems, followed by the direct measurement of a functional activity. Thus, expressed enzymes can be analyzed for activity and specificity. However, technical difficulties with heterologous expression of high molecular mass or membrane-bound proteins, together with associated difficulties in obtaining correct folding of proteins in heterologous systems and the need for ancillary proteins for activity, will often limit the usefulness of this biochemical approach. In recognition of these difficulties, attention is being directed to the development of high throughput cell-free protein translation systems, in which a gene can be transcribed and translated *in vitro* to generate sufficient protein for assays and other analyses.



**Figure 3** Functional analysis of candidate genes. Genetic approaches to functional analysis include loss-of-function and gain-of-function systems, through which genes are silenced or overexpressed, respectively. Silencing techniques include, *inter alia*, virus-induced gene silencing (VIGS), transiently induced gene silencing (TIGS), and stable transformation of cereals with the candidate gene. In the biochemical approach, candidate gene function is investigated through direct expression of the gene in a variety of possible heterologous systems. Subsequently, the 3D structure of the gene product and the degree of allelic diversity of the candidate gene can be addressed.

A second, genetic approach to the nonbiased functional analysis of a large number of genes is to generate mutant libraries, preferably through random insertion of T-DNA or transposons into many genes. The insertional mutagenesis approach results in a library of loss-of-function mutants. Because the sequence of the inserted DNA is known, the silence genes are "tagged" with a sequence from which flanking sequence can be readily determined. Thus, the gene that has been silenced can be identified in a mutant of interest. Several large transposon-tagged or T-DNA insertional libraries for rice have been generated and several groups are actively working on producing equivalent systems for wheat, barley, and maize. In maize, transposon-generated mutant populations have been widely used in gene identification projects. However, loss-of-function, or gene knockout, experiments can also be performed with selected genes of interest, at two levels. One method for functional analysis of candidate genes by lossof-function is known as the double stranded RNA interference (dsRNAi) procedure. Here, dsRNAs with sequence identity with selected endogenous genes are introduced into single cells via microparticle bombardment and results of gene silencing can be observed quickly. Alternatively, the dsRNAi construct can be used to silence a targeted gene after stable integration into transformed plants.

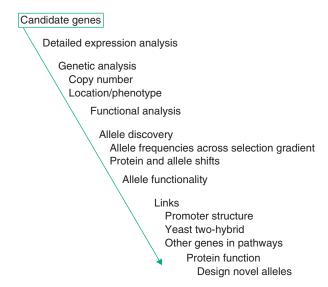
Provided a measurable phenotype is observed, the procedure can be used to test the functions of candidate genes in functional genomics programs.

Loss-of-function systems have been open to criticism because they provide only indirect evidence for the role of a particular gene. Transgenesis can be accompanied by genetic rearrangements that could perturb expression patterns or indirectly silence expression through changes in genes encoding transcription factors. As a result, gain-of-function genetic systems for analyzing cereal genes by transgenesis into *Arabidopsis*, tobacco, and yeast might ultimately prove to be more useful, particularly where the genes of interest are not normally found in the other species. Alterations in phenotype would be used in screening assays prior to more detailed assays of gene function.

# Economic, Social, and Environmental Benefits

A number of commercially relevant outputs can be expected from cereal functional genomics programs. First, novel genes will almost certainly be discovered and many will be valuable for crop improvement programs. The value of a novel gene will vary depending upon the significance of the altered phenotype resulting from transgenesis, the extent to which the phenotype will be modified, and the breadth of application. For example, a gene conferring efficient use of soil Mn in barley only, would be of limited value. However, a gene that stabilized yield under drought to provide an average 10% yield benefit over an extended period (an achievable goal) and was effective not only in wheat and barley but also in other crops would be highly valuable. Both genes and transgenic germplasm can be commercialized through delivery into breeding programs.

Where the gene controlling a trait has been isolated, diagnostic markers can be developed for each allelic variant of the gene (Figure 4). Such markers are 100% accurate in predicting the phenotype of the plants for the target trait and can be readily used in high throughput screening of germplasm in breeding programs. If, for example, a wheat marker tightly linked to drought tolerance were discovered, breeders in large wheat breeding programs would probably all wish to use that marker. Further, once a gene has been cloned, screening for allelic forms becomes possible and a range of alternative alleles could be identified. A diagnostic marker for specific alleles and the associated germplasm can be made available to breeding programs. It is also anticipated that functional genomics programs will develop new technologies



**Figure 4** Additional analysis of candidate genes. Most cereal genes are members of multigene families. The number of genes and genome location of members can be defined, and the degree of allelic variation of individual genes can be investigated. In the latter approach, a gene of interest from different cereal varieties or related germplasm can be examined. If one variant of the gene can be associated with a trait of interest, such as tolerance to a particular abiotic stress, the effects of this variant, or allele, can be followed at the gene product and functional levels and, if necessary, transferred to other species or varieties. Further, interactions of the gene product of interest with other proteins in the cell can be identified through yeast two-hybrid technology and new alleles might be designed through a detailed knowledge of the structure of the gene product.

for gene and allele discovery and functional analysis of genes (Figure 4).

In most cereal functional genomics programs, the generation of products and technologies that can be quickly adopted by rural and related food and manufacturing industries will produce economic benefits in those industries. Let us consider potential benefits arising out of a genomics program on abiotic stress tolerance in the key cereals — wheat and barley. In Australia alone, the area sown to cereal crops in 2001 was close to 17 million hectares (Mha), and the value of those crops is in the \$6–8 billion range annually. Functional genomics programs on abiotic stress tolerance could conceivably deliver an improvement of 10–20% in productivity levels if varieties with tolerance to multiple stresses could be generated.

Benefits accruing from the generation of plant varieties that are more sustainable, require less fertilizer, and have improved water-use efficiency and tolerance to salinity will extend beyond economic benefits to social and environmental benefits. For example, more than 50% of cereal cropping areas in southern Australia suffer regularly from transient salt stress, which is usually linked with moisture stress. Internationally, abiotic stresses represent the major cause of large yield fluctuations. It is estimated that wheat yields in India will drop to one-quarter of current levels by 2020 largely due to these problems. With the developing world accounting for almost half of the world's 550–600 million tons (Mt) annual wheat production, any improvement in yield stability under stress will have major social, economic, and environmental impacts internationally.

#### **Acknowledgments**

The authors' work has been supported over many years by grants from the Australian Research Council and the Grains Research and Development Corporation.

See also: Barley: Genetics and Breeding. Canola: Genetics and Breeding. Genome Mapping. Lupin: Breeding. Maize: Genetics. Plants: Diseases and Pests. Proteomics. Wheat: Genetics.

#### **Further Reading**

Devos KM and Gale MD (2000) Genome relationships: the grass model in current research. *Plant Cell* 12: 637–646.

Fiehn O (2002) Metabolomics – the link between genotypes and phenotypes. *Plant Molecular Biology* 48: 155–171.

Holtorf H, Gutton M-C, and Reski R (2002) Plant functional genomics. *Naturwissenschaften* 89: 235–249.

Roessner U, Luedemann A, Brust D, *et al.* (2001) Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. *Plant Cell* 13: 11–29.

The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408: 795–815.

Yu J, Hu S, Wang J, et al. (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. indica). Science 296: 79–92.

#### **Relevant Websites**

http://wheat.pw.usda.gov. http://rgp.dna.affrc.go.jp. http://www.arabidopsis.org.

### **GLUTEN AND MODIFIED GLUTEN**

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#### What is Gluten?

A nontechnical definition of gluten describes it as "the sticky, viscous residue after removal of starch from flour." This definition would include corn gluten, the protein residue from isolation of starch from corn. However, this material is quite different to wheat gluten, the residue from production of wheat starch from flour. In the technical sense, the term "gluten" usually refers to wheat gluten. However, for people with food intolerance to cereals, especially celiac disease, "gluten" includes the equivalent proteins from rye, triticale, barley, and possibly oats (see Celiac Disease). Thus, "gluten-free foods" refer to food products free from these cereal proteins, or whose cereal protein content is less than a defined amount (usually 200 ppm). In this article, only the properties and uses of wheat gluten will be discussed.

Gluten may thus be defined technically as the "cohesive, visco-elastic proteinaceous material prepared as a by-product of the isolation of starch from wheat flour" (Figure 1). A more theoretical definition may define it as the "storage proteins of the wheat grain." Both definitions are correct but neither



**Figure 1** Gluten prepared from wheat flour, showing its cohesive and visco-elastic nature. (Courtesy of Colin Wrigley.)

tells the whole story. For the purposes of this article, gluten is the commodity isolated on a commercial scale and sold for a variety of purposes in many countries of the world. In particular, the dry form of the product in which the functional properties may be regenerated by rehydration (a material known as "vital wheat gluten") will be mainly considered.

#### **Composition of Gluten**

Although sold as a protein, gluten contains more than just protein. The commodity usually contains approximately 75% protein, 8% moisture, and varying amounts of starch, lipid, and fiber. The starch and fiber become entrapped in the cohesive matrix of the protein and become more difficult to remove as the protein content increases. The amount of starch varies, and more extensive washing can reduce the starch and fiber content and increase the protein content. The extra water needed for this creates its own problems by producing a larger amount of effluent from the process, and increasing the biological oxygen demand (BOD) of that effluent (see below). Consequently, gluten of higher protein content is only produced as a special order and at a premium price. Lipid is unaffected by additional washing. Most of the lipid content of the flour becomes associated with the protein during the washing process. The proteins are hydrophobic and the lipids bind to the hydrophobic areas of the protein as they are repelled by the water used in the washing. Lipids are strongly bound to gluten and are removed with much more difficulty than they are removed from the original flour. The lipid content of gluten is primarily determined by the lipid content of the flour from which it came, and is unaffected by additional washing.

The protein that makes up gluten is actually a complex mixture, containing many, perhaps several hundred, polypeptide species. A typical amino acid analysis of the complex mixture is shown in **Table 1**. The individual proteins are divided into two main classes — monomeric and polymeric. These terms can be confusing in that any protein is a polymer of amino acids. In gluten, monomeric refers to individual, discrete polypeptide species, whereas polymeric refers to chains formed from individual monomeric proteins by cross-linking them with disulfide bonds of cystine residues in adjoining chains. The monomeric proteins are often called gliadin and the polymeric ones are called glutenin. It should

Table 1 Amino acid composition of commercial vital wheat gluten

Amino acid	Content <sup>a</sup>
Alanine	3.0
Arginine	4.3
Aspartic acid <sup>a</sup>	4.8
Cystine	2.6
Glutamic acid <sup>b</sup>	39.0
Glycine	4.6
Histidine	2.7
Isoleucine	4.4
Leucine	8.4
Lysine	2.2
Methionine	2.1
Phenylalanine	7.3
Proline	14.6
Serine	5.6
Threonine	3.1
Tyrosine	4.3
Valine	4.6

Values expressed as q amino acid/100 q protein.

be noted that some of the glutenin subunits do exist in gluten in the monomeric form (see Wheat: Grain Proteins and Flour Quality).

#### **How Is Gluten Made?**

Gluten was first prepared from flour almost 300 years ago by an Italian named Beccari, who conducted a simple water-washing experiment with wheat flour. This discovery, which can be easily reproduced in the home kitchen, has become the basis of a major cereal industry, utilizing millions of tons of wheat annually in North America, Europe, and Australia (Figure 2). The commercial process is basically an efficient repetition of Beccari's experiment. Most commercial operations use variations of either the "batter process" or the "Martin process."

Originally, gluten was produced as a by-product of the wheat starch industry. There is a high demand for starch for both food and nonfood uses, and in those areas where wheat was the major crop, it became the main raw material in the preparation of the starch required for various industrial purposes. Gluten became an embarrassing by-product. In some cases, its disposal was in municipal sewers and drains, but this soon became an undesirable practice. It was also dried in a variety of ways to yield a protein-rich material used in animal feeds, and wet gluten was also used to fortify bread and other baked products made from flour. The shelf life of wet gluten, usually only a few hours, severely restricted its use



Figure 2 Gluten prepared in a commercial process.

in this form. It was not until the application of ring drying that a dry vital gluten product could be prepared in significant amounts, allowing its trade as a valuable commodity.

#### **Martin Process**

In this method, a wheat flour dough is washed with water while it passes through a tumbling cylindrical agitator. The work applied to the dough is not dissimilar to the effect of kneading a dough under water in that starch comes out of the dough and the protein content increases. The tumbling action moves the dough along the cylinder and the starch passes through small holes in the wall while the protein remains inside, receiving further washing until it tumbles out at the end.

#### **Batter Process**

In this process, a thick suspension or batter of flour is stirred slowly in a tank for several hours, during which time the starch separates from the protein. The mixture is then passed through a fine sieve, which allows the starch granules to pass through but retains the curds of gluten on the screen. This gluten is then washed with water to remove further starch in a similar manner to the Martin process and then dried.

The Martin process is a continuous process, while the nature of the batter process makes it more suited to batch operation.

#### Other Processes

Most commercial operators use one or other of the above methods with modifications, but there have been many other processes suggested for production

<sup>&</sup>lt;sup>b</sup>Glutamic acid and aspartic acid are predominantly in amidated form, with ~90% existing as glutamine and asparagine respectively.

of gluten. While most have not made it past the laboratory curiosity stage, others, e.g., the Alfa-Laval Raisio process, have been applied in full-scale production facilities. The basis of these other processes varies and some use centrifugal techniques which may involve either conventional industrial centrifuges or hydro-cyclones to separate the starch from the protein. Many operators use hydro-cyclones as the principal way of cleaning the starch, and, in some cases, in the actual separation of the starch and gluten.

Many of the newer methods utilize whole grain as the raw material, avoiding the production of flour in a dry-milling step. This allows a more complete isolation of the starch fraction from the wheat, but cleaning the protein and starch of residual bran is a major disadvantage of these types of methods. Improved milling processes have reduced the amount of endosperm remaining in the bran and offal fractions in conventional milling, so there is little advantage to be gained in wet milling for starch and gluten recovery. There is also the need to dry the bran unless it can be processed on the spot or at least locally, and this cost will usually exceed the economic benefit of improved starch yield. Thus, despite these newer methods, modifications of the traditional processes have remained the preferred choice for almost all the gluten produced worldwide.

#### **Dry Gluten**

Gluten is very susceptible to heat when wet, and relatively low temperatures destroy the cohesive, viscoelastic properties ("vitality") which make it unique among food proteins. Attempts to dry gluten while retaining these properties were unsuccessful until the application of the ring drier to gluten in the first half of the twentieth century. This process has been the basis of gluten drying since then. The principle is that simple wet gluten with a moisture content of  $\sim 70\%$  is mixed with sufficient dry gluten to reduce the moisture to  $\sim 20\%$ . This lowered-moisture material is comminuted and subjected to flash drying in a ring drier. A portion of the dried gluten is removed and packaged, while the rest is returned to the drying cycle to reduce the moisture content of more wet gluten. The procedure is still very sensitive to excessive heat, but with careful control of the temperature a vital wheat gluten is produced.

An alternative way of drying to prepare a vital gluten is to disperse the gluten in aqueous ammonia or acetic acid and then spray-dry this dispersion. The resulting product retains the visco-elastic properties of gluten, and it may be used for most of the same purposes as normal vital gluten. The cost of this

drying procedure, together with environmental concerns, limit its application except for special reasons. Another dry gluten product is known as "devital gluten." This material has lost its cohesive, viscoelastic properties, but retains the insolubility and water-binding capacity of vital gluten. It is commonly used where the cohesiveness of vital gluten can actually be a disadvantage.

#### **Waste Products**

The amount of water required for each ton of flour varies according to the operator. All processes have a significant waste stream which consists of the wash water plus soluble protein, damaged starch, and sugars plus some fiber. Disposal procedures for this waste vary, depending on the manufacturer, and methods include fermentation to produce ethanol or methane, isolation by drying for use in animal feed, and discharge into the sewerage system. The last option is becoming less common as environmental concerns grow worldwide.

#### **Properties of Gluten**

The most important properties of gluten are its solubility and its rheological functionality. By the nature of its preparation, gluten is a protein that is insoluble in water. While there are small amounts of water-soluble proteins trapped in the gluten matrix, these are essentially not extractable into water under normal conditions. Despite its insolubility and its hydrophobic nature, gluten absorbs approximately twice its dry weight of water to form a hydrated gluten. This material is effectively the same as the wet gluten first isolated from flour. In the case of commercially prepared gluten, drying conditions may cause some deterioration of the functional properties, but gluten prepared in the laboratory shows no change in its properties after freeze-drying and rehydration.

The principal components, gliadin and glutenin, are both insoluble in water. Gliadin can be solubilized in 70% ethanol, one of the steps of the Osborne fractionation of wheat proteins (see Cereals: Protein Chemistry) and the residue after this extraction is considered to be glutenin. Isolation of the dissolved material, however, yields a protein which has lost most of its functional properties. Both gliadin and glutenin may be solubilized to a certain degree by the use of acidic conditions. By careful control of the pH, gluten may be separated into a number of fractions. Reprecipitation by pH adjustment, or by drying the acidic solution directly, gives products which maintain their functional properties.

This can be shown by reconstituting flour through recombining the starch, the isolated protein fractions, and the water-soluble components prepared during the extraction of gluten. Doughs prepared by careful reconstitution show little change in dough strength compared to that of the original flours.

Its rheological properties are the basis of the functional uses of vital gluten. It is these properties that give wheat flour doughs the characteristics that allow the production of breads, cakes, biscuits, and noodles. Thus, gluten can be considered to be like a dough in which the diluting effect of starch is no longer present. In the wet state, the protein molecules form a cohesive matrix which, in dough, also holds the starch granules within it. This matrix is also elastic, allowing it to stretch and expand. In aerated doughs, this elasticity permits the expansion of gas bubbles which produce the texture of bread and cakes. If the gluten matrix is too weak, or the protein content is too low to form an effective matrix, the bubbles expand beyond the elastic limit and burst, reducing the overall volume of a baked product.

#### **How Is Gluten Used?**

The uses of gluten worldwide vary from country to country, but the most common usage in western countries is addition to flour in baked goods of various types (Table 2). The second largest use is in pet foods. Here, gluten is added as a protein source to improve the nutritional quality of the pet food. Its hydration and lipid-binding properties also assist in improving the overall properties of the product. A growing market for gluten is as an ingredient in aquaculture feed, where its cohesive properties hold the feed together when it is put into water.

**Table 2** Usage of gluten in different regions (as percentage of total usage for region)

	N. America (%)	EU (%)	Australia (%)	Japan (%)	Total world (%)
Baking	83	16.5	54	30	63
Flour fortification	0.5	66	9		14
Pet food	12	13.5	13		8
Meats	1		9	25 <sup>a</sup>	5
Breakfast cereals	1		12		2
Noodles				10	
Sausages				12	
Other	2.5	4	3	23	8

<sup>&</sup>lt;sup>a</sup>Includes gluten used for synthetic fish products.

Gluten is used in other countries in a variety of ways. Perhaps the major use is as a meat replacement in vegetarian foods, and in the production of artificial forms of expensive foods such as crab meat. It is also used in the preparation of soy sauce extenders, and the manufacture of mono-sodium glutamate. The high glutamine content of gluten makes it an ideal starting material for this latter product.

#### **Baked Goods**

Wheat-based products, in which gluten is used to fortify flours of lower-than-desirable protein content, have been and continue to be the main application of gluten. There is a concern about the effects of gluten on people with celiac disease and others with wheat protein allergies, but its incorporation into foods that already contain gluten cannot be questioned. Increasing the protein content of a flour by adding vital gluten improves the quality of the flour to be equivalent to one with the higher protein content. This fortification may be necessary because the flour has a naturally low protein and a higher protein content is needed to make quality products, or because the addition of gluten provides a particular property sought in the food by improving the quality of the protein. Addition of gluten to a flour of low protein content improves the texture and shelf life of bread prepared from it, but it is useful in other applications as well. For example, addition of  $\sim 1\%$ of gluten to flour reduces pretzel breakage in the finished product, but the addition of too much gluten may result in pretzels that are too hard to eat. Addition of gluten to pasta-type products will also reduce breakage. However, the desire of consumers for pasta of 100% durum wheat origin means that usage for this purpose is now more limited.

#### **Other Uses**

In addition to its use in pet foods, gluten is commonly added as a binder in meat products for human consumption. Here, the desired property is its ability to bind fat and water while at the same time increasing the protein content. Gluten and modified glutens have also been used in calf-milk replacements.

A number of nonfood uses have also been suggested for gluten. These include adhesives, paper coating, detergent formulations, slow-release pharmaceuticals, medical bandages, construction materials, and binding of heavy metals in industrial processes. Use of gluten in films has also been tried. Gluten-based films may be cast from solutions of gluten in ammonia. Production of gluten films with satisfactory properties could provide a new biodegradable film for widespread use.

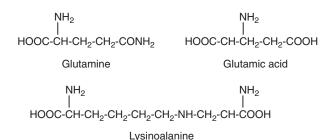
#### **Modifying Gluten**

Gluten is a protein, intermediate in price between low-value commodities suitable only as animal feed without further processing and high-value materials such as casein and soy isolates. This gives significant scope for modification of its properties for value addition. Various threatened surpluses in the market for gluten have turned the attention of manufacturers to ways of converting gluten into products with vastly different properties.

#### **Chemical Modification**

The main modification applied to gluten is solubilization. Gluten becomes soluble in a variety of chemicals including urea solutions, lactic acid, soaps and detergents with or without urea, acetic acid, hydrochloric acid, sodium hydroxide, 70% ethanol, and 2-chloroethanol to name just some. Many of these are incompatible with food products, but for nonfood purposes there are few limitations other than cost, safety, and environmental concerns.

Solubilization by deamidation is the major method applied. This may be achieved with either acid or alkali. Approximately 90% of the glutamic acid in gluten is in the form of glutamine (Figure 3). Removal of the amide group of these residues to form the corresponding carboxylic acid changes the potential ionic charge on the protein, thus increasing its solubility above a certain pH. In acidic deamidation, there is also a degree of peptide hydrolysis to form lowermolecular-weight polypeptides which are also usually more soluble than larger ones. In alkali solutions, peptide hydrolysis does not usually occur as readily as in acid. However, there is the possibility of alkaline attack on the disulfide bonds of cystine, with the subsequent opportunity of creating cross-links due to the formation of lysinoalanine (Figure 3). Use of temperatures close to ambient has shown no formation of lysinoalanine. The reaction mixtures of acid or alkali deamidation require neutralization before the products can be used for their final purpose, a step



**Figure 3** Structures of glutamine, glutamic acid, and lysinoalanine.

which produces significant amounts of salts which usually need to be removed. This can be done by iso-electric precipitation, i.e., adjustment of the pH to the iso-electric point at which proteins are least soluble as they have no net charge. A procedure that uses acidic or basic proteins to neutralize the alkali or acid, respectively, has been reported. No inorganic salt is formed in this process, but the overall proportion of gluten in the final product is very much reduced. Deamidated gluten is easily dispersible which makes it suitable for use in foods for emulsification or foam stabilization. It has been used in meat products, nondairy coffee whiteners, beverages, and milk puddings. No benefits have been reported for the use of deamidated gluten in bread doughs.

Gluten may be treated with sulfuric acid, phosphoric acid, or chlorosulfonic acid to prepare products which bind greatly increased amounts of water. There have been reports that some of these products bind up to 200 times their own weight of water. Other chemical modifications include acylation with carboxylic acid anhydrides. In particular, treatment of gluten with succinic anhydride increases its solubility at pH 7 (close to the point of minimum solubility of native gluten) but decreases its solubility at pH 3 where it is normally quite soluble.

#### **Enzymic Modification**

Hydrolysis of the peptide bonds by enzymes also increases the solubility of gluten. This is achieved by reducing the molecular weight of the polypeptide chains. A number of commercially available enzymes have been used for this purpose, including papain, bromelain, subtilisin, trypsin, and pronase. The enzyme-solubilized gluten has many of the properties of chemically deamidated gluten, such as foam stability and emulsion formation. Unlike deamidated gluten, enzyme-solubilized gluten has beneficial effects on dough properties. Addition at levels of 1-2% reduced dough mixing times by amounts similar to those achieved by chemicals, such as cysteine and ascorbic acid, which are often added commercially to give improved loaf volumes. Many of the reports of enzyme-solubilized gluten refer to it having a bitter taste. This is believed to arise from the formation of small peptides that have been identified with bitter flavors in other proteins. Thus, treatment with enzymes needs to be carefully controlled to minimize the formation of these small peptides.

#### The Future for Gluten

Production of gluten is still driven by the need for starch. Thus, it will always be produced while wheat is a major source of starch. There is the risk that demand for starch will grow faster than the demand for gluten, but to date, this has not happened despite dire predictions on more than one occasion. Although its absolute price has not changed significantly over many years, by becoming relatively cheaper it has found its way into more applications for which it was formerly too expensive. This has served to maintain its value while output has greatly increased. Consumer concerns about gluten-free foods may limit its application in some areas, but the ubiquity of wheat in many foods ensures that gluten will remain an acceptable additive. The greatest threat to the gluten industry has been and will remain the lower cost of preparing starch from sources other than wheat. Gluten has played a major role in keeping the production of wheat starch economically viable in face of cheaper starch from other sources. This situation is expected to be unchanged in the future.

See also: Celiac Disease. Appendix: Foods for Celiac Diets.

#### **Further Reading**

Hoseney RC (1994) *Principles of Cereal Science and Technology*, 2nd edn. St. Paul MN: American Association of Cereal Chemists.

Pomeranz Y (ed.) (1988) Wheat Chemistry and Technology, 3rd edn. St. Paul, MN: American Association of Cereal Chemists.

Shewry PR and Lookhart GL (eds.) (2002) Wheat Gluten Protein Analysis. St. Paul, MN: American Association of Cereal Chemists.

Shewry PR and Tatham AS (eds.) (2000) Wheat Gluten. *The Proceedings of the 7th International Workshop Gluten 2000*. London: Royal Society of Chemistry.

## **GRAIN, MORPHOLOGY OF INTERNAL STRUCTURE**

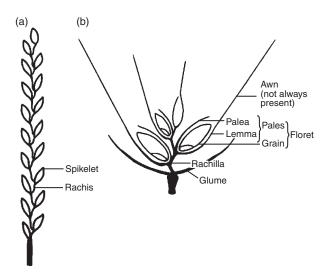
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This is a wide topic because virtually every aspect of grain technology is related to grain structure. Handling, processing, and utilization of grains depend on specific details of their structure. For example, in dry milling of rice and wheat, removal of the bran layers is necessary. The wheat grain is not covered with a tightly attached hull, so unlike in rice initial de-hulling is not required. Bran removal in wheat is part of the process of producing fine particulate flour by crushing the endosperm. Bran removal in rice should leave an unbroken polished rice grain. In both wheat and rice, endosperm should be at a specific hardness to achieve the desired outcome. Wet milling is a fractionation process. In maize, starch, oil, and protein components are separated. Again, the physical structure of the grain is exploited in the design of the milling process. Issues such as these will be covered in detail in this article. More specifically, the following aspects will be covered: wheat grain morphology and its effect on dry and wet milling; rice grain morphology and its effect on dry milling and nutrition; maize grain morphology and its effect on handling and wet milling; barley structure and its effect on brewing; and a comparative summary of general effects of grain structure.

# Wheat Grain Morphology and Its Effect on Dry and Wet Milling

The relationship of the grain to overall spike structure is important in milling (Figure 1). Historically, the wheat ancestors (for example the diploid Triticum monococcum) had a brittle rachis trait. This means that after ripening of the grain and drying of the plant, the rachis (where the spikelet is attached to the axis) breaks naturally and the seed (still surrounded by a tightly bound palea, lemma, and awn) falls from the plant. This is an essential survival feature for a plant whose seeds are dispersed in nature through the action of wind, insects, etc. In a farmer's field, a brittle rachis would not be viable, as harvesting would be extremely difficult. The genetic step from brittle to nonbrittle rachis is the key to domestication of small-grain cereals (e.g., wheat, rice, barley). However, wheat has a further useful adaptation to domestication, which

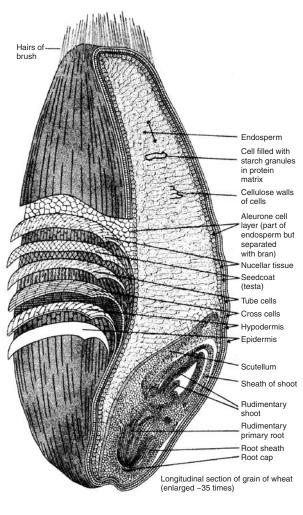


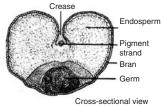
**Figure 1** The structure of the wheat spike (ear). Cultivated wheat requires a nonbrittle rachis so that mechanical harvesting is possible, and a loosely attached palea and lemma, so that a dehulling step is not required. (From Evers AD and Bechtel DB (1988) Microscopic structure of the wheat grain. In: Pomeranz Y (ed.) *Wheat: Chemistry and Technology*, vol. 1, 3rd edn., pp. 47–95. St. Paul, MN: American Association of Cereal Chemists.

differs from rice. In wheat, the palea and lemma naturally separate from the grain – this is called a "naked" grain characteristic. In rice, the grain is "covered," i.e., the hull is attached to the grain and requires a separate processing stage (de-hulling) to remove it.

#### **Bran**

The wheat grain has been described as "a singleseeded fruit called a caryopsis, in which the ripened ovary wall is fused to the seed." The classical diagram for hexaploid common (bread) wheat (Triticum aestivum) grain structure was produced by the Wheat Flour Institute, Washington, DC (Figure 2). The objective of flour milling is to remove the embryo (germ) and the outer layers (epidermis, hypodermis, cross cells, tube cells, seedcoat, and nucellar tissue), which collectively are termed the bran. The aleurone layer, part of the endosperm, is also removed with the bran, and from a miller's point of view may be included as a bran layer. Much of the vitamin and mineral content of the grain is contained in the bran. In many countries, wheat flour is enriched or fortified to replace some of the nutrients lost in milling (see Fortification of Grain-Based Foods). Removal of the bran (which, including the aleurone layer, comprises  $\sim$ 15% of the grain by weight) permits production of white flour. Apart from appearance, white flour has different technological properties to wholewheat meal (that produced by grinding the whole





**Figure 2** The structure of the wheat grain – longitudinal and cross-sectional views. (From Pomeranz Y (1987) *Modern Cereal Science and Technology*, pp. 25–39. Weinheim, Germany: VCH Publishers.)

grain without bran separation). The presence of large amounts of bran dilutes the proportion of gluten in the product, and results in a denser, more fiber-rich product. The presence of small amounts of bran (as bran specks) results in off-white color in flour and in many final products (e.g., steamed bread). In fresh white noodles, discoloration to gray is associated with polyphenoloxidase (PPO) content of flour, and most PPO is present in bran. In Chinese steamed bread, formation of brown spots on the surface may also be associated with PPO effects from bran

contamination (and the use of NaCl in the formulation). Because of the high mineral content of bran relative to endosperm, bran content in flour can be estimated by ashing (furnace heating) a flour sample, and weighing the residue. For the same flour yield, a variety that gives lower ash content (less bran) is of course preferable.

In wheat flour milling, grain is tempered to a uniform moisture content (usually 15.5%). The effect of this is to make the bran layers flexible so that under the crushing action of roller milling, bran will break into large flakes, not be crushed into powder. This formation of flakes of bran results in pieces, which are of different size, shape, and density to the endosperm particles, which on size reduction become flour particles. Without this physical flexibility in bran, milling would not be possible. However, with crushing, some endosperm is likely to be attached to some of the bran flakes. Continuing milling to remove the last particles of endosperm (i.e., aiming for a high flour yield, or percentage yield of flour relative to starting weight of grain) will increase contamination of flour with bran. The flour yield also depends on several other properties of bran:

- 1. the thickness of the bran layers, which is under genetic control,
- 2. the size of the grain bran will be a relatively lower fraction of a larger grain; also a plump well-filled grain will be preferable, and
- 3. the characteristics of the crease.

The crease in a wheat grain is the lateral fold (Figure 2) ending with the pigment strand. A deep crease results in more problems with bran removal in milling — more bran particles attached to endosperm chunks usually result from a deep crease. Also, the crease can trap contaminants, e.g., dust and fungal spores, which can cause problems in storage and in cleaning the grain before milling.

Grain color in wheat, usually due to red pigmentation in the bran (seedcoat) is usually classified as red (presence of pigmentation) or white (absence). White wheat flour, if contaminated with bran, will show less discoloration than red wheat flour. This is why hard white type wheat is becoming more widely accepted as suitable for Asian noodles. Other colors are possible but rare in commercial trade — e.g., blue, purple, or even "black" wheat, with a dark purple pigment in the seedcoat, which is used in some specialty products in China.

#### **Endosperm**

The product of wheat dry milling is flour, the result of crushing endosperm. The endosperm consists of cells

surrounded by a pentosan-rich cell wall (mostly arabinoxylans), and containing starch granules embedded in a protein matrix. The size of the cell and the thickness of the cell wall affect the proportion of cellwall material relative to starch and protein in the endosperm. The cell wall absorbs water during dough mixing, so it is significant in determining optimum water absorption of a flour. The dimensions of the cell wall are genetically variable. The starch granules consist of two types, larger A granules and smaller B granules. Starch composition (proportion of amylose to amylopectin in starch) determines physical properties of any gelatinized starch product resulting from cooking the flour. This is most important in Japanese white-salted noodles ("udon") where a low amylose (partial waxy), high-swelling starch gives favorable texture. In many other products, such as baked bread, the starch composition, within normal limits for wheat flour, does not have much impact on product quality. Starch granules are subject to shear or breakage, termed "starch damage" in milling, if the endosperm is too hard (e.g., genetically hard texture, or too low moisture content in milling) or if milling equipment is not adjusted correctly. A broken granule, exposing the interior, is much more readily subject to hydrolysis by amylases during the bread-making process (during fermentation and early stages of baking before the enzyme is inactivated). Some starch damage may be desirable to provide a substrate for yeast growth, but excessive damage adversely impacts on quality and may result in a sticky texture in the final loaf. Endosperm hardness is largely due to the structure of two puroindolines (components of friabilin), which are amyloplast membrane proteins present in the endosperm. There is a widespread confusion about grain hardness (protein influenced) and noodle texture (starch influenced). A high-swelling starch but not necessarily a soft endosperm is required to make a soft-textured noodle (such as Japanese udon).

#### **Embryo (Germ)**

The embryo consists of the rudimentary structures (shoot and root) of the plant, which can grow from the grain, plus the root and shoot sheathes, the root cap and the scutellar tissue. The embryo,  $\sim 3\%$  of the grain weight, contains  $\sim 8\%$  of the grain protein, but it is mostly in the form of enzymes related to subsequent growth of the seedling, not storage protein (gluten) as in the endosperm. Thus, the wheat germ protein is of higher quality (more favorable amino acid balance, such as higher lysine content) for human nutrition. The lysine level in protein in the embryo is  $\sim 8\%$ ; in endosperm protein  $\sim 2\%$ , and

in bran protein  $\sim$ 4.5%. In addition, 20% of the lipids of the grain are in the embryo (oil-containing spherosomes are present in the aleurone layer and in scutellar cells), but there are also high levels of lipase and lipoxygenase enzymes, which hydrolyze and oxidize oil, causing rancidity. Wheat germ hence has uses as a food material or supplement for human nutrition, and is valuable as an additive to animal feed (particularly for monogastric animals such as pigs and poultry). On the other hand, wheat germ, unless treated, has a short shelf life. Whole-wheat meal also has a shorter shelf life than white flour. Removal of germ to produce flour results in a product that is stable for long periods of time.

#### **Other Features**

Dry milling of durum (*Triticum durum*) wheat (a tetraploid species) to make semolina (larger particle size than flour from common wheat) has a few special features related to the durum endosperm texture. The physical hardness (vitreousness) of durum endosperm makes the grain liable to break during combine harvesting. These broken grains should be separated and recovered during cleaning of grain

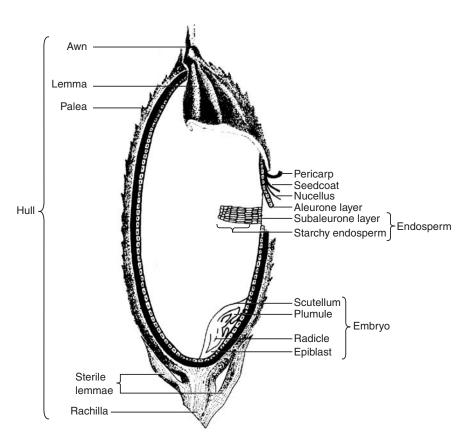
before milling, or a high level of wastage may occur. Optimum tempering of durum grain before milling is also essential, and varies with the vitreousness of the endosperm. Careful milling (e.g., more break rolls than for bread wheat) is needed to achieve suitably fine semolina for high-quality pasta.

In wheat wet milling (to produce wheat starch, gluten, and wheat germ oil), separation is by soaking the grain, crushing, and sedimenting off the starch component. B-type starch granules are too small to easily sediment and their complete removal would require expensive centrifugation steps. Hence, a genetically low proportion of B-type compared to A-type granules would be a strong advantage for increased starch yield in wet milling. Considerable effort is being expended to identify more suitable wheat varieties for this use.

#### Rice Grain Morphology and Its Effect on Dry Milling and Nutrition

#### **Grain Structure**

The anatomical structure of rice (Figure 3) is of course very similar to that of wheat. However, grain shape is



**Figure 3** The structure of the rice grain (From Juliano, JB and Aldama MJ (1937) Morphology of *Oryza sativa* L. *Philippines Agriculturalist* 26: 1–134. Republished in Juliano BO (1993).

of more significance. Short-, medium-, and long-grain rice are common classifications. In the United States, texture can be recognized from grain length – short is low amylose (sticky) and long grain is intermediate amylose (20-25%) (harder cooked texture such that grains are separate after boiling). This is a marketing phenomenon, not a genetic relationship, and in other countries long grain may well be waxy or sticky types, sometimes a source of confusion to traveling Americans. Grain shape (expressed as ratio of length to width) affects milling. A rounded grain with a low length: width ratio will be more resistant to breakage than a slender type of grain. Rice lacks the crease found in wheat grains. This enables abrasive milling to remove the bran and leave only endosperm, greatly simplifying the milling process.

The hull, which covers the rough (also known as paddy) rice, comprises 17–24% of the weight of the paddy rice. It consists of lemma and palea, awn, the rachilla, and lemmae. Varietal differences in percentage of hull affect yield of brown rice, and this in turn affects milled rice recovery (yield of white polished rice). The hull (husk) is silica-rich and indigestible for human nutrition, even after heat treatment, even though it is rich in minerals.

#### Cracking

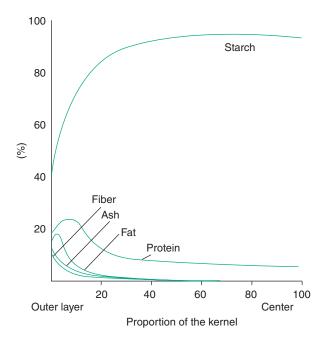
In producing polished white rice, the control of cracking is essential. Cracks caused by poor drying conditions after harvest, for example, will result in grain that breaks easily in milling (de-hulling or polishing) producing a lower yield of head rice and consequently much lower value product. In the production of parboiled rice, rice with the hull still attached is steamed, driving nutrients from the hull and the bran into the endosperm, inactivating enzymes that would cause rancidity, and reducing microbial load. However, after steaming, the parboiled rice must be dried slowly before it is de-hulled and polished, or cracks will form with the same effect as before. Parboiled rice, despite the steaming treatment, does not require less time to cook by the consumer than ordinary white rice, because water penetration to the center of the grain is slow. Instant rice is prepared by steaming polished white rice. It is dried rapidly, forming numerous internal cracks. These cracks, not the starch gelatinization induced by steaming, allow the product to be cooked by the consumer in a few minutes because they aid water penetration. Instant rice is more physically liable to breakage in transportation than raw polished white rice, and the softer texture resulting from the cracks is unattractive to traditional rice consumers in many parts of the world.

#### **Rice Bran Layers**

The bran layers (pericarp, seedcoat, nucellus, and aleurone layer) contain most of the minerals (represented as ash), vitamins, fiber, fat, and much of the protein present in the rice grain (Figure 4). Considerable debate over the years has taken place over the suitability of white polished rice, compared to brown rice, in nutritionally deficient diets in some developing nations. In fact, bran also contains phytate (phytic acid), which binds to available minerals provided by brown rice, and may also bind and remove additional minerals from the gastrointestinal tract. Brown rice consumption may therefore cause a net loss of minerals to the body, and in addition is susceptible to rancidity in storage (lipids and lipases still present) and more susceptible than polished rice to storage pest attack.

#### Rice Endosperm

Rice starch granules are small  $(2-4 \mu m)$ , polygonal and compound, i.e., many small granules are compacted together. Compositionally, rice starch varies from 0% (waxy, sticky, glutinous) amylose to over 35%. This range is much wider than found naturally in hexaploid wheat starch. Expression of naturally occurring waxy mutants is more likely in a diploid cereal (e.g., rice, maize, sorghum) than in a hexaploid



**Figure 4** The effect of progressive surface abrasion of brown rice on content of major constituents. (From Barber S (1972) Milled rice and changes during aging. In: Houston DF (ed.) *Rice: Chemistry and Technology*, pp. 215–263. St. Paul, MN: American Association of Cereal Chemists. Republished in Juliano BO (1993).)

where effect is buffered by the other two sets of chromosomes present. Rice differs from wheat and maize in having crystalline protein bodies rich in glutelin, in addition to the prolamin-rich protein bodies common to all cereal endosperms (Figure 5). The aleurone layer (considered here as botanically part of the endosperm, although it is processed as part of the bran) differs in thickness (usually 1–7 cell layers) depending on variety. The aleurone cells contain protein bodies and lipid bodies.

#### Rice Embryo

The rice embryo is smaller relative to the whole grain than in wheat. It contains several types of parenchymatous cells, containing protein bodies and/or lipid bodies. The main parts of the embryo are the cotyledon and the embryonic axis. The plumule and radicle are similar to other cereal grains. The high lipid content of the embryo contributes to susceptibility to rancidity in storage of brown rice.

# Maize Grain Morphology and Its Effect on Handling and Wet Milling

Maize is monoecious, having separate male and female flowering parts on the same plant. The female seed-bearing part of the plant (called the ear) develops off the lower-middle side of the plant and consists of a central cob with  $\sim\!800$  seeds attached in rows by the pedicel, and covered by a husk. The pedicel acts as a conduit for photosynthetic products supplying the developing grain. The male part of the flower – the tassel – produces pollen and is at the top of the plant. The maize grain (Figure 6) is  $\sim\!10$  times larger than small-grain cereals, an average dent maize grain weighing  $\sim\!300$  mg. The flattened sides of the mature

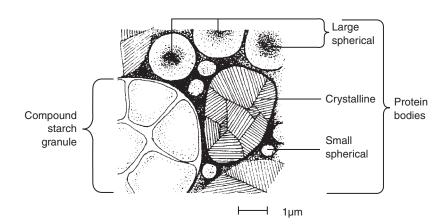
grain are due to pressure from the tight packing in rows on the cob. After removal of the husk, the maize grain is exposed ("naked" characteristic) and easily separated from the cob.

#### Handling

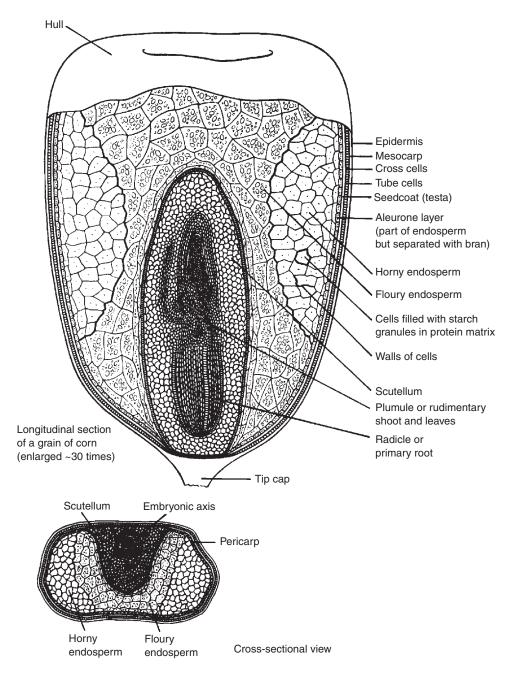
Shelled maize grain (grain separated from the cob and packed in bulk) is more likely than other grains to enter export markets. A high proportion of the US maize harvest is exported, often to highly qualityconscious markets such as Japan. A typical export grain may enter the transportation system by traveling by barge down the Mississippi, entering the Gulf of Mexico for the long voyage to Asian markets. This is physically stressful on the grain, and a poorly dried grain with internal cracks is liable to breakage. This contributes to quality loss and increased susceptibility to insect or fungal attack and moisture damage. The primary criterion for successful shipment of maize is suitable endosperm texture (hard enough) and suitably controlled harvesting and drying to avoid cracking.

#### **Wet Milling**

Most maize which is not fed to animals is processed by wet milling to produce starch (with many diverse applications, such as further processing high fructose corn syrup, fuel alcohol, or modified starch for food and nonfood industries); oil (a high-quality cooking oil); and protein or fiber-rich fractions which are used in livestock feed (*see* Maize: Wet Milling). The composition of these components in the grain is of course of the utmost importance. As the maize seed is much larger than wheat or rice, the relative proportion by weight taken up by the bran layers (including aleurone layer) ( $\sim$ 5%) is less than in these other cereals.



**Figure 5** The rice endosperm indicating structure of spherical and crystalline protein bodies. (From Coffman WR and Juliano BO (1987) Rice. In: Olson RA (ed.) *Nutritional Quality of Cereal Grains: Genetic and Agronomic Improvement.* Madison, WI: Agronomy Society of America. Republished in Juliano BO (1993).)



**Figure 6** The structure of the maize grain – longitudinal and cross-sectional views. (From Pomeranz Y (1987) *Modern Cereal Science and Technology*, pp. 25–39. Weinheim, Germany: VCH Publishers.)

The embryo of maize is a much higher proportion of the grain ( $\sim$ 11%, containing  $\sim$ 33% of the oil) than in other cereals; hence, the overall oil content of the grain is quite high (typically 5%). Also, a higher proportion of the total protein is nonstorage in nature (e.g., enzymes of the embryo), so overall the protein of maize is higher quality for human nutrition than that of wheat flour. However, maize protein, like other cereals, is deficient in lysine. High-lysine mutants (*opaque-2*) give more favorable amino acid balance suitable for feeding people or monogastric

animals even without additional protein sources (*see* Maize: Genetics; Breeding). In practice, spread of *opaque*-2 varieties has been limited because in most cases production of normal endosperm maize, and supplementation with soy protein, is easier for animal feed production. Similarly, a classic long-term selection experiment at the University of Illinois showed that the oil content of maize could be increased to extremes of over 40%. This was basically a result of increased embryo size relative to endosperm.

The maize endosperm, the source of starch and most of the protein, has several interesting features. Maize types are classified by endosperm hardness. "Floury" is a soft endosperm that can be easily crushed to a friable powder. It is physically susceptible to storage pest (mainly insect) and fungal attack. An example of floury maize is the *opaque-2* mutant. "Dent" has a high proportion of floury endosperm but some horny or hard endosperm (Figure 6), and is the most widely produced commercial maize. The "dent" refers to the indentation at the top of the grain on drying when it contracts due to the floury endosperm component. "Flint" maize (of which popcorn is one special type) has only horny and no floury endosperm. The top of the seed is rounded, not indented, and it is physically very hard, even after cooking meal prepared from it.

The outer layers of maize (the seedcoat in particular) contribute the color, which is white or yellow in nearly all commercial maize production. Mass production of maize for feed and industrial use, for example in the US, is predominantly yellow dent. Some white dent is produced for breakfast cereal (e.g., corn flake) production (which follows maize dry milling). The carotenoids (xanthophylls) of yellow maize are nutritionally important and also, in poultry feed, contribute yellow color to egg yolks and to skin and fat of meat poultry. People in regions where maize is a daily food staple may have strong color preferences, e.g., in southern Africa, white (dent) maize is strongly preferred because yellow maize is historically associated with flintiness (although there is no genetic relationship).

#### **Maize Dry Milling**

This also requires a physically sound clean sample with appropriate hardness. Dry milling in the US usually implies use of white maize (a minor proportion of total US production) to make endosperm chunks, which may be steamed and flaked to make corn flakes. Larger dry milling industries exist to serve the needs of maize tortilla production (Mexico) and maize porridge production (a staple diet in much of sub-Saharan Africa) requiring maize meal.

# Barley Grain Structure and Effect on Malting

The barley grain is similar in structure to the wheat grain. For a diagram and further information on this topic, *see* Barley: Malting and Beverages: Distilled.

#### **Overview**

The same principles apply when considering the structure and utilization of other grains, such as sorghum, millets, oats, or even the pseudocereals buckwheat and Amaranthus. First consideration should be the required product, flour, separation of starch/protein/oil, or malt, which drives the choice of process – wet or dry milling, or malting. The size and shape of the grain, particularly the dimensions of the outer layers will most affect dry milling. In some cases, the seed is extremely small (e.g., with Amaranthus) and dry-milling flour yield will be low. For such crops, selection for larger grain size can greatly increase flour yield. For wet milling, the fraction of most value (usually starch) will drive the design of the separation process. Usually the starch-protein binding needs to be loosened by soaking in a solution, such as in alkaline wet milling, where the physical properties of the starch may be affected. Care should be taken that the fractions separated still achieve proper functionality in the intended use.

See also: Barley: Malting. Beverages: Distilled. Fortification of Grain-Based Foods. Grain and Plants, Morphology. Maize: Genetics; Breeding; Wet Milling. Rice: Overview.

#### **Further Reading**

Barber S (1972) Milled rice and changes during aging. In: Houston DF (ed.) *Rice: Chemistry and Technology*, pp. 215–263. St Paul, MN: American Association of Cereal Chemists.

Coffman WR and Juliano BO (1987) Rice. In: Olson RA (ed.) Nutritional Quality of Cereal Grains: Genetic and Agronomic Improvement. Madison, WI: Agronomy Society of America.

Evers AD and Bechtel DB (1988) Microscopic structure of the wheat grain. In: Pomeranz Y (ed.) Wheat: Chemistry and Technology, vol. 1, 3rd edn., pp. 47–95. St. Paul, MN: American Association of Cereal Chemists.

Hoseney RC (1994) Cereal Science and Technology, 2nd edn, pp. 1–28. St. Paul, MN: American Association of Cereal Chemists.

Juliano JB and Aldama MJ (1937) Morphology of Oryza sativa L. Philippines Agriculturalist 26: 1–134.

Juliano BO and Bechtel DB (1985) The rice grain and its gross composition. In: Juliano BO (ed.) Rice: Chemistry and Technology, 2nd edn., pp. 17–57. St. Paul, MN: American Association of Cereal Chemists.

Pomeranz Y (1987) Modern Cereal Science and Technology, pp. 25–39. Weinheim, Germany: VCH Publishers.

Watson SA (2003) Structure and, composition. In: Watson SA and Ramstad PE (eds.) Corn: Chemistry and Technology, pp. 53-82. St. Paul, MN: American Association of Cereal Chemists.

#### **Relevant Websites**

http://www.ricecrc.org - Website of the Australian Cooperative Research Centre for Sustainable Rice Production. Look in the RiceScience Portal for educational resources in rice, including grain structure.

http://www.wheatbp.net - Website from School of Biological Sciences, Bristol University, with interesting images of all aspects of wheat grain development and structure.

http://www.namamillers.org - Website of the North American Millers' Association has useful information on the relationship between grain structure and milling for various cereal grains.

## **GRAINS AND PLANTS, MORPHOLOGY**

R J Jarman, NIAB, Cambridge, UK © 2004, Elsevier Ltd. All Rights Reserved.

#### Introduction

The recognition and specific identification of food and ornamental plants from the morphology of the grains, seeds, and the whole plant still has an important economic role. It is often the main method used to monitor and control the ownership of new varieties of crop plants for Plant Breeders Rights (PBR) and the seed quality as a guarantee of consumer protection.

The methods used follow the basic principles of plant taxonomy and the classification of the plant kingdom established by Linnaeus and subsequent systematic botanists. The study of morphology is used mostly to identify varieties (also called cultivars) which are the lowest known groupings within a single botanical unit after species and subspecies and are the products of plant breeding.

A variety is:

- defined by the (physical) expression of the characteristics resulting from a given genotype or combination of genotypes;
- distinguished from any other plant grouping by the expression of at least one of the said characteristics; and
- considered as a unit with regard to its suitability for being propagated unchanged.

Performance characteristics such as yield or malting or bread-making quality have not been used to define varieties because they are affected by the growing environment and are not capable of precise description. Some would describe the use of morphology of the plant and seed as a traditional method, based on the detailed recognition of botanical structures used by experts, who have developed their own personal experience over many years of practice. Recently the principles of morphological examination have been adapted into more modern methods using digital image analysis that captures, stores, measures, and then analyzes plant structures using computer technology. However, the great advantage of identifying plants from their morphological characteristics is that it is cheap and easy to use and the necessary skills can be quickly learnt. These skills can also be easily applied wherever varieties are being purified, entered for variety registration, or traded commercially using little more than a  $\times$  10 to  $\times$  20 handlens.

Increasingly, the use of morphological characters supports other techniques of plant identification such as DNA technologies and protein electrophoresis. This is because the expression of morphological characteristics is influenced by the local environment in which the plant grows, whereas the expression of molecular and biochemical traits is not. Their expression is independent of the growing environment and therefore much more consistent. The disadvantage of these newer techniques is that they can be more expensive to perform and often require considerable expertise. However, test kits for DNA analyses are becoming more portable and could offer the most accurate method yet for plant identification.

#### **Historical Background**

Breeding new plant varieties by consciously selecting those with improved yield (agricultural crops) or with more ornate flowers (ornamental and horticultural crops) has been taking place for about 150 years. At Debenham in Suffolk, England, in 1825, the Reverend John Chevalier was attracted to the "fine appearance" of the ears of barley growing from the gleanings gathered after harvest in the yard of a farm laborer. It became the variety Chevalier and it was grown widely for malting in England up to the 1930s. Its morphology was distinctive. It had narrow ears that bent over when ripe and the rachilla, the segment of the rachis that remains attached to the grain, had short curly hairs. Chevalier remained true to its description throughout its commercial life. The cultivation of Chevalier was resurrected in 1999 to produce malt for traditional ale to celebrate the millennium.

Bere barley, an ancient landrace, probably brought to Britain during the eighth century by the Viking colonists from Scandinavia, is still grown in Scotland today. It is one of the world's oldest surviving varieties and, as far as we can tell from historical accounts, still retains its unique and distinctive morphology (Figure 1).

Although the morphology of agricultural plants is incidental to characteristics such as yield and disease resistance, it has proved possible to describe the morphological characters of new varieties so they can be accurately recognized and maintained as discrete and unique units.

From the 1940s to the 1960s, a great deal of work was carried out in Europe, North America, and Australia describing the characters of new varieties. The objective was to offer farmers some guarantee that the new varieties they purchased were true to variety and that their agronomic performance, especially yield, would meet the claims made by plant breeders and seed merchants.

There were problems, however. In the rush to improve agricultural productivity and achieve selfsufficiency, there was a proliferation of varieties with "synonyms" (using different names for the

1523 Fitzherbert: Boke of husbandry: described three types of barley

- "Sprott-barley," Spratt or battledore barley, a two-row barley with very broad ears.
- "Longe-eare barley" a general term covering three recognizable two-row barleys with long lax ears: "rath" (early) ripe, "meddle" (middle) ripe, and "late" ripe barleys.
- "Beare barley" a six-row barley still grown in Scotland and the Orkney Islands in the UK.

These were all spring sown.

1757 Lisle: Observations in Husbandry: also described three types of barley

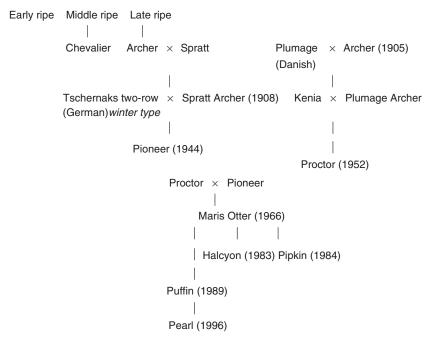


Figure 1 Origins of leading UK winter barleys.

same variety) and "homonyms" (using the same name for different varieties). There was little incentive for private plant breeding because new varieties and seed could be "pirated" and renamed. Most of the breeding for arable crop species was carried out under government subsidy because it was unprofitable. There was no agreed system to define and describe new varieties using morphological characters.

#### **UPOV** and PBR

To address this situation, the International Union for the Protection of New Varieties of Plants (Union Internationale pour la Protection des Obtentions Vegetales (UPOV)) was established in 1961. This convention adopted an international basis for the award of PBR (also called Plant Variety Rights) and agreed guidelines for the conduct of tests and the standardization of variety descriptions based upon the morphology of the grain and plant. PBR is an intellectual property right, often described as a plant "patent."

UPOV's mission is to promote an effective system of plant variety protection with the aim of encouraging the development of new varieties of plants for the benefit of society. PBR enables plant breeders to claim royalties on seed sold to recover profits and breeding costs to reinvest. Once granted, PBR gives the holder exclusive marketing rights and control over a variety's reproduction for sale. PBR has proved to be a profitable incentive to plant breeders, and hence most plant breeding is now carried out by commercial companies.

In the UK, new and improved varieties have contributed to yield improvements of up to 1-2% per year and the cost of royalties to a cereal grower, breeders claim, represents less than 2% of the total input costs. Royalties on a successful winter wheat variety may be worth up to \$2-5 million per year and the commercial life of a variety at this level of royalty return is  $\sim 5$  years, although some varieties are widely grown throughout the entire period of the Rights which expires after 20 years.

The key to PBR is the production of a variety description which describes the morphology of the protected variety using characteristics of the plant and grain.

The morphology of plant and grain is important in the following ways:

- the award of PBR and tests for distinctness, uniformity and stability (DUS);
- the control of seed production by certification to ensure seed sold for agricultural production is true to the variety it is claimed to be; and

• the control of variety purity at the point of final sale, e.g., from the farm to the food processor.

#### **Tests for DUS**

Rights are granted by growing plants in field and laboratory tests to describe the morphology. To be awarded PBR, a new plant variety must be:

- novel new to the market, i.e., not available commercially usually before the date of application for DUS tests;
- distinct have a unique identity;
- uniform must be sufficiently uniform within the limits achievable of the species breeding system, e.g., self-pollinating or partially out-pollinating or obligate out-pollinating etc., from which the new variety was derived; and
- stable capable of reproducing its uniqueness and uniformity over successive generations.

DUS tests take place mainly in field-sown plots, usually over two successive growing seasons. During this period the morphological characters on plants and seeds are recorded. The UPOV guidelines specify which characters should be recorded, at what growth stage records should be taken, the states of expression of individual characters, and example varieties that illustrate specific states of expression (Figure 2).

For the self-pollinating cereals, recording characters is straightforward. Repeated self-pollination after the F2 generation reduces the amount of segregation by half in each successive generation so that at F8 the new variety becomes closely homozygous. At F8 the new variety is usually entered into DUS tests and performance trials (Figure 3). The expression of the morphological characters at this stage is mostly consistent and uniform and failures for lack of uniformity occur in  $\sim 5-10\%$  of new applications. Distinctness is measured by recording the morphological characters on a 1-9 scale of expression: one represents the state of expression at its weakest value, nine represents the strongest state of expression. UPOV calls these scores "notes." For example, anthocyanin color of the awn tips in barley is recorded as 1 = absent to very weak, 3 = weak, 5 = medium,7 = strong, 9 = very strong.

For all the small-grained cereals — wheat, barley, and oats — there are a small group of characters that show discrete differences of expression rather than a continuous range of expression. In barley the rachilla, a vestigial extension of the main stem that is retained on the grain, has either short curly hairs or long silky hairs. The variety Chevalier was distinctive in having a short-haired rachilla, whereas the

Character	Growth stage examined	Description of state	Example varieties	Note or numerical description of state
A "grouping" character showing dis uniformity, and stability for barley."	crete variation. F	From UPOV TG/19/10 (19	994) "Guideline for the conduct of t	tests for distinctness,
26. Barley grain: rachilla hair type	92	Short	Bargeroussel; Atem (Chevalier)	1
, ,		Long	Pastoral; Alexis (Maris Otter)	2
Characters showing continuous var distinctness, uniformity, and stability	•	sion. From UPOV TG/3/1	1 (1994) "Guideline for the conduc	t of tests for
19. Wheat lower glume:	80-92	Absent or very narrow	Courtot	1
shoulder width		Narrow	Forby; Ventura	3
		Medium	Herzog; Prinqual	5
		Broad	Beaver; Adonis	7
		Very broad	Farnese	9

Figure 2 Examples of UPOV morphological characters.

Year	Generation		Selection
1	Initial cross	Malting quality × disease resistance	Choice of parents often based upon existing varieties that are commercially successful
2	F1	resistance	
3	F2	2000 single plants	Selection for disease resistance by deliberate infection
4	F3 and F4	100 lines from selected plants	F4 grown in Australia/New Zealand to achieve two generations in one harvest year; micromalting selection tests
5	F5	8 lines from selected plants	Replicated yield trials, malting tests, purification using morphology and protein electrophoresis
6	F6	4 lines	Further yield trials; purification using morphology
7	F7	Plants from lines harvested and grown as plant or ear rows; harvested seed bulked	Final breeders performance evaluation trials; purification by morphology/protein electrophoresis; seed bulked for official tests and trials
8	F8	First year of official tests and trials	Purification based on morphology to prepare seed for commercial seed production
9		Second year of official tests and trials	Preliminary certification for seed production; purification based on morphology
10		Award of PBR; listed for marketing	Enters commercial seed production and official seed certification
11		Further commercial evaluations for marketing	Limited seed available to farmers
12		Final commercial evaluations for marketing	Seed widely available to meet demand from farmers for new improved variety

Figure 3 Simplified UK cereal variety breeding scheme.

majority of barley varieties grown worldwide have a long-haired rachilla (Figure 4). In wheat there is a similar discrete difference in varieties that have scurs, slender bristle-like projections from the glumes and lemmas that encase the grains and others that do not (Figure 5).

To establish distinctness a profile of morphological characteristics of the plant and grain is recorded. This process records morphological characters from early growth habit of the juvenile plant to characters of the grain at harvest ripeness. This profile is then compared with all other varieties. A consistent difference



Figure 4 Differences in barley rachilla hair type: left, longhaired; right, short-haired. (Illustration published with permission of NIAB.)



Figure 5 Differences in scurs of wheat: left, scurs absent; right, scurs present. (Illustration published with permission of NIAB.)

of at least 1 scale point from the most similar variety is usually enough to confer distinctness. However, each character used to establish distinctness and listed in the final variety description must also show uniformity and stability of expression over the testing period.

The weakness of the UPOV methods using morphological characteristics is that some characters show a continuous range of expression that can vary according to the local environment in which a variety is grown. UPOV provides an international and harmonized DUS testing system but the same variety grown in a range of UPOV member countries may vary by as much as individual varieties.

#### The Characters Used to Describe **Cereal Plants and Grains**

The following morphological characters apply to the small-grained cereals – wheat, barley, and oats – but can also be applied to most other cereal species.

#### **Seasonal Type**

There is a physiological division of the major cereal grain crops, wheat and barley, into winter and spring varieties. Winter-sown varieties require a period of cold weather to trigger the development of meristems that will produce flowers whereas spring varieties do not.

#### **Early Growth Characters**

Early growth characteristics of these two seasonal types can be distinctive. Winter varieties show a prostrate early growth habit and spring varieties have a much more erect growth habit. Individual varieties can show extreme ranges of expression. Winter varieties of barley and oats have distinctive erect hairs on the lower leaf sheath, whereas spring varieties do not. These characters are probably not related to seasonal adaptation but are the result of the heritage of their parent varieties.

In wheat, the anthocyanin color of the coleoptile of 1 week old seedlings is a useful morphological discriminator.

#### **Leaf Characters**

Characters such as leaf color, size, and leaf attitude may also be distinctive in early growth stages. Six-row barleys typically have broad fleshy leaves and a higher leaf area index that may contribute to the higher level of photosynthetic activity that results in higher grain yields compared to two-row barleys. In northern Europe, all wheat varieties have auricles - the small claw-like structures that clasp the stem at the junction of the leaf blade and the leaf sheath – with small hairs; some North American and Australian wheat varieties do not. Barleys do not have hairy auricles and in Europe this is a useful way of distinguishing wheat and barley at the juvenile growth stages before ear emergence.

#### Ear Emergence

The appearance of the ears or panicles as they burst out of the flag leaf sheath in 50% of visible tillers defines ear emergence and is a valuable character that distinguishes many varieties. To an experienced observer a difference of 2 days is enough to identify closely similar varieties. Anthesis is closely linked to ear emergence and in wheat most varieties have



**Figure 6** Side-by-side plots of two different barley varieties showing differences in ear emergence: left, ears emerged; right, ears not yet emerged. (Illustration published with permission of NIAB.)



**Figure 7** Wheat ear at anthesis with yellow-colored anthers. (Illustration published with permission of NIAB.)

yellow-colored anthers but some have red anthers (Figures 6 and 7).

#### **Plant Height**

This is an important discriminating character. Some varieties have been bred to express dwarf characteristics but are often oversensitive to growth factors such as temporary drought; others are too tall,

especially in response to applications of standard dressings of nitrogen fertilizers and the stems buckle reducing harvest yield. A repeatable difference of 5–10 cm in height can be enough to differentiate varieties.

#### Glaucosity

From ear emergence onwards many cereals develop a waxy "bloom" that covers the leaves and infloresences. Glaucosity appears as a layer of exuded wax particles that coat the surfaces of the plant. It is probably a residual adaptive characteristic that prevents water loss. Some wheat varieties are very strongly glaucous with a heavy blue-gray waxy covering over the leaves and all the floral bracts. Other varieties can be distinguished by the varying degrees of glaucosity on the leaves, the exposed neck, or culm of the tiller and the ears. Some varieties have no glaucosity at all which gives the crop a distinctive clear bright green appearance. Glaucosity characteristics are valuable in identifying contamination with other varieties. They are easily recognized at ear emergence and 10-14 days after ear emergence and are used by plant breeders and seed producers to purify crops or lines that are to be submitted for official test and trials. Glaucosity characters are fickle and are easily lost in windy conditions; as plants rub together the wax is rubbed off.

#### **Anthocyanin Pigmentation**

In barley the lower leaf sheaths, the auricles, the tips of the awns and the nerves on the ripe grains may either show presence or absence of pigment or show varying degrees of pigmentation when it is present. These are useful discriminating characters but the degree of pigmentation is affected by the environment within crops, between crops, and between years of the same variety. Wheat pigmentation, when present, is confined to the auricles and in some varieties from North America and northern Russia it is present in the awns.

#### The Morphology of the Ear

Fully developed ears of the major cereals crops show a range of characters (Figure 8). Ear length, the density or compactness of grains on the ear, the attitude of the ear, and the presence or absence of awns and scurs are important morphological features. In barley a major division is either six-row or two-row varieties. Both types are treated as *Hordeum vulgare* L. but in six-row varieties all the florets produce a viable grain whereas in two-row barley only the median grains are viable. In two-row barley the size and attitude of the sterile spikelets is a useful character (Figure 9).



**Figure 8** Differences in the "collar" of the first rachis segment of barley ears: left, "platform" collar; right, "cup" collar. (Illustration published with permission of NIAB.)



**Figure 9** Differences in the attitude of the sterile spikelets of two-row barley: left, sterile spikelets divergent; right, sterile spikelets parallel. (Illustration published with permission of NIAB.)

The most important characters are those associated with the small bracts that enclose the grains: glumes, lemma, and palea. The high-protein North American bread wheats are typically red chaffed – these floral bracts become reddish brown at maturity – whereas in Europe they are whitish. In some oats the lemmas and paleas that tightly enclose the kernel of the grain can be black, brown, gray, white, or yellow, each state is discrete and diagnostic. In wheat the internal and external characteristics of the glumes are very important. Identification is based on a combination of individual structural features such as width of the glume shoulder, shoulder



Figure 10 Contrasting shapes of the lower glume of wheat.

shape and shoulder length, length and curvature of the beak, and degree of hairiness of the internal surface. Many experts could extend this list of characters. For example, in the UK the structures of the glume have been used to support forensic evidence in a murder enquiry. Glume characteristics can be used with confidence from ear emergence to harvest ripeness (Figure 10).

#### Morphology of the Grain

In many grasses such as wheat, the lemmas and paleas thresh-free from the ripe grain. In others such as oats and barley, the lemma and paleas are tightly wrapped round the kernel of the grain and the entire structure is shed as a unit.

In barley the lemma and palea are fused to the grain and only loosely attached above the germ area. In these species, the morphological characters of the grain are defined by the surface features of the lemma and palea. These characters are best observed on the ripe grain. In barley, the lemma nerves may have pigment absent or present and if present then the degree of expression may vary. The nerves at the top of the lemma just before it extends into the awn can show varying degrees of spiculation and the base of the lemma can vary in shape. On the ventral surface of the grain, the palea view, lying in the furrow is a minute extension of the rachis called the rachilla, which has either short woolly hairs or long silky hairs and along the margins of the furrow some varieties have minute spiny hairs. Rachilla hair type and the presence or absence of ventral furrow hairs are two discrete state characters that are inherited independently of the environment and can be used to classify barley varieties into groups. Within these groups, other characters such as degree of pigmentation in the lemma nerves, which shows a continuous range of expression and is affected by the environment, can be used.

Most international protocols used to harmonize cereals variety descriptions use only a shortened list of characters. For specialist purposes, the number of characters used to describe some plant structures can be extended, e.g., barley rachilla characters:

- top level: rachilla hair type short or long;
- additional characters: length of rachilla very short to very long; and
- length of hairs at tip of rachilla extending to tip of rachilla only to extend well beyond tip of rachilla.

These additional characters can be used to clinch the identification of some malting barley at the point of sale or intake for processing and can have considerable commercial value in negotiating commodity price.

Other characters of the barley grain include the shape of the lodicules, the two vestigial structures lying beneath the lemma next to the embryo, and the color of the aleurone layer. The latter is an important feature in malting and distilling. Some processors prefer varieties with a "white" aleurone layer for some malt products whereas others will accept varieties with a colored or "blue" aleurone. The color is caused by the presence of pigmented organelles in the cells of the aleurone layer — the layer of cells just beneath the surface of the kernel of the grain. Five genes control aleurone expression and the strength of pigmentation depends on the dosage, or number, of these genes present.

The morphology of wheat grains is more difficult. Without the attached lemmas and paleas, grain characters depend upon color of the grain (either red or white and the vast majority are red-grained), shape of the germ area and embryo, and length of the brush hairs. Other characters include a physiological reaction that develops when grains are immersed in 1% phenol solution. Image analysis techniques have been tried on wheat varieties but grain shape is affected by seasonal growth factors and variation within varieties can be greater than variation between varieties.

Oats are even more difficult to discriminate using grain characters even though the lemmas and paleas remain attached. Oats have a limited and more specialized market share and the few varieties that are bred tend to use parent varieties that are interrelated. All varieties have either a white or yellow colored lemma and the only other characteristics of use in identification are the presence or absence and length of hairs on the rachilla at the base of the grain. A small number of newer varieties thresh-free from the lemmas and paleas and here varieties are described and identified by the characters of the plants such as

height, date to ear emergence, and glaucosity characteristics.

#### The Future

The General Agreement on Tariffs and Trade (GATT) that established the World Trade Organisation in 1995 contains important provisions covering the protection of intellectual property in the agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS). The TRIPS agreement requires that members of the WTO who have ratified the GATT treaty have compatible legal systems in place that grant protection of plant varieties either by patents or a sui generuis system, or a combination of these methods. For most member countries, this means membership of UPOV and a PBR system based upon plant morphology. In Europe this has become a legal requirement as part of European Union Directives. Studies in plant and seed morphology are set to remain important in the foreseeable future. Plant breeders will still use morphological characters to purify candidate varieties before submitting them for tests and trials, and seed traders will still use morphological characters to verify variety at the point of sale.

However, the science of systematics is being revolutionized by molecular technologies. New species are being revealed and new ancestral relationships are being identified. Early work using biochemical and molecular markers for variety registration studies used nonfunctional markers. However, recent work has identified functional markers from parts of the genome that are known to be expressed. This has linked with studies to identify performance traits such as resistance to barley yellow mosaic virus and malting quality in barley and bread making in wheat. At the UPOV convention in 1991, the concept of dependent or essentially derived varieties was incorporated. An essentially derived variety is one that derives from an initial variety and that retains the essential characteristics of the initial variety but is clearly distinguished from the initial variety. It was accepted that the breeders of the initial variety could claim ownership or part-ownership of the essentially derived variety. "Genetic engineering" techniques may in future reduce the difference or "distance" between varieties and, if so, molecular techniques will be used to assess any claims of essential derivation.

This may enable tests and trials to move away from field sown plots to evaluations in the laboratory that will be independent of the local environment. It will enable beneficial traits to be recognized and transferred into new varieties and offers a new way of managing genetic resources that the studies of morphology cannot achieve. Molecular techniques may

also have the power to accelerate the production of new varieties into commerce by identifying performance trails and offer a way of managing variety registration methods much more efficiently and within legal requirements and boundaries. For the time being the future belongs to morphological and molecular techniques running in parallel.

See also Barley: Genetics and Breeding. Oats. Variety Identification of Cereal Grains. Variety Registration and Breeders' Rights.

#### **Further Reading**

tion of Cereal Chemists.

Cooke RJ (1988) Electrophoresis in plant testing and breeding. Advances in Electrophoresis 2: 171–261.

Cooke RJ and Reeves JC (2003) Plant genetic resources and molecular markers: variety registration in a new era. In: Plant Genetic Resources: Characterisation and *Utilisation* (in press).

Hervey-Murray CG (1980) The Identification of Cereal Varieties. Cambridge: Cambridge University Press.

Hubbard CE (1992) Grasses. London: Penguin Books. Jarman RJ (1995) Visual identification by grain characters. In: Wrigley CW (ed.) Identification of Food Grain Varieties, pp. 35–55. St. Paul, MN: American AssociaMauria S (2000) DUS testing of crop varieties. In: Plant *Varieties and Seeds*, vol.13, pp. 69–90. Cambridge:

Percival J (1921) The Wheat Plant. London: Duckworth. Silvey V (1986) The contribution of new varieties to cereal yields in England and Wales between 1947 and 1983 Journal of the National Institute of Agricultural Botany 17: 155–168 (National Institute of Agricultural Botany, Cambridge).

Stanton T (1955) Oat Identification and Classification. Washington: USDA.

#### **Relevant Websites**

http://www.upov.int – Website of The International Union for the Protection of New Varieties of Plants (Union Internationale pour la Protection des Obtentions Vegetales - UPOV). This gives news and information of developments in plant variety protection and access to the Guidelines for testing all the species covered.

http://www.cpvo.eu.int - Website of the European Union (EU) Community Plant Variety Office (CPVO). This gives news and developments of plant variety protection in Europe and test guidelines for species of plants covered by EU directives.

## **GRAIN CROPS, OVERVIEW**

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#### Introduction

Plants considered "grain crops" are those producing small, hard dry seed or fruit consumed by man or his domesticated animals as a foodstuff, or processed for food or industrial purposes. "Grain crops" as a grouping, is, however, largely artificial. Plants producing useful grains have evolved in a number of plant families, and these families are not always closely related. Grains themselves are heterogeneous. Grains of cereal grasses represent an entire fruit (caryopsis), while those of other grain crops are the dry seeds of various types of fruits including legumes (pulses), achenes (sunflowers, buckwheats), siliques (canola), capsules (cotton), etc. Grains, therefore, are the result of convergent evolution, or the development of similar structures (grains) in diverse organisms. The actual genes involved in the formation of these structures might be different in each family of grain crops.

Grain crops of significance are listed in Table 1 according to their common English names and the equivalent botanical names (as Genus and species). When such lists are formulated, it is valuable to arrange species in a systematic fashion. This has been accomplished in Table 1 by showing taxonomic relationships, indicating those with close or distant relationships by grouping them in Orders, Families, and at times, Tribes. Taxonomy is the science of the identification, classification, and nomenclature of organisms. It is used to insure proper identification of organisms under study, to provide scientific accuracy to organisms in published works, to provide a universal system for the naming of organisms through use of scientific (Latin) names, and to define genetic and evolutionary relationships (or the lack thereof) between organisms via classification into groups of related species. Taxonomists have been

Table 1 The grain crops, their family groupings, and botanical names (genus and species), plus common names

Family groupings	Genus	Species (accepted name <sup>a</sup> )	English common names
Class Liliopsida			
Order Cyperales			
Family Poaceae (Gramineae)			
Subfamily Pooideae			
Tribe Aveneae	Avena	Avena abyssinica Hochst. in Schimper	Abyssinian oat
		Avena brevis Roth	Short oat
		Avena nuda L.	Naked oat
		Avena sativa L.	Oats, common oat
		Avena strigosa Schreb.	Black oat, small oat
Tribe Agrostideae	Phalaris	Phalaris canariensis L.	Canary grass
Tribe Triticeae	Hordeum	Hordeum vulgare L.	Barley
	Secale	Secale cereale L.	Rye
		Secale × derzhavinii Tzvelev	Perennial rye
	×Triticosecale	×Triticosecale Wittm.	Triticale
	Triticum	Triticum aestivum L. subsp. aestivum	Common wheat, bread wheat
		Triticum aestivum L. subsp. compactum (Host) Mackey	Club wheat
		Triticum aestivum L. subsp. spelta (L.) Thell.	Spelt, faro
		Triticum monococcum L. subsp.	Small spelt, einkorn, farro
		Triticum timopheevii (Zhuk.) Zhuk. subsp. timopheevii	Zanduri wheat, Georgian whea
		Triticum turgidum L. subsp. durum (Desf.) Husn.	Durum, durum wheat
		Triticum turgidum L. subsp.  abyssinicum Vavilov	Ethiopean wheat
		Triticum turgidum L. subsp. carthlicum (Nevski) A. & D. Löve	Persian wheat
		Triticum turgidum subsp. dicoccum (Schrank ex Schübler) Thell.	Emmer
		Triticum turgidum L. subsp. polonicum (L.) Thell.	Polish wheat
		Triticum turgidum L. subsp. turanicum (Jakubc.) A. & D. Löve	Khorasan wheat
		Triticum turgidum L. subsp. turgidum	Cone wheat, pollard wheat, riv
Subfamily Panicoideae			
Tribe Maydeae	Coix	Coix lacryma-jobi L.	Coix, Job's tears
Table Andrews	Zea	Zea mays L.	Maize, Indian corn, corn
Tribe Andropogoneae Tribe Paniceae	Sorghum Pennisetum	Sorghum bicolor (L.) Moench Pennisetum glaucum (L.) R. Br.	Sorghum Pearl millet
Tribe Famiceae	Panicum	Panicum miliaceum L.	Proso millet, common millet, French millet, hog millet, broomcorn millet
		Panicum sonorum Beal	Sowi millet, sauwi
		Panicum sumatrense Roth	Little millet, blue panic
	Setaria	Setaria italica (L.) P. Beauv.	Foxtail millet, Hungarian millet
	Echinochloa	Echinochloa esculenta (A. Braun) H. Scholz	Japanese millet, Japanese barnyard millet
		Echinochloa frumentacea Link	Sawa millet, sawa, billion dolla grass
	Paspalum	Paspalum scrobiculatum L.	Kodo millet
	Digitaria	Digitaria exilis (Kipp.) Stapf	Fonio, white fonio, white fonio millet
		Digitaria iburua Stapf	Black fonio
	Brachiaria	Brachiaria deflexa (Schumach.) C.E. Hubb. ex Robbyns	Guinea millet, animal fonio

Family groupings	Genus	Species (accepted name <sup>a</sup> )	English common names
Subfamily Chloridoideae			
Tribe Chlorideae	Eleusine	Eleusine coracana (L.) Gaertn.	Finger millet. Birdsfoot
Tribe Eragrosteae	Eragrostis	Eragrostis tef (Zuccagni) Trotter	Tef
Subfamily Bambusoideae	Ü	· · · · · · · · · · · · · · · · · · ·	
Tribe Oryzeae	Oryza	Oryza sativa L.	Rice
,	- ,	Oryza glaberrima Steud.	African rice
	Zizania	Zizania palustris L.	Northern wild rice
		Zizania aquatica L.	Wild rice
Subfamily Stipoideae		Zizaina aquanoa L.	TTIIG 1100
Tribe Stipeae	Oryzopsis	Oryzopsis hymenoides (Roem. &	Indian rice grass
Tibe dipeac	O1y20p3i3	Schult) Ricker	maian nee grass
Class Magnoliopsida		Condity Flicker	
Order Fabales			
Family Fabaceae (Leguminosae)	A wa alai a	Avantia tumanana I. autam	Viuninia manust
Tribe Aeschynomeneae	Arachis	Arachis hypogaea L. subsp.	Virginia peanut
		hypogaea	D . O
		Arachis hypogaea L. subsp. fastigiata	Peanut, Spanish or Valencia
		Waldr.	types
Tribe Cicereae	Cicer	Cicer arietinum L.	Chickpea
Tribe Genisteae	Lupinus	Lupinus luteus L.	Yellow lupin
		Lupinus angustifolius L.	Blue lupin
		Lupinus albus L.	White Iupin
Tribe Indigofereae	Cyamopsis	Cyamopsis tetragonoloba (L.) Taub.	Guar
		(L.) Taub. in Engl. & Prantl	
Tribe Phaseoleae	Cajanus	Cajanus cajan (L.) Millsp.	Pigeon pea
	Canavalia	Canavalia ensiformis (L.) DC.	Jack bean, horse bean
		Canavalia gladiata (Jacq.) DC.	Sword bean
	Glycine	Glycine max (L.) Merr.	Soybean, soya
	Lablab	Lablab purpureus (L.) Sweet	Hyacinth bean
	Macrotyloma	Macrotyloma geocarpum (Harms)	Kersting's groundnut
	macrotytoma	Marachel & Baudet	resisting a grounding.
	Mucuna	Mucuna pruriens (L.) DC. var. utilis	Velvet bean
	Mucuna	(Wall. ex Wight) Baker ex Burck	verver bearr
	Phaseolus	Phaseolus acutifolius A. Gray	Terpary bean
	rnaseoius	•	
		Phaseolus coccineus L.	Runner bean, scarlet runner bea
		Phaseolus lunatus L.	Butter bean, lima bean
		Phaseolus vulgaris L.	Dry edible beans, kidney bean,
			navy bean, wax bean, green
			bean, field bean, etc.
	Psophocarpus	Psophocarpus tetragonolobus	Winged bean
		(Stickm.) DC.	
	Vigna	Vigna aconitifolia (Jacq.) Marechal	Moth bean
		Vigna angularis (Willd.) Ohwi &	Adzuki bean
		Ohashi	
		Vigna radiata (L.) R. Wilczek	Mung bean
		Vigna mungo (L.) Hepper	Black gram
		Vigna subterranea (L.) Verdc.	Bambara groundnut
		Vigna umbellata (Thumb.) Ohwi &	Rice bean
		Ohashi	
		Vigna unguiculata (L.) Walp.	Black eye pea, cowpea
Tribe Vicieae	Lathyrus	Lathyrus sativus L.	Chickling pea, chickling vetch
	Lens	Lens culinaris Medik.	Lentil
	Pisum	Pisum sativum L. subsp. sativum	Pea, field pea, sugar pea
	i louill	Pisum sativum subsp. abyssinicum	Abyssinian pea
		(A. Braun) Berger (A. Braun)	Abysolillati pea
		, , , ,	
		Berger in Hedrick	Aciatia nao
		Pisum sativum L. subsp. asiaticum	Asiatic pea
		Govor.	
		Pisum sativum L. subsp.	Caucasus Mountain pea
		transcaucasicum Govor.	

Table 1 Continued

Family groupings	Genus	Species (accepted name <sup>a</sup> )	English common names
	Vicia	Vicia ervilia (L.) Willd.	Bittervetch
		Vicia faba L.	Broad bean, fava
Order Caryophyllales			
Family Amaranthaceae	Amaranthus	Amaranthus hypochondriacus L.	Grain amaranth
		Amaranthus caudatus L.	Amaranth, Inca wheat
		Amaranthus cruentus L.	Purple amaranth, red amaranth, amaranth
Family Chenopodiaceae	Chenopodium	Chenopodium quinoa Willd.	Quinoa
		Chenopodium pallidicaule Aellen	Canihua
Order Asterales			
Family Compositae (Asteraceae)	Carthamus	Carthamus tinctorius L.	Safflower
	Guizotia	Guizotia abyssinica (L.f.) Cass.	Niger thistle, niger
	Helianthus	Helianthus annuus L.	Sunflower
Order Euphorbiales			
Euphorbiaceae	Ricinus	Ricinus communis L.	Castor bean
Order Linales			
Family Linaceae	Linum	Linum usitatissimum L.	Linseed, flax, linola
Order Scrophulariales			
Family Pedaliaceae	Sesamum	Sesamum indicum L.	Sesame
Order Polygonales			
Family Polygonaceae	Fagopyrum	Fagopyrum esculentum Moench	Buckwheat, Japanese buckwheat
		Fagopyrum tataricum (L.) Gaertn.	Tartary buckwheat
Order Capparales			
Family Brassicaceae (Cruciferae)	Brassica	Brassica napus L.	Canola, rape, oilseed rape
		Brassica nigra (L.) Koch (L.) Koch in	Black mustard
		Röhling	
		Brassica juncea (L.) Czern	Brown mustard, sarson
		Crambe abyssinica Hochst. ex R.E. Fries	Crambe
Order Malvales			
Family Malvaceae	Gossypium	Gossypium arboreum L.	Tree cotton
		Gossypium barbadense L.	American Pima cotton, Sea Island cotton
		Gossypium herbaceum L.	Arabian, Levant or Maltese cotton
		Gossypium hirsutum L.	American cotton, upland cotton

<sup>&</sup>lt;sup>a</sup> From Mansfeld's Encyclopedia of Agricultural and Horticultural Crops.

engaged for several centuries in the naming and classification of all organisms populating our globe. Taxonomy, therefore, allows grain scientists knowledge of which grain crops are closely related (in genetic and evolutionary senses) and which are not.

# **Grouping Grain Crops into Hierarchical Categories**

All plants are assigned to a specific species, or a group of closely related, morphologically similar individuals, capable of intermating, and reproductively isolated from members of other such species. Groups of closely related species are placed within a larger group known as a genus (pl. genera). Closely related genera are placed within families. Families sometimes are split into smaller groups known as tribes. In practice, however, the use of tribes tends to be applied only when discussing large (for example the grasses)

families. Related families are grouped together in orders, related orders into classes, and related classes into divisions. Plant scientists tend to use the term "division" as the equivalent of the animal scientists' "phylum." Finally, divisions are grouped into kingdoms. All flowering plants belong to the Kingdom Plantae, Division Anthophyta. Division Anthophyta is divided into two classes, the Liliopsida (monocotyledonous plants) and the Magnoliopsida (dicotyledonous plants). Grain crops are found in both divisions, indicating their common ancestors diverged quite early in the evolution of flowering plants.

While plant species are the fundamental unit of taxonomy, they may themselves be divided into smaller units. Morphological variants of a species with a unique geographic or ecological distribution are termed "subspecies." Some taxonomists have used the term "variety" rather than "subspecies" but "variety" often has been applied indiscriminately for any morphological variant regardless of its

geographic or ecological distribution. The term "race" also is used, often to designate groups within subspecies or varieties. Unfortunately, there seems to be no unanimity on the use of these terms, and one taxonomist's "subspecies" might be another's "variety." The present treatment follows Mansfeld's Encyclopedia of Agricultural and Horticultural plants, and uses the term "subspecies." "Cultivar" designates a cultivated variety of a species or subspecies. Grain crop species might contain both cultivated and noncultivated subspecies, and typically numerous (as many as thousands) cultivars will exist.

# **Rules for Allocating Names**

All plant species are assigned a scientific or Latin name that designates both the species itself, the genus to which it belongs, and the authority responsible for the naming of the species in question. For example, in the scientific name of cultivated barley, Hordeum vulgare L., Hordeum is the generic name, vulgare is the specific epithet, and L. stands for Linnaeus, the scientist responsible for first publishing the name of this particular species. Scientific names always are printed in italics, and always contain both the generic name, and the specific epithet. The specific epithet is never used alone. Within a published work, the generic name is used at the first mention of a species. Thereafter, the generic name may be abbreviated. Thus, H. vulgare may be used throughout the remainder of this article to designate barley, but vulgare may not be used alone. This "binomial system of nomenclature" traces its origin to the Swedish botanist Carolus Linnaeus, who employed it in his work Species plantarum, published in 1753. Species plantarum is viewed by botanists as the starting point of plant nomenclature and classification.

Linnaeus was so well known, and named so many species, that scientific names he coined carry only the abbreviation "L." to designate him as the authority. In most cases, the entire family name of the authority is given, although abbreviations are used for a few wellknown authorities. Various permutations on the authority name will be encountered. For example, the authority Host applied the scientific name Triticum compactum to club wheats, a morphologically distinct type of wheat grown for use in pastry and other low-protein flours. However, the authority Mackey decided that T. compactum was really not a distinct species from common or bread wheat, T. aestivum L., but was rather, merely a subspecies. Thus, the scientific name for club wheat became T. aestivum L. subsp. compactum (Host) Mackey indicating that while Host named the species, Mackey believed the assignment of specific rank to be unjustified. At times, an authority might prepare a voucher specimen and a name for a species, but fail to publish it. If a second authority later agrees, and publishes the name, both names appear, separated by the term "ex." If such a case occurred with Host and Mackey, the authority would be written Host ex Mackey. Other arrangements exist and are described in many of the references listed below.

#### **Common Versus Scientific Names**

Common names are the local, regional, or national names for plants. With the multitude of spoken languages in existence, common names for many crop species often are numerous. In scientific literature, common names for plants should be used only when the scientific name is presented at first mention of a plant species. Use of common rather than scientific names can lead to needless confusion. Some plant species are known, even within a single language, by a multitude of common names. For example, the grain crop proso millet (*Panicum miliaceum* L.) is also known by the English common names common millet, French millet, hog millet, broomcorn, and broomcorn millet. Also, some common names have been applied to totally different species. In the Americas, "corn" refers to Zea mays L., while in Europe the term has been applied to barley (H. vulgare L.) or to spelt wheat [T. aestivum L. subsp. spelta (L.) Thell.]. The use of the scientific name would avoid the obvious confusion that might result from use of common names.

#### The Species Concept and Grain Crops

Species have been defined as groups of morphologically similar individuals sharing a common ancestor, capable of interbreeding, and reproductively isolated from all other such groups. While such a definition might appear concise, concrete, and easily applied, in practice, boundaries between species are not always clear. In addition, many plant species named over the course of taxonomic history have been found to be undeserving of this rank. Early taxonomists relied primarily on morphological features as a means of recognizing species. To some authors, any difference in plant appearance or morphology was justification for a new species assignment. Thus, some experts recognized more than 30 species of cultivated grain sorghum, while modern taxonomic treatments designate but one, Sorghum bicolor (L.) Moench. Exercise of natural selection by man on grain crops has led to the preservation of a plethora of morphologically distinct forms. However, genetic studies have shown that many of these unique morphologies are due to the presence of only one or a few genes. Hybridization studies also have shown that many morphologically

distinct forms can freely interbreed. For example, club wheat differ from common or bread wheat by having a more compact, triangular shaped inflorescence. To a morphologically based taxonomist, club wheat would appear to be a distinct species. However, genetic studies established that the compressed spike morphology was conditioned by a single genetic locus, and club wheat will freely hybridize with bread and spelt wheat. Thus, recognition as a distinct species was not warranted.

Reproductive isolation from other closely related groups is key to most species concepts. However, many grain crop species can successfully be mated with related species, and set viable seed. Often the F1 generation of such matings will be sterile, but, in some cases, limited female fertility will occur, and second generation progeny can be obtained via backcrossing. In addition, geneticists and breeders have developed sophisticated techniques such as embryo rescue and doubled-haploidy that allow gene transfer between even more distantly related species. To avoid confusion and taxonomic "lumping" it is best to consider two species distinct if, under normal circumstances (i.e., without the intervention of man) they will not produce viable offspring.

Early, morphologically based taxonomic treatments resulted in numerous scientific names being published for various forms of grain crop species. Later treatments, based more on genetic principles and evolutionary relationships, led to numerous changes in grain crop nomenclature. As scientists in the twentieth century became more aware of the work of scientists from other nations, numerous names were found published for the same species. Thus, numerous synonyms exist for most of our grain crops, and will be encountered in the literature. In such cases, the earliest published name takes precedence, and references such as Mansfeld's Encyclopedia of Agricultural and Horticultural Crops and others provide extensive lists of synonyms and accepted names.

Due to extensive morphological variation, numerous subspecific names have been assigned to grain crops and their relatives. Subspecies names are useful as a means of differentiating various forms of cultivated crops, especially when distinct market classes exist for each type. For example, common or bread wheat is classified as *T. aestivum* subsp. *aestivum*. Two morphologically distinct forms of *T. aestivum*, club wheat (*T. aestivum* subsp. *compactum*) and spelt (*T. aestivum* subsp. *spelta*) are grown and marketed for different end-uses. Use of the subspecies names, in such cases, is useful as it differentiates the various cultivated forms. Subspecies names also are useful to differentiate cultivated forms of plant species from wild ones. For example, durum wheat

(*T. turgidum* subsp. *durum*) has several wild, semi-wild, and formerly cultivated relatives including *T. turgidum* subsp. *dicoccoides*, *T. turgidum* subsp. *dicoccoum*, and others. In both examples, use of the subspecies names serves to differentiate the morphologically different forms, while retaining an indication of the close genetic relationships between the various types. Such information is useful as an indication to geneticists and breeders as to the existence of close relatives of grain crops for use as gene donors in crop improvement programs. It also is useful as an indicator to people with dietary restrictions (e.g., those afflicted with celiac disease) of the true genetic relationship between grain crops.

# The Major Families of Grain Crops

Plants classified as grain crops are found in both classes of the Division Anthophyta (Table 1), and include representatives of ten orders and eleven families. The families with the largest number of members are the Poaceae (Gramineae), also known as the grass or cereal family, and the Fabaceae (Leguminosae), the legume or bean family. These two families contain over ~70% of the species considered grain crops. Five species alone, including four grasses, wheat (T. aestivum and T. turgidum subsp. durum), corn (Z. mays), rice (Oryza sativa), and barley (H. vulgare), and the legume soybean (Glycine max) also provide over 70% of the world's metric tonnage of food from annual plants.

# The Grasses

The grass family (Poaceae) contains the largest and, in terms of annual production, most important grain crops, the cereals. The Poaceae is one of the largest plant families, commonly divided into subfamilies, each, in turn, containing one or more tribes. Cereal grains (Table 1) are found in the subfamilies Pooideae, Bambusoideae, Chloridoideae, and Panicoideae, with the Pooideae and Panicoideae containing the largest numbers of grain crop species. Early systems of grass taxonomy relied heavily on morphological features, especially spikelet and inflorescence morphology. However, beginning in the 1950s, information regarding chromosome numbers, embryo structure, leaf anatomy, interspecific hybridizations, types of photosynthetic systems, etc. began to accumulate, and it became clear that the early arrangement of grass genera into subfamilies and tribes was largely artificial. In the 1960s, grass taxonomy was completely revised. Many earlier volumes still in wide use (e.g., Hitchcock and Chase's "Manual of the Grasses of the United States") reflect the original

groupings of grass genera. More recent references (e.g., Watson and Dallwitz's "Grass Genera of the World"), followed herein, should be consulted for proper tribal affiliation of grass genera.

The wheat and other members of the tribe Triticeae (subfamily Pooideae) are among the most important grain crops. Common or bread wheat, T. aestivum L. subsp. aestivum, and durum wheat, T. turgidum (L.) subsp. durum (Desf.) Husn., are the two most widely cultivated. Two additional subspecies of *T. aestivum*, T. aestivum L. subsp. compactum (Host) Mackey (club wheat), and T. aestivum L. subsp. spelta (L.) Thell. (spelt or farro), also are cultivated. Club wheat are used to produce pastry and other low protein flours, and spelt is grown largely as an heirloom crop, often in organic production systems. Some authors consider these to be separate species, but they both differ from typical bread wheat only in some aspects of spike morphology controlled by one or a few genes, and the forms will all freely hybridize, so recognition as separate species is unwarranted. T. monococcum L. subsp. monococcum (small spelt, einkorn, or faro) was once grown as a grain crop in most states bordering the Mediterranean Sea, and throughout Europe. T. timopheevii (Zhuk.) Zhuk. (Georgian or zanduri wheat) was cultivated in what is now the nation of Georgia. Both T. monococcum and T. timopheevii have all but disappeared from modern cultivation. Several additional subspecies of T. turgidum have been cultivated in historical times, but their importance either has diminished, or they are cultivated in local, isolated areas as relic crops. These include T. turgidum L. subsp. abyssinicum Vavilov (Ethiopean wheat), T. turgidum L. subsp. carthlicum (Nevski) A. & D. Love (Persian wheat), T. turgidum L. subsp. dicoccum (Schrank ex Schübler) Thell. (emmer), T. turgidum L. subsp. polonicum (L.) Thell. (Polish wheat), T. turgidum L. subsp. turanicum (Jakubc.) A. & D. Love (Khorasan wheat) and T. turgidum L. subsp. *turgidum* (cone, pollard or rivet wheat).

Other important members of the tribe Triticeae include barley (*H. vulgare* L.) and rye (*Secale cereale* L.). Triticale (X *Triticosecale* Wittm.) is a man-made crop, grown primarily as forage but also as a grain in some parts of the world. Triticales were developed via hybridizations between bread or durum wheats, and rye. Octoploid triticales contain all the genes of bread wheat and rye, while hexaploid triticales contain the genes of durum wheat and rye. *Secale* × *derzhavinii* Tzvelev, another man-made crop, has been developed as a perennial grain crop. Its cultivation is limited.

The oats, *Avena*, are more distantly related, being placed along with the wheats in the subfamily Pooideae, but in a different tribe, the Aveneae. Common

oat, Avena sativa L., is the most commonly cultivated species. Its importance has diminished in historical times with the change from animal-driven to mechanized agriculture, but oats are still an important grain crop for human consumption, especially in the northern hemisphere. Other species of Avena have been grown as grain crops, but they either are no longer cultivated, or have been reduced to the status of relic crops. These included A. abyssinica Hochst. in Schimper (Abyssinian oat), A. brevis Roth (short oat), A. nuda L. (naked oat), and A. strigosa Schreb. (black or small oat). Phalaris canariensis L., the only cultivated member of the tribe Agrostideae, subfamily Pooideae, primarily is grown as a source of feed for caged birds.

Rice and its relatives Rice (O. sativa L., tribe Oryzeae) is the major food of nearly 50% of the world's population. Two forms or races of rice are recognized, "indica" types (race *indica*), grown in the tropics, and "japonica" types (races japonica and javonica), the temperate forms. These types are not recognized as subspecies. African rice (O. glaberrima Steud.) long has been cultivated in western and central Africa, but it has largely been replaced by O. sativa. Zizania palustris L., Northern wild-rice and Z. aquatica L. also members of the tribe Oryzeae, are cultivated and gathered from wild-populations in North America. Oryza and Zizania are the only grain crops assigned to the subfamily Bambusoideae. Indian rice-grass [Oryzopsis hymenoides (Roem. & Schult.) Ricker], formerly was gathered as a grain crop by Native Americans, and there have been some recent attempts to cultivate and market it. Indian rice-grass is so named for its superficial resemblance to rice, but it actually is placed in a different subfamily, the Stipoideae.

Maize, sorghum, and job's tears Maize (Z. mays L.) and sorghum [S. bicolor (L.) Moench.], are both members of the subfamily Panicoideae. Maize is placed within the tribe Maydeae, while sorghum belongs to the tribe Andropogoneae. Job's tears (Coix lacryma-jobi L.), rarely cultivated as a grain and ornamental crop, also is a member of the Maydeae. Many morphological types of both maize and sorghum exist, but, in both cases, only a single species and subspecies are recognized.

The millets and tef The millets are a taxonomically confusing group. The term "millet" evidently has been applied to any grass with a small round seed. Thus, the term is used to describe a number of, at times, only distantly related species. Most of the millets are placed in the subfamily Panicoideae, tribe Paniceae.

The most important millet in terms of annual production is pearl millet [Pennisetum glaucum (L.) R.Br.], most commonly grown in sub-Saharan Africa and in India. The remaining millets often are termed "minor millets." Of these, proso millet (P. miliaceum L.) is the most widespread, grown as a grain crop throughout Asia and into Eastern Europe. In North America it is largely grown as a feed for caged and domestic birds. Several additional millets are grown, primarily in Asia. The following also are members of the tribe Paniceae: Setaria italica (L.) P. Beauv. (foxtail millet), Echinochloa esculenta (A. Braun) H. Scholz (Japanese millet), and E. frumentacea Link. (sawa millet), Paspalum scrobiculatum L. (kodo millet), Digitaria exilis (Kipp.) Stapf (fonio or white fonio) and D. iburrua Stapf (black fonio), and Brachiaria deflexa (Schumach.) C.E. Hubb. ex Robbyns (Guinea millet). Panicum sonorum Beal (sowi millet) has seen limited cultivation in northern Mexico. Eleusine coracana (L.) Gaertn, known as finger millet or birdsfoot, actually is a member of the subfamily Chloridoideae, tribe Chlorideae, indicating that it is more closely related to tef than it is to the rest of the millets.

Tef [Eragrostis tef (Zuccagni) Trotter], subfamily Chloridoideae, tribe Eragrosteae, is cultivated as a grain crop in Ethiopia, and as a forage grass elsewhere. It is the only grain species in a fairly large genus of grasses.

#### The Legumes

The Fabaceae (legume or bean family) is second only to the Poaceae in terms of economic importance to man. This large family is divided into three subfamilies, the Papilionoideae, Caesalpinioideae, and Mimosoideae. Some authors treat these as separate families. All of the grain legumes are members of the Papilionoideae. Most grain legumes are members of the tribes Phaseoleae and Vicieae, although some also are assigned to the tribes Aeschynomeneae, Cicereae, Genisteae, or Indigofereae (Table 1).

Soybean, or soya (*G. max* L.) is the most important, in terms of annual metric tonnage of production. *G. soja* Siebold & Zucc., the wild progenitor of soybean, is a close relative, and the two are, at times, treated as one species. Intermediate types exist, likely the result of hybridizations between the two species. Intermediate types have been designated *G. gracilis* Skvortzov, but they now are recognized as representatives of the subspecies *G. max* subsp. *gracilis* (Skvortzov) Enken.

Edible dry beans are assigned to the species *Phaseolus vulgaris* L. A large number of morphological variants exist for this species, some being consumed as

pulses, other for the edible pods. Common names include dry edible beans, kidney bean, navy bean, wax bean, green bean, field bean, etc. All are considered members of the same species. Other species of *Phaseolus* are cultivated, including the terpary bean, *P. acutifolius* A. Gray, *P. coccineus* L. (scarlet runner bean), and *P. lunatus* L. (butter bean or lima bean).

Arachis hypogaea L. (peanut) is widely cultivated in both Old and New Worlds. Two subspecies are grown, A. hypogaea L. subsp. hypogaea (Virginia peanut) and A. hypogaea L. subsp. fastigiata Waldr. (the Spanish or Valencia peanut). Other important grain legumes include *Vicea faba* L. (broadbean or fava) Cajanus cajan (L.) Millsp. (pigeonpea), Cicer arietinum L. (chickpea), Lens culinaris Medik. (lentil), and Pisum sativum L. (field or sweet pea). Several subspecies of *P. sativum* are cultivated, with *P. sativum* L. subsp. sativum, the common garden pea, being the predominant form. Other less common forms include P. sativum subsp. abyssinicum (A. Braun) Berger (A. Braun) Berger in Hedrick (Abyssinian pea) grown in Ethiopia and Yemen, P. sativum L. subsp. asiaticum Govor. (Asiatic pea), cultivated in Asia, and P. sativum L. subsp. transcaucasicum Govor., cultivated in the Caucacus mountain region.

Legumes of lesser importance include: Cyamopsis tetragonoloba (L.) Taub. (L.) Taub. in Engl. & Prantl (guar), cultivated at times as a grain but now more important as a source of guar gum. Other less common grain legumes are three species of the genus Lupinus, L. luteus L. (yellow lupin), L. angustifolius (blue lupin), and L. albus (white lupin). The lupins also are grown as ornamental plants. Widely cultivated, though of only local importance as grain crops are two species of the genus Canavalia (jack bean and sword bean), Lablab purpureus (L.) Sweet (Hyacinth bean), Lathyrus sativus L. (chickling pea), Macrotyloma geocarpum (Harms) Marachel & Baudet (Kersting's groundnut), Psophocarpus tetragonolobus (Stickm.) DC. (winged bean), Vicia ervilia (L.) Willd. (bittervetch), Mucuna pruriens (L.) DC. var. utilis (Wall. ex Wight) Baker ex Burck (velvet bean), and several species of the genus Vigna, V. angularis (Willd.) Ohwi & Ohashi (adzuki bean), V. aconitifolia (Jacq.) Maréchal (moth bean), V. radiata (L.) R. Wilczek (mung bean), V. mungo (L.) Hepper (black gram), V. subterranea (L.) Verdc. (Bambara groundnut), and V. umbellata (Thunb.) Ohwi & Ohashi (rice bean), and V. unguiculata (L.) Walp. (black-eye pea or cowpea).

# **Oil-Seed Crops and Pseudocereals**

Several crops of various families produce grains from which edible or industrial oils are processed.

The mustard family (Cruciferae or Brassicaceae) contains several oil-seed species, most important of which is Brassica napus L. (rapeseed or canola). B. nigra (L.) Koch in Röhling (black mustard), and B. juncea (L.) Czern. (brown mustard) occasionally are cultivated for oil and to produce mustard. Crambe abyssinica Hochst. ex R.E. Fries (crambe) is another member of the mustard family used as a source of oil for various industrial purposes. One member of the family Pedaliaceae, Sesamum indicum L. (sesame) is cultivated as both a spice and as a source of sesame oil. Ricinus communis L. (castor bean), of the family Euphorbiaceae, is the source of castor oil, used in industrial applications. It is also infamous as the source of the toxin ricin, a seed protein. Linseed oil is derived from Linum usitatissimum L., a member of the Linaceae. L. usitatissimum also is the source of flax.

The Compositae (Asteraceae), or sunflower family, probably is the world's largest family, in terms of number of species. However, it contains only three members cultivated as grain crops, Helianthus annuus L. (sunflower), Guizotia abyssinica (L.f.) Cass. (Niger thistle), and Carthamus tinctorius L. (safflower). Sunflower and safflower primarily are grown as oil seed crops, but sunflower also is used as a grain for human and avian consumers. Niger thistle also is grown for birdseed.

Cotton species (Family Malvaceae, genus Gossypium) independently were domesticated both in the Old and New Worlds. G. herbaceum L. (Arabian or Egyptian cotton) was of Old World origin, while G. hirsutum L. (American upland cotton) and G. barbadense L. (American Pima cotton) and G. arboreum L. (tree cotton) were domesticated in the New World. Over 90% of the world's cotton production now is from G. hirsutum L. While primarily grown as a fiber crop, both oil and protein are processed from cotton seeds.

Pseudocereals are nonmembers of the grass family often milled to flour and used to produce flat breads and other products similar to those derived from true cereals. This is another artificial grouping, containing members of several plant families. Two members of the Chenopodiaceae are cultivated, Chenopodium quinoa Willd. (quinoa) and C. pallidicaule Aellen (canihua). The grain amaranths, Amaranthus hypochondriacus L., A. caudatus L., and A. cruentus L., are assigned to the Amaranthaceae. These five species all were components of the ancestral cultivated flora of Meso- and South America. Additional pseudocereals include Fagopyrum esculentum Moench. (buckwheat) and F. tataricum (L.) Gaertn. (Tartary buckwheat), both members of the Polygonaceae. Both probably were first cultivated in western China.

See also: Amaranth. Barley: Genetics and Breeding. Beans. Buckwheat. Canola: Genetics and Breeding. Cereals: Overview; Evolution of Species. Chickpea: Overview. Lentil: Breeding. Lupin: Overview. Maize: Genetics. Oilseeds, Overview. Pea: Overview. Pseudocereals, Overview. Pulses, Overview. Rice: Overview. Sorghum: Breeding and Agronomy. Soybean: Germplasm, Breeding, and Genetics. Taxonomic Classification of Grain Species. Wheat: Genetics.

# **Further Reading**

Belton PS and Taylor JRN (2002) Pseudocereals and Less Common Cereals: Grain Properties and Utilization Potential. Berlin: Springer.

Brummitt RK and Powell CE (eds.) (1992) Authors of Plant Names. Kew, UK: Royal Bot. Garden Kew.

Cronquist A (1981) An Integrated System of Classification of Flowering Plants. New York: Columbia University Press.

Cronquist A (1988) Evolution and Classification of Flowering Plants. New York: New York Botanical Gardens.

Duke JA (1981) Handbook of Legumes of World Economic Importance. New York: Plenum Press.

Gould FW (1968) Grass Systematics. New York: McGraw Hill.

Hanelt P (ed.) (2001) Mansfeld's Encyclopedia of Agricultural and Horticultural Crops. Heidelberg: Springer. Harlan JR (1992) Crops and Man, 2nd edn. Madison, WI: American Society of Agronomy.

Hitchcock AS (1971) Manual of the Grasses of the United States, 2nd edn. New York: Dover (revised by A. Chase).

Lawrence GHM (1951) Taxonomy of Vascular Plants. New York: Macmillian.

Mabberley DJ (1997) The Plant-Book. Cambridge: Cambridge University Press.

Pohlhill RM and Raven PH (1981) Advances in Legume Systematics. Kew, UK: Royal Botanic Gardens Part 1.

Smartt J (1990) Grain Legumes. Evolution and Genetic Resources. Cambridge: Cambridge University Press.

Wiersema JH and Blanca L (1999) World Economic Plants: A Standard. Boca Raton, FL: CRC Press.

#### **Relevant Websites**

http://biodiversity.uno.edu - This website provides a complete taxonomic description of the worlds grass genera, including information on assignment to tribes, descriptions, etc. Watson L and Dallwitz M I (1992 onwards) Grass Genera of the World.

http://www.ars-grin.gov - On-line searchable version of "World Economic Plants: A Standard Reference." This version includes all plant species listed in the reference and maintained in the United States Department Agriculture's National Plant Germplasm Collection. Searches can be performed on scientific names and plant uses or other attributes of economic importance. Also includes complete lists of common names and synonyms.

http://mansfeld.ipk-gatersleben.de — On-line version of "Mansfeld's Encyclopedia of Agricultural and Horticultural Crops." Searchable, includes scientific names, synonyms, common names, discussion, and references for what seems to include every plant species ever cultivated by man.

http://biodiversity.soton.ac.uk - LegumeWeb from the International Legume Data and Information

Service World Database of Legumes, a searchable database providing complete information on legume taxonomy, including accepted names, assignment to tribes, references, etc.

http://www.botany.hawaii.edu — Complete listing of families of flowering plants, arranged in orders according to Cronquist's system of classification.

http://www.bgbm.fu-berlin.de – International Code of Botanical Nomenclature – accepted rules for naming plant species.

Grain Legumes see Pulses, Overview.

# GRAINS OTHER THAN CEREALS, NONSTARCH POLYSACCHARIDES

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#### Introduction

All plant tissues contain a large variety of polysaccharides many of which serve structural or reserve functions in the plant. However, the most important polysaccharide obtained from grain crops is starch and the focus in starch-producing grain crops is naturally on the yield of starch. The other polysaccharides present are granted seeming secondary importance as "nonstarch polysaccharides (NSPs)" a term, which covers a great variety of biological functions and chemical structures. There is a growing awareness that these polysaccharides can have considerable value both in human nutrition and for the food industry. In particular, noncereal grains show an interesting diversity of polysaccharides that are less familiar than the better studied NSP of the cereal grain. Noncereal grains include a great variety of potential species, many of which have not been analyzed for NSP content. Consequently, here it is only possible to present a limited selection together with some details of the structure and their value in products derived from grains.

Polysaccharides in a grain can serve two major biological functions. They can act as the energy reserve in the endosperm or cotyledons or they may be involved in forming the structure of the grain tissues. Other functions in which polysaccharides can play essential roles are in the regulation of water balance during dormancy and imbibition, and in the protection of the seed against predation and attack by pathogens. In cereals, only starch has amajor reserve function but in dicotyledonous grains other polysaccharides are found to act as reserves, often together with starch.

In common with all plant tissues, the tissues forming a grain are composed of cells, each of which is defined by a cell wall. The cell is the basic structural unit of plants and cellulose is the basic structural component of the plant cell wall. All higher plant cell walls contain not only cellulose but also a number of other polysaccharides that are important in maintaining the structural integrity of the cell wall and controlling cell wall permeability. Other components may be involved in interactions with neighboring cells and the environment.

The cell walls of plants display a composition which is characteristic of the taxonomic group in which the plant is found. In the angiosperms, there are consistent differences in the pattern of cell-wall polysaccharides present between dicots and

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monocots. Thus, cereal plant cells possess cell walls typical of monocot grasses and this also applies to the endosperm cells in the cereal grain. The cell wall is formed from two types of wall, the primary and the secondary. Primary walls are those first laid down after a cell is formed and are thinner and more flexible than secondary cell walls which are found in mature cell types. Both primary and secondary cell walls contain cellulose but the cellulose fibrils of the primary cell wall are thinner and more loosely arranged; in the secondary cell wall the cellulose fibrils are closely packed into organized layers that are linked to each other by cross-links. Endosperm cells are typically larger and have thinner cell walls than other tissues in the plant, so the proportion of cellulose present can be lower than in other tissues.

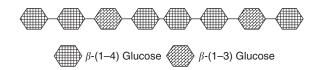
Seed-bearing plants deposit energy-containing reserves to support growth of the embryo within the seed. While the major reserve polysaccharide is indisputably starch, there are a number of other polysaccharides, which are used particularly within the plant family of the leguminoseae. Starch reserves are stored in the cytoplasm in amyloplasts, but the nonstarch reserve polysaccharides in seeds are deposited in highly thickened walls of endosperm cells without a structural function.

#### **Distribution of Grain NSPs**

There is a clear distinction between monocot and dicot grain nonstarch polysaccharides, which reflects the differences between the monocot and dicot cell wall. The actual levels present in any grain sample can also vary depending on the growth conditions and particular genotype. The distribution of NSPs in dicot grains is more difficult to assess due to the variability in designation of what constitutes a dicot grain. In dicots, mixed linkage  $\beta$ -glucans (Figure 1) are absent and arabinoxylans, though they may be present in dicot cell walls, should generally be expected to be largely replaced by xyloglucan which is the main hemicellulose in dicots. Xyloglucans can also be found as a reserve polysaccharide in a number of seeds. Galactomannans are found as seed-reserve polysaccharides in several dicot families, though their commercial importance as food additives is derived from their presence in large tree seeds, they are also present in some smaller grains.

#### **Uses of Grain NSPs**

The presence of high levels of NSPs in grain products will reduce the nutritional value of the grain, a factor that is of significance in the animal feed industry. An important feature of NSPs is their ability to bind



**Figure 1** The sequence of glucose units in a mixed linkage of  $\beta$ -(1-3) and  $\beta$ -(1-4) glucan.

water, which allows them to act as hydrocolloids and disproportionately influence the rheology of aqueous systems. In baking, the NSPs of wheat can enhance water retention and texture of bread and cakes. Purified NSPs can be used as raw materials for the food-additive industry, which is a major user of polysaccharide texture modifiers, stabilizers, and gelling agents. Currently, traditional grains are not major sources of industrial polysaccharide hydrocolloids but high demand on traditional supplies makes this a competitive area for development. However, one of the major benefits of NSPs is that on consumption they contribute to the dietary fiber content of the food.

#### **Dietary Fiber**

The NSPs together form the major part of the dietary fiber of grain crops. Dietary fiber is the fraction of a consumed food which is not degraded in the gut. As human digestive enzymes can only cleave  $\alpha$ -(1–4) glucan bonds, polysaccharides other than starch are part of the dietary fiber. Dietary fiber can be both soluble or insoluble. There are a smaller number of other polymers present in plant tissues, such as lignin, which also form part of the dietary fiber. However, the simplest available measure of the quantity of NSP in grains is the level of dietary fiber present (Table 1) and often this is the only figure available for many minor grain crops that have not been analyzed in detail for polysaccharide composition.

Although unable to contribute to human nutrition in terms of provision of energy, the dietary fiber is recognized to form an important component of our diet for the correct functioning of the digestive system. Polysaccharides through their high water-binding capacity play an important part in providing bulk to the gut contents to allow easy passage through the intestine. The human digestive system developed to cope with a diet rich in high fiber plant material with a large volume and is less well suited to the modern high fat, energy rich, low volume diets of industrialized countries. This deficiency in the modern diet can be overcome through the consumption of grain products high in dietary fiber. Polysaccharides can also bind to dietary lipids and reduce their uptake into the body, a factor which has been observed in the ability of high fiber diets to lower blood lipid levels

Table 1 Dietary fiber in cereal and legume grains

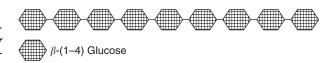
Cereals and grains	Total fiber
Wheat	14.6
Barley	22.6
Rice	2.4
Sorghum	10.1
Millet	8.5
Maize	13.4
Oats	9.6
Rye	14.6
Triticale	18.1
Chickpea	13.5
Black gram	7.1
Mung bean	12.8
Pigeon pea	8.1
Soybean	4.96
Lupin	4.0
Lima bean	6.3
Kidney bean	6.2
Faba bean	8.0
Sunflower	4.2

Figures given are the highest levels reported. Actual values may vary considerably depending on the variety grown and the cultivation conditions. Sources: Sathe SK (1996) In: Nwokolo E and Smartt I (eds.) Food and Feed from Legumes and Oilseeds, pp. 12–32. London: Chapman and Hall; and Shelton DR and Lee WJ (2000) In: Kulp K and Ponte JG (eds.) Handbook of Cereal Science and Technology, 2nd edn., pp. 385–415. New York: Marcel Dekker.

with potentially beneficial consequences for the incidence of cardiovascular disease.

#### **Insoluble Cell-Wall Polysaccharides**

The main structural component of any cell wall is cellulose, a  $\beta$ -(1-4) linked polymer of glucose (Figure 2). It is the world's most abundant polymer, closely followed by the related glucan starch, but with the key difference that cellulose cannot be degraded by human digestive enzymes and cannot therefore contribute directly to our nutrition. Cellulose chains are long flat linear ribbons of glucose units, the number of which can exceed 10 000 and with molecular weights of over 1 000 000. Because the  $\beta$ -(1-4) linkage between the glucose units holds the chain in a flat conformation, it is possible for cellulose chains to align next to each other and form numerous hydrogen bonds between the sugar hydroxyl groups. The chains can stack together to form larger microfibrils which make cellulose highly insoluble and tough, an ideal building material for plants. The quantity of cellulose that is found in whole grains can vary from species to species and is largely a consequence of the thickness of the husk and seedcoat, which tends to have stronger, thicker cell walls that contain more cellulose. The cells of seed endosperms have only thin cell walls and in a well-filled grain the proportion of cellulose



**Figure 2** The sequence of sugar units in the  $\beta$ -(1-4) glucan cellulose.

to starch, or other reserve polysaccharide, should be low. Other largely insoluble polysaccharides such as glucomannans can occur in the walls of some plants, but quantities are typically low and although levels of up to 2% in some minor grain walls have been indicated, these would not have a major impact on grain properties.

# Soluble Cell-Wall Polysaccharides

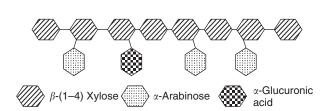
All plant cell walls contain a class of more soluble polysaccharides known as hemicelluloses which have a variety of different structures that serve various functions. These polymers contain a number of different sugar units and are classified according to their composition and solubility. The pattern of soluble polysaccharides present is characteristic of groups and species of plants. Purified hemicelluloses can show varying degrees of water solubility dependent on their size and structure, but all hemicelluloses are strongly bound to the intact cell wall, either by hydrogen bonding or by cross-linking to cellulose. The major function in the plant of hemicellulose is to increase the rigidity and impermeability of the cell wall.

The pectins are another class of highly soluble, cell wall related polysaccharides that are accumulated to high levels in some fruits but not to a great extent in most seed grains. Pectins are charged, acidic polysaccharides with a variety of structures based on rhamnogalacturonans, polymers of rhamnose and galacturonic acid with a number of other sugar substituents.

# **Dicot Arabinoxylans**

Arabinoxylans can be found in many dicots but usually only at low levels. An exception is in linseeds, the seeds of the flax plant (*Linum usitatissimum*). Linseed is a traditional crop valued for the oil which can be expressed from the seeds. The seeds readily hydrate to form an arabinoxylan mucilage that can easily be extracted by incubation in cold water.

High levels of arabinoxylans are known to occur in seeds of various species of genus *Plantago* and one species, psyllium, has been used in commercial preparations of arabinoxylans. The arabinoxylan is present in the seedcoat and can be extracted with



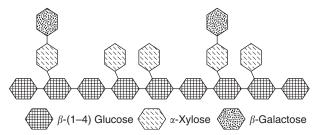
**Figure 3** The arrangement of sugar units in arabinoxylan.  $\alpha$ -Arabinose and  $\alpha$ -glucuronic acid linked 1–2 or 1–3 to a backbone chain of  $\beta$ -(1–4) xylose units.

boiling water and yields are increased under mild alkaline conditions. The structure of the psyllium arabinoxylan is based on a xylan backbone substituted with arabinose and some uronic acids (Figure 3). Solubility in water is not high and the polysaccharide swells to give a weak gel. The gel shows a broad melting range ~80°C and is susceptible to syneresis on freeze/thawing but shows good stability over a range of ionic concentrations. Psyllium arabinoxylan is a soluble dietary fiber which is effective in lowering plasma cholesterol levels and which has traditionally been a component in laxatives where its high swelling power and mucilaginous gel eases the passage of gut contents.

Another abundant arabinoxylan is obtained from seeds of the quince tree (*Cydonia oblonga*). Seeds can be directly extracted with hot or cold water and a mucilage is readily released which is a mixture of cellulose microfibrils dispersed in a matrix formed principally of xylose and arabinose. The polysaccharide is easily soluble in cold water and produces a highly viscous mucilage but is not gel forming. Dispersions show good stability with respect to pH, salt concentration, and temperature. Quince seed arabinoxylan is mainly used for cosmetics applications in Middle Eastern countries, a number of food uses have been suggested, such as for use as a stabilizer in ice cream, but these have not been commercialized due to the high cost, poor availability, and variable quality of the gum.

# **Dicot Xyloglucans**

The major hemicellulose of the dicot cell wall is a xyloglucan formed from a  $\beta$ -(1-4) linked chain of glucose units substituted with  $\alpha$ -(1-6) xylose units. Galactose may be present linked  $\beta$ -(1-2) to some of the xylose units (Figure 4). Most legume grains contain a major proportion of xyloglucan in the cell wall as a structural hemicellulose. Less frequently high levels of xyloglucans are found in the seeds of some plants where they appear to have a reserve function such as in nasturtium (*Tropaeolum majus*) and the tamarind tree (*Tamarindus indica*).



**Figure 4** The arrangement of sugar units in xyloglucan.  $\alpha$ -Xylose linked 1–6 to a backbone chain of  $\beta$ -(1–4) glucose units, some  $\beta$ -galactose linked 1–2 to the xylose units.

Tamarind seeds can be ground to a hot-watersoluble flour to form a mucilaginous gel. The main polysaccharide is a xyloglucan with a  $\beta$ -(1-4) glucan backbone carrying 1–6 linked xylose and arabinose and galactose substituents in a ratio of  $\sim 1:2:3$  for galactose: xylose: glucose. Small amounts of arabinose (and fucose) are found as alternate substituents to galactose on the xylose units. However, the proportion is low and would not be expected to exert a major influence on the properties of the polysaccharide. Tamarind xyloglucan can form a gel under acid conditions which remains stable at alkaline pH. Gels can also form in the presence of ethanol where crosslinks arise due to the low solubility of some chain regions allowing aggregates to form. Tamarind gels are used in India for a range of traditional confectionery products. Xyloglucans are used as food additives in Japan for a wide range of products.

#### **Reserve Polysaccharides**

Seed-bearing plants deposit energy-containing reserves to support growth of the embryo within the seed. While the major seed reserve polysaccharide is starch, there are a number of other polysaccharides which are used as reserves, particularly within the legume family. Unlike starch, which is stored in amyloplasts, it can be inferred from the anatomy that in the cytoplasm the major nonstarch reserve polysaccharides in seeds are deposited in the cell wall but do not serve a structural function.

Apart from starch there is only one other major polysaccharide used by plants as a reserve outside the cell wall. These are polymers of fructose, the fructans which can have chain lengths of up to 250 sugar units, but are generally shorter and highly water soluble. Many grasses are known to use fructans for storage and for frost resistance. Potentially fructans may occur in the grains but there is little evidence for significant quantities and most sources of fructans are from tubers or stem tissue.

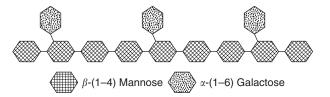
#### Galactomannans

Many seeds of the legume family contain high levels of reserve galactomannans. The seed galactomannans all possess the same basic structure of an  $\alpha$ -(1-4) linked mannan backbone chain with varying degrees of substitution with  $\alpha$ -(1–6) linked galactose residues (Figure 5). The ratio of galactose to mannose is roughly constant for a given species. The distribution of galactose along the mannan chain is not uniform but tends to be clustered in blocks of high substitution (rough regions) which are separated by intervening stretches with few galactose residues (smooth regions). Note that the arrangement of galactose substituents on the mannan backbone is neither regular nor uniform and clustering can occur with the effect stated but this is not intended to imply a block co-polymer structure as is observed for some other polysaccharides.

Purified galactomannans for industrial usage are obtained from four main plant sources in the subfamily Ceasalpinioideae which are well characterized. There is Guar gum from the seeds of Cyamopsis tetragonoloba, Locust bean gum from seeds of Ceratonia siliqua (Carob tree), Tara gum from seeds of Cesalpinia spinosa, and Cassia gum from seeds of Cassia obtusifolia. Extraction of galactomannans involves de-hulling of seeds, crushing to remove the embryo, followed by milling of the endosperm to produce crude flour. The flour can be purified by dissolving in hot water followed by filtration and precipitation with isopropanol to remove impurities. The properties of galactomannans from different sources varies depending on the structure of the galactomannan which is characteristic of the source species.

The use of Locust bean galactomannan (LBG) in the Mediterranean and the Middle East has been part of traditional food preparation for hundreds of years. LBG has a galactose to mannose ratio of 1:4 and molecular weights ~300 000. The cold-water solubility of LBG is low and dispersions need to be heated to 85°C to achieve good dissolution, concentrations of up to 5% w/v being possible. Low solubility is due to the tendency for the linear mannan chains to strongly hydrogen bond to each other in unsubstituted regions of the chain, limiting opportunities for interaction with water molecules.

Though LBG does not itself form gels it can be used together with other hydrocolloids to provide gel formation. This synergestic gel formation can be observed with other nongelling polysaccharides such as xanthan gum. This property is attributed to the ability of the nonsubstituted regions of the linear mannan backbone being able to hydrogen bond to helical regions of the other hydrocolloids and provide



**Figure 5** The arrangement of sugars in galactomannan.  $\alpha$ -Galactose linked 1–6 to backbone chain of  $\beta$ -(1–4) mannose units.

cross-linking. LBG is widely used as a thickener and stabilizer in many foods such as ice cream, cheese spreads, salad creams, processed meat products, and pie fillings. A major use of LBG, and Guar, is in ice cream where it can act as an effective stabilizer to prevent ice crystal growth at low temperatures by restricting accretion of liquid water to existing ice crystals. The presence of large ice-crystals leads to a significant deterioration in ice-cream texture.

Guar galactomannan has long been used as a food ingredient in India. Guar is a linear  $\beta$ -(1–4) mannan with a higher proportion of galactose substituents than LBG, having a glactose:mannose ratio of 1:2 and this is reflected in the easier dispersion of Guar when compared with LBG. Guar can be dissolved at lower temperatures (20°C) than LBG as the extent of unsubstituted regions of mannan chain is smaller, reducing opportunities for interchain hydrogen bonding that would lead to aggregate formation and prevent hydration. The rheology of Guar is similar to that of LBG - pseudoplastic and decreasing with temperature, with good pH stability. Gel formation is again only observed when other polysaccharides are added, but the ability of Guar to participate in such synergistic actions is weaker than that of LBG, and this is also attributed to the reduced extent of galactose free regions of the mannan chain, which could form close hydrogen bonding with another polysaccharide.

Galactomannan from Tara seeds has a galactose mannose ratio of 1:3, intermediate between those of LBG and Guar. The rheological properties are similar to those of LBG. Cassia galactomannan has an average galactose/mannose ratio of 1:5 and can only be solubilized after boiling when a high viscosity solution can be obtained. The structure of the gum is rather variable and fractions of different solubility are obtained due to varying degrees of galactose substitution.

Like the above tree seeds of the legume subfamily Ceasalpinioideae the major grain legumes in the subfamily Papilionoideae can also contain galactomannans. Fenugreek is a legume from the Mediterranean region, which contains high levels of a seed endosperm galactomannan with a galactose mannose ratio approaching 1:1. Fenugreek galactomannan is cold-water soluble, forming solutions with a lower viscosity than the other galactomannans. It has potential to act as an emulsifier and can show a good ability to stabilize oil/water interfaces. Soybeans contain a potentially useful seed galactomannan with a galactose/mannose ratio approaching 1:2. Lupin seeds are unusual in containing a reserve polysaccharide which is structurally unrelated to other legume reserve galactomannans, this is a galactan (or arabinogalactan) with a main chain of  $\beta$ -(1-4) linked galactose and low levels of arabinose substitution.

# **Identifying NSPs**

As new species are tested for potential as grain crop production, investigation of the presence of any NSPs should be an essential component in the study. It is relatively simple to ascertain the presence of any unusual polysaccharides where these contain different sugar units from those in the known polymers. Analysis of the monosaccharide composition of a grain sample after total acid hydrolysis will often be sufficient to infer the presence of polysaccharides from the ratios and quantities of monosaccharides present. Monosaccharide analysis can be conducted directly by high-performance liquid chromatography or by gas chromatography after generation of volatile sugar derivatives. Where the polysaccharides differ more subtly by variation in the linkages between the monosaccharide units, then the method of choice is usually methylation analysis, requiring the production of methylated sugar derivatives reflecting the positions of glycosidic linkages which can then be analyzed by GC/MS. Where soluble polysaccharides are under investigation, clear signals reflecting the position of glycosidic linkages can sometimes be obtained relatively rapidly by NMR analysis in solution.

The above analytical techniques will usually be complemented by prior separation of polysaccharides present into different fractions depending on their solubility in solvents of different ionic strength and pH. Using the water solubility/insolubility of a polysaccharide is a rapid way, which can simultaneously extract polysaccharides from a sample and distinguish among those present in the grain.

#### Conclusion

There exists an enormous diversity of NSPs which are potentially present in seeds. The polysaccharides of

the major cereal crop grains are now well known but as the exploitation of grain resources expands to include new and often diverse dicot plant resources new nonstarch polysaccharides will be encountered. Many of these new grains have not been fully investigated for the composition of minor polysaccharides present in the endosperm, which may have a significant impact on the properties of grain-derived products. Similarly there are considerable opportunities for the development of new grain crops to exploit NSP resources that are presently obtained from related species with much lower yields. Eventually we may see nonstarch polysaccharides no longer being regarded as a nuisance by reducing the value of a grain, but instead representing the major economic product.

See also: Grain, Morphology of Internal Structure. Starch: Uses of Native Starch; Analysis of Quality; Chemistry; Modification; Synthesis.

# **Further Reading**

Brett C and Waldron K (1996) *Physiology and Biochemistry of Plant Cell Walls*, 2nd edn. London: Chapman Hall.

Cui SW (2001) Polysaccharide Gums from Agricultural Products. Pennsylvania: Technomac.

Eliasson A-C (ed.) (1996) Carbohydrates in Food. New York: Marcel Dekker.

Hedley CL (ed.) (2001) Carbohydrates in Grain Legume Seeds. Wallingford: CABI Publishing.

Loewus FA and Tanner W (eds.) (1982) Encyclopedia of plant physiology, vol. 13A. In: *Plant Carbohydrates I*. Berlin: Springer.

Phillips GO and Williams PA (eds.) (2000) Handbook of Hydrocolloids. Florida: CRC Press.

Sathe SK (1996) The nutritional value of selected Asiatic pulses: chickpea, black gram, mung bean and pigeon pea. In: Nwokolo E and Smartt J (eds.) Food and Feed from Legumes and Oilseeds, pp. 12–32. London: Chapman and Hall.

Shelton DR and Lee WJ (2000) Cereal carbohydrates. In: Kulp K and Ponte JG (eds.) *Handbook of Cereal Science and Technology*, 2nd edn., pp. 385–415. New York: Marcel Dekker.

#### **Relevant Websites**

http://www.fao.org. http://food.oregonstate.edu. http://www.hort.purdue.edu.

# GRAIN PRODUCTION AND CONSUMPTION

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# **Overview**

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#### Introduction

The term grain is used to include cereals (wheat, rice, and the coarse grains such as maize, barley, sorghum, oats, and rye), oilseeds, and pulses. Wheat, rice, and maize are the leading grains in terms of global production and area planted. Production of oilseeds and pulses has been driven by the world demand for high protein, vegetable oil, and animal feeds. World grain production has fallen short of consumption since 2000. The annual deficits for 2000–02 have caused a precipitous drop in grain stocks, reaching the lowest level in 30 years. The carryover stocks for wheat, rice, and maize at the end of the 2002 crop year amounted to 23%, 28%, and 15% of annual consumption respectively, the lowest in 28, 18, and 40 years, respectively.

The leading grain producers are the US, China, and India. Grains are produced in different geographical regions and either consumed in areas where they are produced or exported to countries where there is deficit in production. For example, the US produces maize for industrial processing but also exports to Japan for the meat industry. China produces most of the world rice but exports very little. Major grain exporters are the US, Canada, Australia, Argentina, and EU. Countries in NE Asia and North Africa import large quantities of grain. The grains are used for human food, livestock feed, and industrial processing other than food or feed.

# World Grain Production of Cereals, Oilseeds, Pulses

World grain production, particularly in wheat and rice, increased substantially between 1961 and 1980 due to the increase in yields per ha or the "Green Revolution" (Figure 1). Since then, per capita (per person) grain production has not increased much as most of the easily realizable benefits of plant breeding, fertilizer, machinery, and irrigation have already been achieved. Production of grain per ha is close to the maximum obtainable through photosynthesis, hence, world grain production has not matched increases in the world's population. Low grain prices at planting time, high temperatures, and water shortages are the major factors contributing to reduced grain harvests. The fall in production has triggered an increase in the prices of wheat and maize and a corresponding increase in products (bread, breakfast cereals, pasta, and livestock products, including meat, milk, and eggs) derived from these grains.

World production of cereals (wheat, rice (paddy), and coarse grains) in 2002 was 572, 576, and 880 million tons (Mt), respectively (Table 1). Total cereal production is shown in Figure 2 as an average of 10 year periods beginning 1961. There was a 30% increase in cereal production during the period between 1971–80 and 1981–90. The rate of increase in cereal production has been slowing since the days of the Green Revolution in the 1970s. Table 1 shows world production of cereals, wheat, rice, maize, barley, sorghum, millet, and rye for the period 1992–2002. Data on yields and area harvested is also listed.

The world's wheat was produced in Asia (44%), Europe (37%), and North and Central (NC) America (12%) in 2002. About 91% of the world's rice was

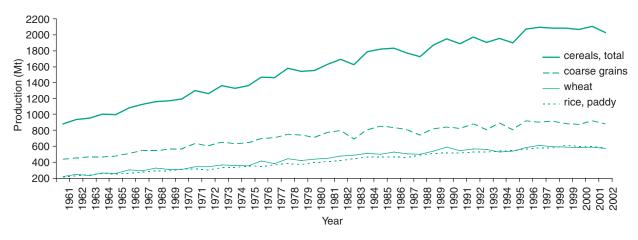
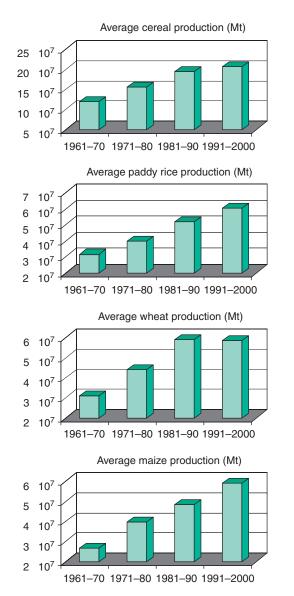


Figure 1 World grain production for the period 1961-2002. (Source FAOSTAT Database.)

**Table 1** World cereal production for the period 2000–02

	(Mha)	(kg ha <sup>-1</sup> )	Production (Mt)
Cereals, total			
2000	673	3064	2064
2001	676	3114	2106
2002	658	3083	2029
Wheat			
2000	215	2719	585
2001	214	2748	590
2002	210	2720	572
Rice, paddy			
2000	153	3916	602
2001	151	3952	597
2002	147	3916	576
Maize			
2000	138	4284	592
2001	139	4417	614
2002	138	4342	602
Barley			
2000	54	2455	133
2001	56	2565	144
2002	52	2534	132
Sorghum			
2000	41	1372	56
2001	44	1345	59
2002	42	1280	54
Millet			
2000	37	746	27
2001	37	786	29
2002	33	698	23
Oats			
2000	12	2049	26
2001	13	2058	27
2002	13	1897	25
Rye			
2000	9	2045	19
2001	9	2362	23
2002	9	2228	21



**Figure 2** World cereal production, averages for 10 year periods. (Source FAOSTAT Database.)

Source: FAOSTAT Database.

produced in Asia. Africa and South America contributed  $\sim$ 3% each to the world's rice production. NC America, Asia, Europe, and South America contribute 43%, 27%, 13%, and 10%, respectively of the world's maize production. Wheat, rice, and maize contributed over 86% of global cereal production. World exports and imports of cereals by region in 2000 and 2001 are given in Figures 3 and 4, respectively. Pulses (including dry beans, dry peas, chick pea, and pigeon pea) were  $\sim$ 3% of total cereal

production. Among oilseeds, soybean was  $\sim$ 9% of world cereal production.

#### Wheat

Wheat is produced under diverse climate conditions ranging from dry land with limited moisture (US, Australia, Former Soviet Union (FSU), West Asia, North Africa) to land that either has adequate moisture (Western Europe) or needs irrigation (FE

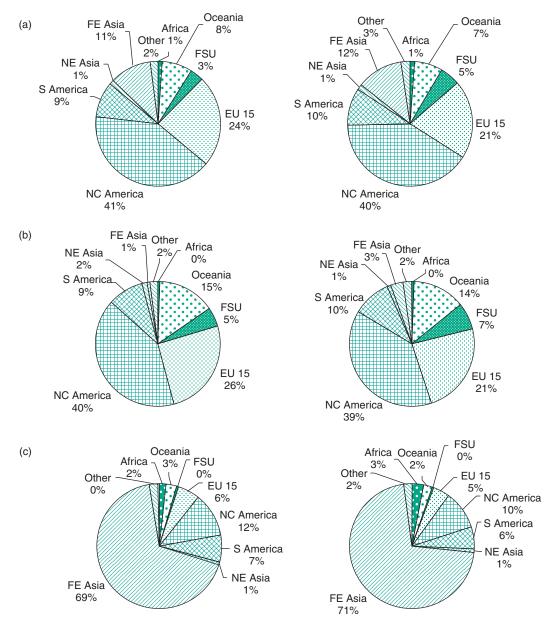


Figure 3 (a) World exports of cereals by region for 2000 (272 Mt) and 2001 (263 Mt); regional totals exclude processed secondary products. (b, e) World exports of wheat by region for 2000 (116 Mt) and 2001 (113 Mt); regional totals exclude processed secondary products. (c) World exports of milled rice by region for 2000 (23 Mt) and 2001 (26 Mt); regional totals exclude processed secondary products. (d) World exports of maize (corn) by region for 2000 (82 Mt) and 2001 (78 Mt); regional totals exclude processed secondary products. (Source FAOSTAT Database.)

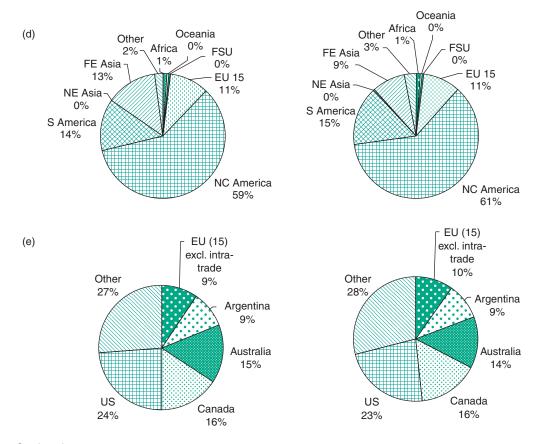
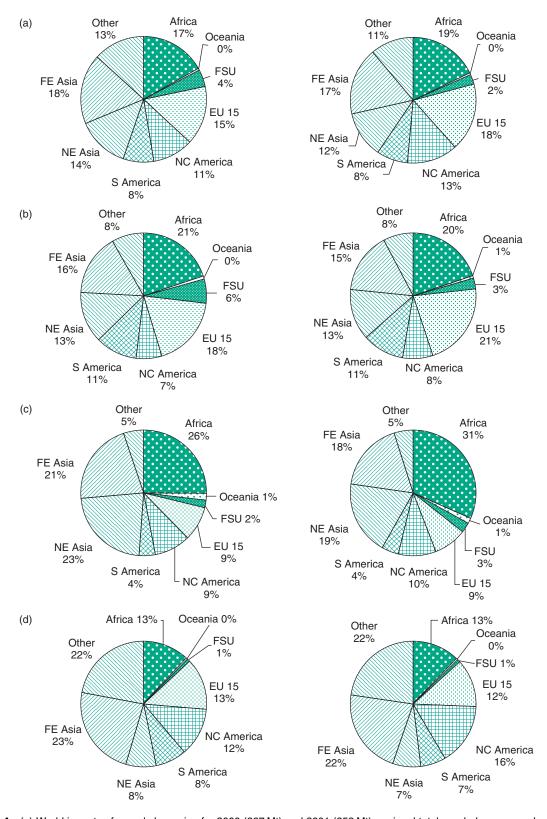


Figure 3 Continued.

Asia – China, India, Pakistan). Data on world wheat production, area harvested and yield for the period 2000–02 is given in **Table 1**. World wheat production decreased by 18 Mt in 2002 from the previous year (Table 1). The area harvested declined by 4 million ha (Mha) but yields remained unchanged. Yields are forecast to decline in 2003 due to insufficient rains in some areas in China and Canada, continued drought in New South Wales, Australia, and hot, dry weather in the Russian Federation, Ukraine, and many parts of Europe. The average global wheat production increased tremendously during the period 1960-80 largely due to an increase in yield. In developed countries, wheat yields tripled between 1950 and 1996 due to new wheat varieties and improved technologies including methods of sowing, irrigation, fertilizer application, moisture retention, and integrated pest management. Potential yields are still to be realized in African and Asian developing countries due to several constraints including inadequate water, infertile soils, poor weather, and lack of inputs. The major wheat producing countries by regions are China, India, Pakistan (FE Asia), Iran and Turkey (NE Asia), US and Canada (NC America), Argentina (South America), Australia (Oceania), Russian Federation, and Ukraine (FSU), and France, Germany, and UK (EU 15).

Bread wheat is planted on 93% of world wheat growing area while durum and soft wheat occupy the remainder. Spring types are sown on two-thirds of the land devoted to wheat in developing countries. Winter wheat is largely cultivated in Turkey, Iran, China, Europe, and US. The area planted in US is expected to increase by 6% due to strong wheat prices. Higher wheat production is expected in US due to support under the Farm Security and Rural Investment Act. EU wheat area is forecast to decline by 4% from 2002 due to low prices. The latter has been partly attributed to the near-record imports of wheat from Eastern Europe and the FSU.

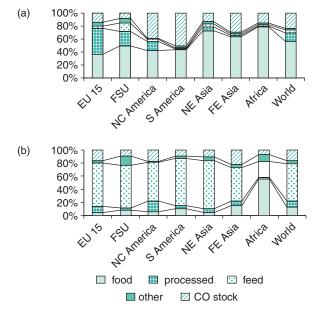
The five major wheat exporters are Argentina (South America), Australia (Oceania), EU (15), Canada, and US (NC America) (Figure 3). The Russian Federation and Ukraine (FSU), both nontraditional exporters are expected to continue their wheat exports in 2003. India and Pakistan produce enough wheat to meet their country needs. Developing countries in Africa, Asia, South America accounted for ~60% of world wheat imports in 2000 and 2001 (Figure 4). Imports have risen over the



**Figure 4** (a) World imports of cereals by region for 2000 (267 Mt) and 2001 (252 Mt); regional totals exclude processed secondary products. (b) World imports of wheat by region for 2000 (115 Mt) and 2001 (110 Mt); regional totals exclude processed secondary products. (c) World imports of milled rice by region for 2000 (22 Mt) and 2001 (23 Mt); regional totals exclude processed secondary products. (d) World imports of maize by region for 2000 (81 Mt) and 2001 (82 Mt); regional totals exclude processed secondary products. (Source FAOSTAT Database.)

years. Major wheat importers include North African countries (Algeria, Morocco, and Tunisia), FSU, West Asia, and the tropical belt. World wheat stocks are forecast at 133 Mt, reflecting smaller output from the EU, FSU, and Australia. Projected total carry-overs for the five majors are at 37 Mt, 3 Mt more than at the end of 2002. This is due to increase in US carryover stocks.

Figure 5 shows consumption of wheat in the EU, FSU, NC America, South America, NE Asia, FE Asia, and Africa in 2000. Most wheat is used for human consumption with a limited amount going into livestock feed. Projected world wheat consumption in 2003 is at 596 Mt with lower feed use in the EU and FSU. About 65-70% of world wheat flour is consumed as bread. The demand is higher for semolina than flour in some European countries. Wheat dry milling produces flour, semolina, bran, and germ. The primary products of milling are used largely in baking and extrusion to produce breads and pastas. Wheat germ oil and meal are valuable co-products. The bran, a by-product of milling, is used in high fiber foods and as an animal feed ingredient. Wheat is also used as a valuable feed ingredient for milk and beef livestock as it is more nutritious than maize, sorghum,



**Figure 5** Wheat and coarse grain consumption and carryover (CO) stocks by regions for 2000 in Mt. (a) Total wheat consumption and CO stocks in selected regions were 91 and 15 (EU 15), 65 and 5 (FSU), 54 and 34 (NC America), 23 and 24 (South America), 46 and 7 (NE Asia), 224 and 93 (FE Asia), 43 and 8 (Africa), and 582 and 175 (world). (b) Total coarse grain consumption and CO stocks in selected regions were 98 and 19 (EU 15), 52 and 5 (FSU), 283 and 60 (NC America), 59 and 6 (South America), 29 and 3 (NE Asia), 229 and 63 (FE Asia), 86 and 7 (Africa), and 888 and 169 (world). (Source FAOSTAT Database.)

and barley. Whole grain wheat is fed to animals in developing countries only if it has been damaged (sprouted or shriveled).

#### **Rice**

World production of paddy rice (unmilled or rough rice) was 576 Mt in 2002, a decrease of 21 Mt from the previous year. The area harvested decreased from 151 to 147 Mha during the same period. World rice production doubled from 216 to 448 Mt during the period 1961–83. The increase in average rice production was ~15% between the 10 year periods 1981–90 and 1991–2000 compared to 30% for the periods 1971–80 and 1981–90 (Figure 2).

Most of the rice is grown in developing countries. In 2002, Asia was responsible for 91% of the world rice production, 51% of the total crop being produced by China and India. Only small amounts (less than 5%) of world rice produced are traded internationally. World exports of rice by region for 2000 and 2001 are given in Figure 3. The major rice exporters include Thailand, United States, Vietnam, China, Pakistan, and India. Australia produces small quantities of rice but has large surpluses for export due to its small population. China produces most of the rice for domestic consumption. Myanmar (formerly Burma) is an emerging exporter of rice. "Basmati" rice is a high-quality product grown in Pakistan and Northeast India for sale at four times the price of local rice. Primary and secondary products from rice milling find way into the export market. For example, Thailand exports broken rice while China, Indonesia, Malaysia, Sri Lanka, Thailand, and Vietnam export edible rice bran oil. Many countries in NC and South America, Europe, and Africa are net importers (Figure 4). Africa imports 80% of its rice requirements.

Rice is a staple food, providing  $\sim 56-70\%$  of total calories consumed in Bangladesh, Cambodia, Indonesia, Lao Peoples Democratic Republic, Myanmar, Thailand, and Vietnam. It provides less than 10% in most African and Latin American countries with the exception of Guinea, Guyana, Surinam, Liberia, Madagascar, and Sierra Leone where 31-45% of total calories consumed come from rice. It is consumed mostly as white polished grain that has been obtained by milling. Parboiled rice is popular in parts of Asia and Africa, and to a limited extent, in some European and American countries. Parboiling paddy rice concentrates nutrients, enhances flavor, and corrects some defects of the harvested crop. Popped or flaked rice is used for production of breakfast cereals. In some European and North American countries, health-conscious consumers prefer brown

rice in which the bran has been retained after dehulling to remove the husk. Other products such as the Japanese rice wine or "sake" or Laos rice "toddy" and liquor are obtained after fermentation of rice. In the US, brewers' grits produced from rice milling are used as a raw material for beer production.

#### **Coarse Grains**

World production of coarse grains (including maize (corn), barley, sorghum, oats, rye, millet, triticale, buckwheat, and quinoa) was 880 Mt in 2002 compared to 918 Mt in 2001. Among the coarse grains, maize, barley, and sorghum are most important in terms of total cereal production. World production of maize, barley, and sorghum was 602, 132, and 54 Mt respectively in 2002 (Table 1). Production of other coarse grains was below 30 Mt each. Projected production of coarse grains in 2003 is at 924 Mt. Increase in production of maize and sorghum crops is expected in US due to favorable weather conditions. Spain also expects a good barley crop. However, hot weather and dry conditions stressed maize crops in Central and Eastern Europe and in China.

World trade in coarse grains totaled 107 Mt with US, Argentina, China, EU, Australia, and Canada exporting 55, 12, 9, 9, 4, and 3 Mt, respectively. FE Asia, NE Asia, NC America, and Africa imported 42, 15, 20, and 12 Mt, respectively. Countries that imported large quantities (in Mt) of coarse grain included Japan (20), South Korea (9), Saudi Arabia (6), Mexico (11), and Egypt (5). There is a slight reduction in barley imports as North Africa's needs are smaller than previously expected for 2003. Figure 5 shows consumption (total 888 Mt) and carryover stocks (total 169 Mt) of coarse grains in the EU, FSU, NC America, South America, NE Asia, FE Asia, and Africa in 2000. World consumption and carryover stocks of coarse grains other than maize and barley were only 145 and 21 Mt, respectively for the same year. Coarse grains find greater use as animal feed compared to wheat. Maize and barley have been in abundant supply replacing wheat in animal feed rations in the EU, FSU, and Australia. Projected world coarse grain carryover stocks are at 151 Mt for 2003.

Maize Maize is the third most widely grown cereal crop after wheat and rice, comprising ~22-25% of total cereal production in the world. Maize is the preferred crop in Africa and Latin America. In Asia, it occupies the third position after wheat and rice. World maize production fell by 12 Mt from 614 Mt in 2001 (Table 1). The area harvested in

2002 was 139 Mha, 70% of which is in developing countries. However, only 50% of world maize production is harvested in developing countries. Yields have remained relatively low in developing countries (2.5 tons ha<sup>-1</sup>) compared to developed countries (7.9 tons ha<sup>-1</sup>) due to environmental, technological, and socio-economic factors. Developed countries make use of adequate inputs and a well-mechanized system for maize production. In 1961, world maize production was  $\sim$ 205 Mt. The figure rose to  $\sim$ 615 Mt in 1998 and after this peak in world maize production, rising production costs and shortage of foreign exchange in many developing countries have led to diminished production, and consequently, maize trade. There was a 50% increase in average global maize production between the period 1961-70 and 1971-80 (Figure 2).

World exports and imports of maize by region for 2000 and 2001 are given in Figures 3 and 4, respectively. The main maize exporters are the US, Argentina, France, China, Hungary, Canada, South Africa, and Germany. The US accounts for over half of the world maize exports. China supplies grain to its neighboring countries. China still has high (but declining) grain stocks enabling it to compete effectively with US for Asian export markets. More than 0.5 Mt on average were imported by 28 countries (Russian Federation, United Kingdom, China, Egypt, Israel, Netherlands, Indonesia, Venezuela, Saudi Arabia, Portugal, Peru, Malaysia, Italy, Iran, Syria, Spain, Japan, Morocco, Mexico, Turkey, South Korea, Dominican Republic, Colombia, Chile, Brazil, Belgium, Luxembourg, and Algeria) each during the period 1999–2002. Industrialized countries imported 80% of their maize requirements. Japan and South Korea are the largest importers, each importing 16 and 9 Mt respectively, in 2000.

Maize consumption was 607 Mt in 2000. The primary product of maize milling is the meal used for feed and food purposes. At least 65% of world maize production is used to feed livestock and 19% is used for human food. Other uses include industrial processing and seed. Secondary and derived products are obtained through dry milling and wet milling processes. Products include tortillas, maize flours, chips, snacks, breakfast cereals, starch, thickeners, pastes, syrups, sweeteners, grits, maize oil, soft drinks, beer, and whisky. In US, domestic consumption of maize has been increasing due to increased supplies, continued strong livestock feed demand, lower wheat feeding, and increased use of maize in ethanol production.

Barley World barley production was 132 Mt in 2002, down by 12 Mt from 2001 (Table 1). The

area planted was 52 Mha in 2002. World barley production is expected to increase by 7% in 2003 due to increase in production in Canada and Australia. Both have increased area planted and are forecast to achieve higher yields as they recover from drought. In the EU and Australia, supply of barley is expected to increase. In 2000, the major producers by region included EU (Germany, Spain, and France >10 Mt each), FSU (Russian Federation 14), NC America (Canada 13, US 6), NE Asia (Turkey 7), and Oceania (Australia 6). The EU is a major exporter of barley. The Russian Federation and Ukraine have become competitors with the EU in export markets. Exports from the EU also face competition from Australia and Canada especially for supply of malting barley. NE Asia and FE Asia imported 8 and 3 Mt, respectively of the total 18 Mt traded in 2000. Saudi Arabia, China, Japan, and Iran were the major importers. World barley consumption totaled 135 Mt in 2000. Feed use accounted for 72% of barley consumption while food use remained low. The latter is likely to increase due to the promotion of the soluble and insoluble fiber in barley and the healthful benefits of whole grain diets. Domestic consumption in the EU is expected to increase due to reduced supplies of feed wheat. Carryover stocks for 2000 were  $\sim 20 \,\mathrm{Mt}$ .

**Sorghum** Sorghum occupies the fifth position after rice, wheat, maize, and barley. Area under production was 42 Mha in 2002 compared to 44 Mha in 2001 (Table 1). Global yield and production were 1 tons ha<sup>-1</sup> and 54 Mt, respectively in 2002. US, Nigeria, India, Mexico, Argentina, China, and Australia are the leading producers. Most sorghum is produced by small-scale and subsistence farmers in semitropical regions of Africa and Asia and by other farmers in US and Latin America. Africa produced 37% of the world total in 2002 on 23 Mha, more than half the world total area. India has the largest area harvested to sorghum, although, there has been a significant reduction from 16 Mha in 1989 to 9.5 Mha in 2002. Production decreased in China from a peak of 6 Mt in 1994 to 2 Mt in 2002 due to a decline in area harvested. Argentina and the US have the highest average yield in excess of 4 tons ha<sup>-1</sup>. Area under production as well as sorghum utilization has been increasing in Brazil. World trade of sorghum was 8 Mt in 2000 with US supplying 6 Mt to the export market and Mexico and Japan importing 5 and 2 Mt, respectively. Mexico, the fourth most important producer of sorghum at 5 Mt in 2002 is also the largest importer. FSU and Venezuela are also main importers. Sorghum is also an important commercial and export crop for Australia and Argentina. The crop is utilized for feed (51%), food, and other uses (41%). The US, Mexico, Argentina, and Japan are principal feed users.

Millets World production of all millets was  $\sim$ 23 Mt in 2002 (Table 1), 35% of which was produced in Africa. West Africa (Nigeria, Niger, Burkina Faso, Chad, Mali, and Senegal) produces ~70% of the millet output in Africa. Asia and Africa account for  $\sim$ 94% of global output of millet. India, China, Nigeria, Russian Federation, and Niger are the leading producers of millet. Small-scale farmers produce almost all millet for household consumption and localized trade. Very little millet ( $\sim 0.2-0.3$  Mt or 1% of world millet production) is traded internationally. India, US, Argentina, and China are the major exporters of millet. The European community accounts for more than 50% of the global imports. Millet is primarily dry-milled to produce cracked grain, grits, meal, and flour from which a number of secondary and derived products are made.

Oats World oat production in 2002 was 25 Mt, down by 2 Mt from 2001 due to a decrease in crop yields (Table 1). The total area harvested was 13 Mha with an average yield of  $\sim 1.9$  tons ha<sup>-1</sup>. World oat production for 2000 by region, in Mt, was 6.9 (EU), 8.5 (FSU), 5.7 (NC America), 1.2 (Oceania), 0.7 (FE Asia), and 0.3 (NE Asia). The Russian Federation and Canada are the leading producers. US oat production continues to decline as area allocated to oats is drawn to soybeans and maize, both of which offer relatively strong returns. Oat production in Canada is forecast to increase sharply from 2002. EU oats face competition from Canadian oats, the latter being able to serve requirements for US and Canadian mills. Carryover stocks are projected to increase from a record low of 2002. Exports are expected to increase considerably in 2003 due to an increase in production and improved quality. Oats is used for both human food and animal feed. High levels of soluble fiber in oats have contributed to its promotion as a healthy food ingredient.

Rye, triticale, buckwheat, fonio, quinoa, canary seed, and tef World production of rye, triticale, buckwheat, fonio, quinoa, and canary seed was 21, 10, 2, 0.25, 0.05, and 0.20 Mt, respectively in 2002. These grains comprised only 2% of total cereal production. World production of rye fell by 2 Mt in 2002 due to a small decrease in yields (Table 1). The Russian Federation, Poland, and Germany are

**Table 2** World pulse and oilseed production (Mt) for the period 2000-01

Year	Total pulses	Dry beans	Dry peas	Chickpeas	Cow peas	Pigeon peas	Soybean	Rapeseed
2000	54	16	10	7	3	3	161	39
2001	53	16	10	6	3	2	176	35
2002	55	18	9	7	3	3	179	33

Source: FAOSTAT Database.

the major producers of rye. World buckwheat production declined from 3 Mt in 2000 to 2 Mt in 2002 as area harvested and yields decreased slightly. Fonio production fell slightly in 2002 for the same reasons. World quinoa production has remained unchanged from 2000 to 2002. Canary seed was planted on a global area of 0.26 Mha in 2002. Canada contributed 74% of world canary production in 2002. About 90% of the seed is exported mostly to Mexico, Brazil, and Belgium. Canary seed is currently used almost exclusively as birdseed. Rye, triticale, buckwheat, fonio, and quinoa are used for human food mostly as ingredients in baked products. It is projected that the demand for these grains will grow as whole grains continue to be promoted as healthy foods. Feed use, industrial use, and exports are forecast to increase with increased supplies.

World tef production fluctuated between 1.2 and 2.0 Mt during the period 1992–97. Tef is a staple food crop of Ethiopia as well as an export crop. Small-scale production of tef has begun in the US, Canada, Australia, South Africa, and Kenya. Tef is exported to the Middle East, North America, and Europe and others mainly for Ethiopians who immigrated to these regions. Tef is utilized for food in the form of flour that is then used to make "injera" (pancake-like bread).

#### **Pulses**

Table 2 gives data on world pulse production for the period 2000–02. FE Asia contributed  $\sim$ 40% of world pulse production. Pulse production tripled in NC America and Africa and doubled in the EU since 1961. India and Brazil are the leading producers of dry beans. China, France, and Canada compete for the dry pea export market. Major chickpea producers are India, Pakistan, Turkey, and Canada. Canada is the global leader in the export of dry peas and chickpeas. Other chickpea exporters include Turkey, Mexico, and Australia. Spain, India, and Pakistan form the major import markets. North Americans consume more beans than any other pulse. Peas are used for both livestock feed and human food. The proportion for the latter use is higher in Asia and Latin America than in Europe.

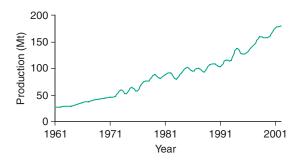


Figure 6 World soybean production for the period 1961–2002.

#### **Oilseeds**

World oilseed production was 194 Mt with soybean, cottonseed, rapeseed/canola, peanut, sunflower seed, palm kernel, and copra making up 60%, 10%, 10%, 9%, 7%, 2%, and 2%, respectively of the total production. World oilseed production has risen tremendously since the 1970s due to expansion of the area planted. Soybean, palm, rapeseed, and sunflower oil each accounted for 32%, 28%, 12%, and 9% respectively of the world vegetable oil consumption (93 Mt) in 2002. However, soybean made up 70% of the world protein meal consumption (187 Mt).

Soybean World soybean production was 179 Mt in 2002 (Table 2) compared to 26 Mt in 1961 (Figure 6). The US, Brazil, Argentina, and China accounted for  $\sim$ 39%, 26%, 18%, and 8% of total production in 2002. Production has been increasing since the 1950s due to increase in global area planted and yields. In Europe, production is limited due to poor climate and soil conditions. Production is now worldwide due to its ability to adapt to different types of soils and climates and versatile end uses. Brazil and Argentina continue to expand soybeanprocessing capacity as domestic policies continue to encourage value-added activities. World soybean exports were at 63 Mt in 2002. Major exporters were the US (44%), Brazil (33%), Argentina (14%), and Paraguay (4%).

Soybean is grown primarily for its meal in order to satisfy the feed protein requirement of broilers,

pork, and aquaculture producers, which are not met by meat and fish meals and meals from other oilseeds. Soybean oil is a secondary product. Derived products (made from soybean, soybean meal, or soybean by-product) include fermented foods (e.g., tofu, soymilk, and soy sprouts) and nonfermented foods (soy sauce, "miso," "tempeh," and "natto"). Soybean can be used to produce products differing in protein content. Other derived products include soy ice cream, yogurt, burgers, cheese, and meat analogs and salad oil, cooking and frying oils, shortening and margarines.

Rapeseed/canola Rapeseed/canola production was 33 Mt in 2002 compared to 26 Mt in 2001 (Table 2). About 90% was produced in FE Asia, EU, and NC America, that is 16.0, 9, and 4 Mt, respectively. China contributed one-third of total rapeseed production. Canada produced 84% of the crop in NC America. Canola is processed into vegetable oil for human consumption and meal for livestock feed.

See also: **Grain Production and Consumption**: Africa; Asia; Europe; Cereal Grains in North America; Oilseeds in North America; Oceania; South America.

# **Further Reading**

Agricultural Statistics (2000) National Agricultural Statistical Service. United States Department of Agriculture. Washington: US Government Printing Office.

FAOSTAT (2003) FAO statistical databases. Food and Agriculture Organization of the United Nations. Rome, Italy (website: http://faostat.fao.org).

Halweil B (2003) Grain production drops. In: Starke L (ed.) Vital Signs 2003. New York: Norton.

IGC (2002) World Grain Statistics 2000/01. London, England: International Grains Council.

Signposts (2002) *Envisioning the Future*. A Worldwatch CD-ROM Resource. Washington, DC: Worldwatch Institute.

#### **Relevant Websites**

http://www.angoc.ngo.ph - Agrarian Reforms in Asia.

http://www.igc.org.uk - International Grain Council market reports and grain statistics.

http://www.irri.org — International Rice Research Institute world rice statistics.

http://www.soystats.com and http://www.unitedsoybean.org — Oilseed and soybean statistics.

http://www.fao.org and http://faostat.fao.org - United Nations Food and Agriculture Organization, probably the best single source of general articles,

and FAOSTAT, online databases covering international statistics in agriculture.

http://usda.mannlib.cornell.edu — USDA Economics, Statistics and Market Information System.

http://www.worldwatch.org — Worldwatch research on food and agriculture.

# **Africa**

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# Introduction

Africa is the second largest continent with an area of 30.3 million square kilometers. It is the only continent that straddles both tropics. Hence, Africa has a predominantly subtropical and tropical climate. Most of northern Africa and much of southwestern Africa has a desert climate, with less than 250 mm of rainfall a year. The climate in the African tropics varies from warm and dry (rainfall 250-500 mm a year), through hot with a dry season (rainfall  $500-1000 \,\mathrm{mm}$ ) to hot with rain all year (1000-3000 mm) towards the Equator. Only on the northwestern Mediterranean seaboard, the southern Cape and South African eastern seaboard is there a mild, warm, and wet climate (rainfall 500-1000 mm). Africa's natural vegetation mainly ranges from desert and semi-desert, though savanna, to sub-tropical and tropical broadleaf forest. One significant exception is the central part of South Africa, which is natural grassland, the "grassveld."

As of 2003, Africa's population is  $\sim$ 850 million (Figure 1). It has been growing very rapidly at  $\sim$ 3% a year, and has doubled since the mid-1970s. The population is still increasing rapidly but the rate

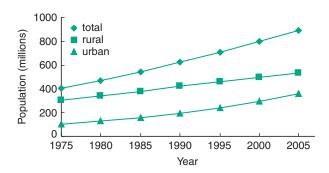


Figure 1 Africa's population.

of growth is slowing and is now  $\sim 2.5\%$  per year. As can be seen, both the rural and urban populations are increasing, but urbanization is taking place much more rapidly. In fact, in some countries (e.g., South Africa and Zambia) well over 50% of the population now live in towns and cities. Obviously, Africa's high population growth rate, coupled with rapid urbanization is having a great impact on food security and food utilization.

This article examines the food situation in Africa with particular reference to the importance of grains as foodstuffs, grain agriculture, Africa's major grains and their production, grain consumption with respect to the types of grain foods and beverages in Africa, and the challenges and opportunities facing Africa regarding grain production and grain food processing. The data are from the Food and Agriculture Organization (FAO) of the United Nations.

# **Importance of Grains**

Africa is an exception compared to the rest of the world in that quantitatively cereals are only the second largest foodstuff. Starchy roots are the largest, in particular cassava. The quantities are  $\sim$ 148 million tons (Mt) of cereals and 175 Mt of starchy roots (Table 1). However, it should be taken into account that, since cereal grains contain only  $\sim$ 12% moisture as against the  $\sim$ 23% for starchy roots, this translates to  $\sim$ 130 Mt dry weight of cereals and 40 Mt dry weight of starchy roots. With respect to cereals, Africa is also unusual in that only a relatively small proportion is used as animal feed,  $\sim$ 12.5%. Also of note is the fact that plant protein foods (pulses) at  $\sim$ 10 Mt

are almost as important as meat (11.9 Mt). In fact, plant foods are far more important to Africa than animal foods, as seen by the fact that vegetable oils amount to  $\sim$ 9 Mt, whereas animal fats only 0.8 Mt.

# **Grain Agriculture**

Whereas Africa produces essentially all its requirements for starchy roots, oil crops, and pulses, today nearly one-third of its cereal requirements have to be imported (Table 1). As can be seen from Figure 2, cereal production in Africa has been steadily increasing, from 66 Mt in 1977 to 117 Mt in 2001. However, as Figure 2 also shows, much of this increase has been due to the increasing land area under cultivation. There has only been a modest increase in yield, from 1.03 t ha<sup>-1</sup> in 1977 to 1.24 t ha<sup>-1</sup> in 2001. Increasing agricultural land has involved farming more marginal areas where the soil is poor and rainfall is intermittent, further exacerbating the situation. As a result, yields have generally remained low. Obviously, increasing cultivation area is unsustainable and it can be seen that there is evidence that the area of land being devoted to cereal cultivation is now increasing only slowly. This suggests that the practical limit of arable land area may be being reached.

Despite the increase in cereal production in Africa, it has not kept pace with the continent's population growth. In 1977, some 154 kg of cereals were produced per person. By 2001 this had fallen to 143 kg – hence the increasing quantity of cereals that has to be imported (Figure 2). Cereal imports increased from 16 Mt in 1977 to 46 Mt in 2001, an increase from 19% to 28% of the continent's cereals supply.

Products	Production	Imports	Stock changes	Exports	Total
Cereals (excluding beer)	111 032	46 062	3910	2923	148 082
Starchy roots	173 933	702	-43	436	174 157
Sugar crops	88 405	0	0	4	82 407
Sweeteners	9564	6391	608	3367	13 196
Pulses	9187	940	19	163	9983
Oil crops	17 582	1131	203	861	18 055
Vegetable oils	5229	4219	233	669	9012
Vegetables	46 254	1378	8	1148	46 491
Fruit	60 067	904	402	4 154	57 223
Stimulants (tea, cocoa, coffee)	3566	483	184	2887	1347
Spices	621	64	7	68	624
Alcoholic beverages	25 673	457	5	658	25 478
Meat	11 270	749	27	128	11 918
Offal	1193	88	0	1	1280
Animal fats	487	364	47	46	842
Milk	27 538	5164	<b>-74</b>	326	32 302
Eggs	2046	329	0	8	2076
Fish (seafood)	7094	2441	11	1666	3242

Figure 2 Cereal production and imports in Africa.

The need to import an increasing proportion of cereal grains is related to the fact that much of Africa's agriculture is still subsistence farming. However, smallscale commercial and large-scale mechanized farming are widespread and well established. Unfortunately, the so-called Green Revolution has barely impacted on the subsistence and small-scale commercial farming sectors. Here, agriculture is characterized by traditional farming practices using manual labor and some draught animal power (Figure 3a), low inputs (no inorganic fertilizer or pesticides), and the use of low-yielding traditional varieties or landraces. Taking the example of sorghum, yields in Africa have remained more or less constant since the mid-1970s at less than 1 t ha<sup>-1</sup>. This compares to the sorghum yield of more than 3 t ha<sup>-1</sup> in the USA. However, where mechanized, high-input agriculture using hybrid seeds on good soil exists in Africa, for example, in South Africa (Figure 3b), sorghum yields are much higher,  $\sim 2.4 \, \text{t ha}^{-1}$ .

#### **Grains Produced**

Cereal production in Africa is dominated by maize, which is a tropical (C4) cereal, at  $\sim$ 42 Mt (Table 2) (2002), some 37% of total cereals. As can be seen, production is very widespread across the continent both geographically and climatically. The top three producing countries are Nigeria, Egypt, and South Africa. In fact, maize production is recorded for 51 countries in Africa. The next most important cereals, in descending order, are sorghum, rice, and wheat, accounting for 17%, 15%, and 14% of total cereal production. Production of these three cereals is also widespread across Africa, with 42 producing countries being recorded for sorghum and rice, and 33 for wheat. However, there are some important differences with respect to their cultivation. Africa's production of sorghum,  $\sim$ 23 Mt is approximately one-third of world sorghum production. The area





**Figure 3** Cereal agriculture in Africa: (a) subsistence – harvesting teff in Ethiopia and (b) commercial – harvesting sorghum in South Africa.

under cultivation in Africa, 23 Mha is almost half the world total. The major production is in the semi-arid tropics of northern Africa, from Nigeria and Burkina Faso in the west to Sudan and Ethiopia in the east. This is probably on account of the fact that sorghum, a tropical cereal, is indigenous to Africa and is very well adapted to harsh climatic conditions, being able to withstand periods of drought and water-logging. Sorghum requires a minimum of only 400 mm of water for cultivation, as opposed to the 500-600 mm needed by maize. In contrast, the production of rice, which is also a tropical cereal but requires much more water, is concentrated in areas of high water availability: the Nile valley of Egypt, tropical Nigeria, and Madagascar. Concerning wheat, which is a temperate (C3) cereal, its production is highest in countries in

Table 2 Grains produced in Africa (2002 data)

Grain	Production $(t \times 10^3)$	Production area (ha×10³)	Yield (t ha <sup>-1</sup> )	Top three producing countries (descending order)
Cereals				
Cereals (total)	115 757	94 049	1.23	Nigeria, Egypt, South Africa
Barley	3545	3626	0.98	Morocco, Ethiopia, Algeria
Fonio	252	347	0.73	Guinea, Nigeria, Ivory Coast
Maize	42 561	26 936	1.58	South Africa, Egypt, Nigeria
Millet <sup>a</sup>	13 633	20 626	0.66	Nigeria, Niger, Mali
Oats	136	155	0.88	Algeria, Ethiopia, South Africa
Rice (paddy)	17 034	8555	1.99	Egypt, Nigeria, Madagascar
Rye	34	40	0.84	Egypt, Morocco, South Africa
Sorghum	20 309	23 585	0.86	Nigeria, Sudan, Ethiopia
Wheat	16 287	7992	2.04	Egypt, Morocco, South Africa
Pulses (grain legumes)				
Pulses (total)	9208	17 136	0.54	Nigeria, Ethiopia, Uganda
Bambara bean	59	77	0.76	Burkina Faso, Mali <sup>b</sup>
Drybeans (Phaseolus beans)	2417	3605	0.67	Uganda, Tanzania, Burundi
Broad bean	1132	835	1.36	Ethiopia, Egypt, Morocco
Chickpea	354	494	0.72	Ethiopia, Morocco, Malawi
Cowpea	3441	8759	0.39	Nigeria, Niger, Burkina Faso
Lentil	90	157	0.57	Morocco, Ethiopia, Egypt
Peas (dry)	293	531	0.55	Ethiopia, Congo (DRC), Burundi
Pigeon pea	206	269	0.77	Malawi, Uganda, Tanzania
Oil-rich legumes				
Peanuts (groundnuts) (in shell)	8486	9975	0.85	Nigeria, Sudan, Senegal
Soybean	989	1090	0.91	Nigeria, South Africa, Uganda
Oilseeds				
Rapeseed	118	44	2.65	Algeria, Ethiopia, Tunisia
Sunflower seed	1042	944	1.10	South Africa, Egypt, Tanzania

<sup>&</sup>lt;sup>a</sup>Predominantly pearl millet.

Africa outside the tropics. The top three producers are Egypt, Morocco, and South Africa. Substantial quantities of wheat are also grown in countries in the tropics, in particular Ethiopia and Kenya, with cultivation being carried out at elevated altitudes to attain cooler conditions.

Africa is the home to a number of millet species. Millets are, by definition, small grained cereals. The FAO data (Table 2) only records the millet species fonio (Digitaria sp., also known as acha), with the other millets being lumped together under the general heading "millet." Millet production accounts for more than 20% of the total land area under cereal cultivation in Africa. By far the most important millet is pearl millet (Pennisetum glaucum), accounting for  $\sim$ 87% of millet production in Africa, and  $\sim$ 8% of the continent's total cereal production. Pearl millet is uniquely able to produce a crop under very low rainfall conditions, with a minimum water requirement of only 300 mm. The other economically important millets in Africa are, in descending order of production: finger millet (*Eleucine coracana*), teff (tef) (*Eragrostis*  tef), and guinea millet (Brachiaria deflexa). Finger millet, so-called because the grains are borne on finger-like panicles (Figure 4a), is grown throughout sub-Saharan Africa. It is an especially important grain with regard to rural food security on account of its excellent storability, being slightly subject to insect attack.

As can be seen from Table 2, there is also substantial production of barley in Africa, which is produced for both brewing and as a food. There is also limited production of oats and rye.

Production of other grains is dominated by peanuts (groundnuts) at  $\sim$ 8.5 Mt (Table 2). This is related to the fact that peanuts are used both as a food and a source of cooking oil. Production takes place throughout Africa, with 48 countries recorded as significant producers. However, Nigeria produces by far the largest quantity of peanuts, some 2.7 Mt. Concerning the pulses, quantitatively the most important is cowpea (*Vigna unguiculata*), which is indigenous to Africa, with a production of some 3.4 Mt. The major producing countries are those in

<sup>&</sup>lt;sup>b</sup>Only countries listed.





**Figure 4** Examples of indigenous African grains important in rural food security: (a) finger millet and (b) bambara bean.

tropical west Africa, in particular Nigeria, although production is widespread throughout Africa. Other quantitatively important pulses are drybeans (*Phaseo*lus beans), which originated in South America, and the broad bean, which probably originated in Eurasia. The origin of these beans is reflected by where they are produced. Drybeans are produced very widely across Africa, with significant production taking place in at least 29 countries. However, the major production is in the tropics, with Uganda being the largest producer. In contrast, significant production of broad beans is only in north Africa. The production of other pulses, including chickpea, peas, pigeon pea, and bambara bean, is also of significant importance, especially with regard to rural food security. Of particular interest is the bambara bean (Vigna subterranea) (Figure 4b), also known as the African groundnut. The bambara bean, which is indigenous to Africa, can produce a reasonable crop under extreme conditions (drought and poor soil) and is considered by some to be one of the world's most underestimated and underdeveloped crop plants.

Significant quantities of oil-bearing grains, other than peanuts, are also produced in Africa (Table 2). The most important are sunflower and soybean, with a total production of  $\sim$ 2 Mt. There is also some production of rapeseed (canola).

As can been seen from Table 2, agricultural production efficiency in Africa varies very considerably

between the grains, with a low of millet at  $0.66\,\mathrm{t}\,\mathrm{ha}^{-1}$  among the cereals, and an overall low of only  $0.39\,\mathrm{t}\,\mathrm{ha}^{-1}$  for cowpea and a high of  $2.04\,\mathrm{t}\,\mathrm{ha}^{-1}$  for wheat among the cereals and an overall high for rapeseed of  $2.65\,\mathrm{t}\,\mathrm{ha}^{-1}$ . Production efficiency appears to depend, to a considerable extent, on whether the grain is primarily home-processed or industrially processed. In other words, production is driven by the existence and needs of a linked food processing industry. For example, in the case of maize, where in Africa it is mostly milled and made into porridge at home, average yield is only  $1.6\,\mathrm{t}\,\mathrm{ha}^{-1}$  compared to  $8.1\,\mathrm{t}\,\mathrm{ha}^{-1}$  in the USA. In contrast, in the case of wheat, which essentially is only milled industrially, the yield in Africa at  $2\,\mathrm{t}\,\mathrm{ha}^{-1}$  compares reasonably well with the  $2.4\,\mathrm{t}\,\mathrm{ha}^{-1}$  in the USA.

# Consumption

The variety of African grain foods and beverages is huge. There is a vast range of traditional African products, as well as increasingly popular "western" products such as spaghetti, pizza, baked beans, and lager beer.

Traditional African cereal foods include:

- roasted snack foods, e.g., "kollo" barley (Ethiopia),
- leavened baked wheat flour breads, e.g., "geish baladi" (Egypt),
- leavened steamed wheat flour breads, e.g., "ledom-bolo" (South Africa),
- flat breads, e.g., "kisra" sorghum or millet (Sudan),
- pancakes, e.g., "injera" tef, sorghum, finger millet, wheat or maize, or combinations (Ethiopia),
- dumplings, e.g., "kenkey" maize (Ghana),
- whole or de-hulled boiled grains, e.g., "supa mtama" sorghum (Kenya),
- steamed, granulated foods, e.g., "couscous" wheat, sorghum, or pearl millet (north and west Africa),
- stiff porridges, e.g., "sadza" maize (Zimbabwe), and "ugali" maize, sorghum, and millet (east Africa).
- soft porridges and gruels, e.g., "uji" maize, sorghum, or millet (East Africa),
- porridge cooked with wood ash extract making it alkaline, e.g., to sorghum or millet (Mali),
- porridge, lactic-acid-fermented, making it sour, e.g., "ogi" – maize, sorghum, or millet (Nigeria), and "ting" – sorghum (Botswana and South Africa), and
- porridge flavored with tamarind or lemon juice, making it acidic, e.g., to sorghum (Burkina faso).

Traditional African cereal beverages are both non-alcoholic, e.g., "mageu" (southern Africa) and alcoholic beers, e.g., "pito" (Nigeria) and sorghum beer (central and southern Africa). They are generally opaque and viscous in consistency, due to the presence of semi-suspended starch. Beverages are produced variously from sorghum, maize, pearl millet, and finger millet, either singly or in combination. In the beers, a portion of the cereal is in the form of malt, in order to provide amylase enzymes to hydrolyze the starch into fermentable sugars.

A characteristic of many traditional African cereal foods and beverages is that they have undergone a lactic acid bacterial fermentation during processing. This gives the product a characteristic sharp, sour taste, and helps preserve it against microbial spoilage; examples include the pancake "injera," the dumpling "kenkey," the firm porridge "ting," the thin porridge "ogi," the nonalcoholic beverage "mageu" and sorghum beer.

Legume-based traditional African foods include:

- blanched and roasted whole grain snacks, e.g., "kollo" chickpea (Ethiopia),
- boiled whole grain snacks, e.g., "nifro" chickpea, broad bean, or lentil (Ethiopia),
- boiled whole grains foods, e.g., "dikgobe" cowpea (Botswana), "mayengele" drybeans (Uganda), and "mtakura" cowpea, peanuts, or bambara bean (Zimbabwe), all often mixed and served with boiled maize grains,
- steamed pastes, e.g., "moi-moi" drybeans (Nigeria) and "okpa" – bambara bean (Nigeria),
- deep fried pastes, e.g., "kose" cowpea or drybeans (Ghana),
- deep fried bean balls, e.g., "akara" drybeans (Nigeria),
- fermented pastes, e.g., "siljo" broad bean (Ethiopia),
- boiled grains made into a sauce, e.g., "magila" cowpea (Uganda),
- germinated and boiled grains made into a sauce, e.g., "azifa" – chickpea, broad bean, or lentil (Ethiopia), and
- dry roasted grain milled into powder, spiced and made into a sauce, e.g., "shiro" – pea, broad bean, or chickpea (Ethiopia).

In most countries in Africa, home processing of grains into foods or the purchase of food produced by vendors "street foods" is the norm. Industrial processing of grains into foods and beverages is in general not as well established or advanced in Africa, as in much of the rest of the world. The exceptions are wheat milling and lager beer brewing, where there are large-scale industrial operations in most countries

on the continent. Probably the African countries with the most advanced food processing industries are Egypt, Kenya, Nigeria, South Africa, and Zimbabwe. However, as can be seen even in these countries the scale and sophistication of grain food and beverage processing varies enormously (Figure 5).

# **Challenges and Opportunities**

To prevent increasing poverty and malnutrition, it is essential that the effectiveness of African grain agriculture is improved. Growing dependence on imported grains has a very adverse effect on the fragile economies of many African countries. As an illustration, in 2001 Africa imported some \$7.7 billion worth of cereals and cereal products. This was equivalent to nearly \$10 per person. This may not seem a lot of money, but it has to be seen in the context that the vast majority of African countries have annual per capita incomes of less than \$1000.

The improvement of agriculture in Africa faces a number of severe challenges. These include the acute need to introduce modern agricultural production technologies and chronic environmental problems such as decreasing soil fertility and desertification, resulting from farming more marginal land. There are also socio-political issues. Governmental policies are often not conducive to local agricultural development. Food prices are in some cases kept artificially low, subsidizing the urban consumer, at the cost of the farmer's livelihood. Large-scale farming operations have been, or are threatened with disruption, through inadequate land reforms.

However, probably the greatest challenge is human immunodeficiency virus/acquired immuno deficiency syndrome (HIV/AIDS). For example, at the time of writing (August 2003) the FAO is calling for \$43 million to help save and enhance the livelihoods of 6.5 million people in southern Africa affected by the pandemic. The high rates of HIV/AIDS related sickness and death among young adults in southern Africa mean that farming has increasingly to be done by children orphaned by the pandemic and the elderly. Children and the elderly are obviously less capable of hard, physical labor, resulting in declining farming activity.

The application of biotechnology to grain agriculture in Africa has the potential to provide many benefits that could help the continent leapfrog the first Green Revolution. To this end, several African countries have invested very heavily in agricultural biotechnology research and development, and genetically modified organism (GMO) crops are currently (2003) being commercially cultivated in some countries. However, some African countries









Figure 5 Cereal food and beverage processing in Africa: (a) commercial sorghum milling in Nigeria, (b) industrial maize and wheat mill in South Africa, (c) entrepreneur sorghum beer brewery in South Africa, and (d) industrial sorghum beer brewery in Zimbabwe.

take a different view and refuse to even import GMO grains. Notwithstanding this, GMO plants with "built-in" specific resistant to pests, thereby reducing agricultural input labor requirements and costs; with tolerance to environmental stress enabling crops to be produced on marginal land; and with improved nutritional value, thus combating malnutrition, will be of tremendous value.

Biotechnology will not, however, be a panacea. To improve African agriculture will also require that countries dramatically improve their grain handling systems. Lack of grain storage facilities in many countries causes ruinously low prices for farmers when crops are good and acute shortages for consumers when crops are poor. The almost complete absence of railways and lack of good roads in much of Africa means that the costs of transporting grain from the point of production to where it is processed are very high. This is compounded by high costs of grain assembly, where many small consignments of grain from different small-scale farmers have

to be brought to one place to make up a truckload. As a result, locally produced grain is often more expensive than imported grain. There can also be severe problems with quality that render small-scale farmer produced grain unsuitable for industrial processing. For example, consignments of grain may comprise different varieties with differing properties such as grain size, color, and hardness. Worse, the grain may be contaminated with dirt and stones, as a result of threshing on the ground, which damage food processing machinery and contaminate the product.

Rapid urbanization in Africa may be seen as challenge facing agriculture, but it is also presenting an opportunity. The growing urban population is creating a market for quality, value-added, convenience food products. Demand has led to the development of processing industries to produce traditional African foods. The development of the sorghum beer industry in southern Africa is an often-quoted example. More recent examples are the production of instant soy-ogi

in Nigeria and fermented pearl millet flour in Namibia. A perhaps even more exciting development is the manufacture of "western" type products using local grains. Notable examples are the manufacture of malted nonalcoholic beverages in Nigeria, e.g., "Milo" and the brewing of stout and lager beer in Nigeria and also very recently in Uganda, all using locally produced sorghum. In these instances, major multinational companies have been responsible. In doing so, they have created systems for the production of quality grain and more stable markets for the grain, thus helping to develop more efficient and profitable local agriculture.

See also: Fermentation: Foods and Nonalcoholic Beverages. Grain Production and Consumption: Overview; Asia; Europe; Cereal Grains in North America; Oceania; South America. Millet: Pearl; Minor. Sorghum: Breeding and Agronomy; Harvest, Storage, and Transport; Utilization. Teff.

# **Further Reading**

Food and Agriculture Organization (1998) African Experience in the Improvement of Post-Harvest Techniques. Rome: Food and Agriculture Organization.

Food and Agriculture Organization (1999) *Biotechnology Development and Potential Impact on Trade in Cereals*. Rome: Food and Agriculture Organization.

Food and Agriculture Organization (2003) Food Outlook No. 5, November 2003. Rome: Food and Agriculture Organization.

Group for Assistance on Systems Relating to Grain After Harvest (GASGA) (1992) *Policy Issues on the Small Grains Sector of Southern Africa*. Ottawa: International Development Research Centre.

National Research Council (1996) Lost Crops of Africa, vol. I, Grains. Washington, DC: National Academy

Proctor DL (1994) FAO Agricultural Services Bulletin No. 109. Grain Storage Techniques: Evolution and Trends in Developing Countries. Rome: Food and Agriculture Organization.

Taylor JRN, Randall PG, and Viljoen JH (eds.) (1993) Cereal Science and Technology: Impact on a Changing Africa. Pretoria: The CSIR.

Van Wyk B-E and Gericke N (2000) *Peoples Plants: A Guide to Useful Plants of Southern Africa*. Pretoria: Briza Publications.

#### **Relevant Websites**

http://www.africabio.com — Website of AfricaBio, an organization that seeks to promote the safe, ethical and responsible research, development and application of biotechnology and its products in Africa.

http://www.afripro.org.uk — Website of the proceedings of a workshop on sorghum and millets in Africa held in South Africa in 2003, which brought together African, European, and American grain scientists. Of particular note is the paper by Rohrbach, DD, "Improving the commercial viability of sorghum and millet in Africa.".

http://www.icrisat.org — Website of the International Crops Research Institute for the Semi-Arid Tropics. ICRISAT is the CGIAR Future Harvest Center for sorghum, millet, groundnut, chickpea and pigeon pea. http://www.fao.org — Website of the Food and Agriculture Organization of the United Nations. The FAOSTAT data bases are an invaluable and amazingly comprehensive resource.

# **Asia**

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Without agriculture, there is no stability, without grain, there is chaos.

Deng Xiaoping

#### Introduction

Several factors make Asian grain production and consumption key to understanding the global balance of grain exports, imports, and food sufficiency. Asia has the world's two most populous countries, China with  $\sim 1.2$  billion people, and India with  $\sim 1$  billion and on track to exceed China in population within 20-30 years. Asia is the dominant producer of rice, yet an insignificant amount enters world grain trade. Asia is a major net importer of wheat and soybeans, thus influencing decisions about quality priorities in exporting countries. Asian countries have tended to aim for grain self-sufficiency for reasons of agrarian stability, foreign currency conservation, and independence from political restrictions on their imports from major western producers. Major successes in grain production over recent decades have increased confidence in regional capability to seek continuing technological solutions to create ever-increasing productivity. This confidence has been dented by stalling yield gains, especially in overexploited rice soils, and by industrialization and urbanization, which absorb agricultural land and compete for water resources. This article will discuss these issues, focusing on three major themes: (1) statistical patterns of grain production, import, and export; (2) the impact and aftermath of the Green Revolution in wheat and rice production; and (3) the role of China as a consuming giant in an era where small-scale grain production makes less economic sense, and where increasing prosperity drives demand for grain-intensive animal product consumption. Asia is a diverse region with many countries of widely differing land area, population, and specific problems. These will not be discussed separately, but the main issues will be presented by discussion of the few of the biggest countries.

#### **The General Problem**

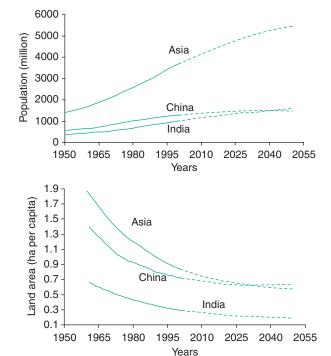
Although rice is the staple crop for half the population of the world, only 2% of total production is traded on international markets. The principle of national production for domestic consumption has driven most Asian countries to aim primarily for self-sufficiency (although some countries such as Thailand and India are rice exporters). In many countries, such as Philippines, self-sufficiency is difficult to achieve. Populations are increasing; people are increasingly moving off the land to the cities; water supply is not sufficient to produce the possible three harvests per year on the rice paddies; every flat arable piece of land is already in production, so major land-use changes tend to be those taking land out of production. In the Philippines and elsewhere, rice has in many areas been grown continuously in the same fields for centuries. It had long been supposed that atmospheric and waterborne mineral and trace element additions to the soil are sufficient to allow indefinite maintenance of soil fertility, requiring only macronutrients and water for each new crop. But results of long-term field experiments analyzed at the International Rice Research Institute (IRRI) near Manila showed that across Asia, for irrigated rice systems, the soil base has been depleted of nutrients by continuous monoculture. The result of this is that the same variety responds less to the same level of added nitrogen. In other words, improved varieties and production methods are needed simply to maintain current yield levels. In future, expanded use of hybrid rice varieties using Chinese technology may increase yield per unit area but also further increase mineral depletion and biomass removal from the field. Philippines has achieved approximate self-sufficiency in rice in 2004 but this may be transient; demand will continue to increase, and energy-intensive inputs will still be required. Farmers who produce rice on small pieces (typically 2 ha) of rented land will continue to seek lives with less physical hardship and better incomes elsewhere.

In a 1996 interview, K. Lampe, then Director General of IRRI, summarized the problems of developing tropical Asian economies such as Philippines.

In 30 years from now we will have for example in the Philippines 120 million people, 60 million more than today. How to feed them nobody seems to care. Over the last 30 years rice has had to increase to such an extent that 600 million people can eat rice today who otherwise would not be able to. Another 30 years and we are predicting a world population of more than 8 billion people. More than half of them will be rice eaters. A prediction from United Nations data is that about 400 million people will move over the next 10 years from rural areas in Asia to the big cities. What will happen is that the belt of poverty around these mega-urban conglomerations will become most probably unbearable. And what we will face is most probably social unrest of unprecedented magnitude. Our task is to grow more rice on less land with less fertilizer, less pesticides, less labor, because producing rice is one of the most tedious tasks for a farmer.

#### **Production Statistics**

Using the Food and Agriculture Organization of the United Nations (FAO) definition of "Asia," the population of Asia has more than doubled in the past 40 years (Figure 1), and is dominated by its two most populous countries (China and India). The population of China is more or less under control, such that India is likely to overtake it as the world's most populous nation in the not too distant future. The limited land supply (an index of population density) in China is going to be matched across Asia as a whole as the population of the rest of Asia continues to increase (Figure 1). Although the population density of India is greater than China, the supply of arable land is also greater (Figure 2). Widely quoted figures are that China has 22% of the world's population but only 7% of the world's arable land. This creates a stark problem – how to feed  $\sim$ 5 billion Asians in 25 years time without increased supply of high-quality land (indeed, with high-quality land continuing to be lost to urbanization and industrialization). Six Asian countries had populations of 100 million or more in 2001 (Figure 2). Their agricultural populations accounted for 43–66% of their total population, except for Japan, which had only 3.6% of its people engaged in agriculture. Japan, as a highly advanced economy which is already committed to food import rather than food self-sufficiency, has substantially different problems from other Asian countries. It focuses on managing the quality and suitability of its imports,

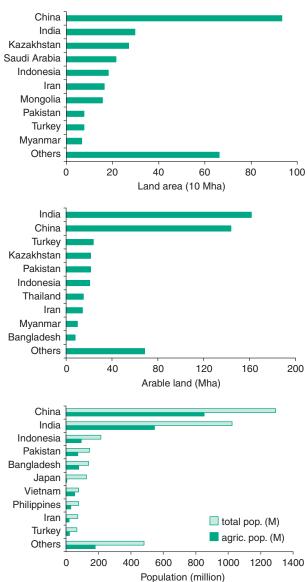


**Figure 1** Estimated population (1950–2001) and land area per capita (1961–2000) of all Asia, China, and India, with projections to 2050.

and on maintaining a domestic agricultural sector (e.g., a meat industry using imported grain), which competes poorly on price but which is politically and socially important.

China produces 41% of Asian grain on 26% of the area under cultivation; India produces 22% on 30% of the area (Figure 3). This reflects a wider adoption of intensive cultural practices and some favorable environmental factors in China. Indonesia, another highly populous country, also has greater production per unit area than average for Asia, whereas Thailand shows below-average production. These figures can be used to indicate possible areas for greater technology adoption and hence increased production using existing land under cultivation.

How is Asia's one billion ton (Gt) annual grain production distributed across crops? Asia is well-known for rice production, and for the widespread dietary cultures based on rice consumption. Indeed, more than half of Asian grain production is rice (Table 1, Figure 4) with 523 million ton (Mt) in 2002. Other than for India, Asia is less well known as a wheat producer, yet more than 44% (Table 2) of global wheat production is from Asia, and China is by far the largest wheat producer in the world. Maize production at 166 Mt, again predominantly in China, is substantial. China ranks second to the US in maize production. It is interesting that the center of origin of

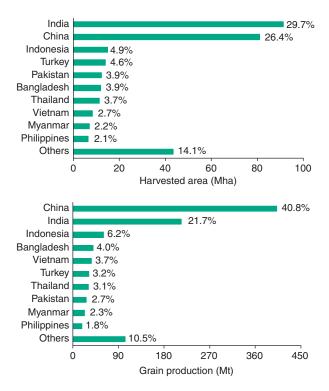


**Figure 2** Land area, arable land, and total and agricultural populations of top ten population countries in Asia in 2001. (Data from FAOSTAT Database.)

maize is in America (Mexico) but it has become globally widespread and successful, including in Asia. However, soybean, a crop originating in Asia, is more commercially significant in the US than in Asia. Sorghum and millets are significant crops in arid parts of Asia, typically areas of China and India too dry for maize production. Many local traditional uses of these cereals have developed, for example typical use of sorghum in China is in distilled alcoholic beverage production. Buckwheat also has a significance beyond its relatively small production, with uses in traditional "soba" (Japanese buckwheat noodles) and some Chinese traditional foods such as noodles, vermicelli, and fermented vinegars. Barley is

a substantial crop, fourth after maize, partly reflecting China's rise to rank first in the world for beer production.

Asia's grain imports in 2001 were worth about US\$25 billion, and exports about \$8 billion (Table 2). Imports were dominated by wheat, maize, and soybean, and exports by rice. Industrial,



**Figure 3** Harvested area and grain production of top ten grain-producing countries in Asia in 2002. Data from FAOSTAT Database. Percentage (%) is based on total harvested area or total grain production in Asia.

processed food, and feed uses predominate for maize and soybean. The unique properties of wheat flour make it impossible to substitute with any other grain. Strong demand for wheat supplies exists for traditional uses (e.g., noodles and steamed bread in northern China and other temperate Asian countries). Economic development also tends to increase demand for wheat products (e.g., baked bread) at the expense of rice consumption. Thus, many tropical Asian countries that do not produce any wheat require substantial imports (e.g., Philippines, Indonesia, Malaysia). Country data (Table 3) show that Japan is the largest net importer of grain in Asia. Its demand is likely to be stable, due to constant (or slightly declining) population and its advanced economic status (far past the rapid growth stage) and therefore stable dietary patterns. China was a major importer but also a significant exporter (in 2001). But its import export pattern is highly variable, as slight percentage changes in its large production base, will cause dramatic changes in its import needs and export capacity (Figure 7). India was a net exporter in 2001, and should be able to maintain this capability in the short term. Other countries have adopted a policy that will see sustained dependence on imports, some not having any economic reason or climatic suitability to pursue self-sufficiency (e.g., Saudi Arabia). Sustained import markets are the easiest for exporters to deal with – they can develop relationships with the buyers to understand quality needs and develop long-term policies to meet these needs. Erratic, but large purchases, on international markets are more difficult to handle efficiently.

Total cereal production has risen fairly steadily across Asia as a whole since 1960 (Figure 4) (despite

**Table 1** Area, production, and yield of grains (cereals, soybeans, and pulses) in Asia (2002)<sup>a</sup>

Crop	Are	ea harvested				Countries	
	Mha	% of Asia total	Mt	% of Asia total	% of world total	(kg ha <sup>-1</sup> )	(number)
Cereals, total	307	100	986	100	48.6	3209	47
Paddy rice	131	42.6	523	53.1	90.8	3998	30
Wheat	96.1	31.3	253	25.6	44.1	2629	37
Maize	42.7	13.9	166	16.8	27.5	3884	39
Barley	11.8	3.8	19.4	2.0	14.7	1645	33
Millet	11.4	3.7	9.1	0.9	39.0	803	20
Sorghum	11.4	3.7	11.0	1.1	20.2	959	20
Buckwheat	0.9	0.3	1.4	0.1	65.2	1451	6
Rye	0.7	0.2	1.1	0.1	5.2	1467	11
Oats	0.7	0.2	1.1	0.1	4.3	1543	15
Triticale	0.5	0.2	1.2	0.1	10.9	2320	1
Others	0.2	0.1	0.2	0.0	2.6	2641	11
Soybeans	17.1		23.7		13.2	1385	25
Pulses, total	36.3		25.6		46.4	706	40

<sup>&</sup>lt;sup>a</sup>Data from FAOSTAT Database.

Chinese production in the last 5 years, although high, has been more variable than in the past). This reflects improved varieties and agronomic practices (under the Green Revolution, discussed below), but also expansion of cultivated area under the multipronged approaches adopted to cope with vast increases

in demand. The situation has become rather less stable in more recent years (**Figures 4** and 5), with substantial drops in production of cereal grains other than the big three, namely, rice, wheat, and maize. In fact, between 1992 and 2002 total production (output) increased only  $\sim$ 6%, whereas area harvested

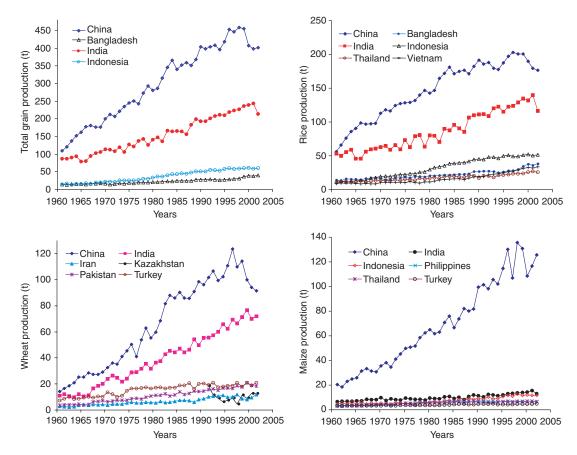


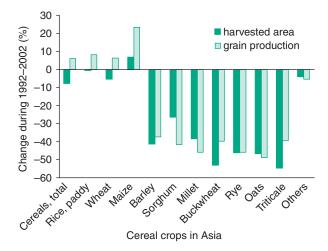
Figure 4 Trends of yield production in major Asian countries producing principal cereal crops from 1961 to 2002. (Data from FAOSTAT Database.)

Table 2 Quantity and value of grain imports and exports to/from Asia in 2001

Crop	Imports – qty (Mt)	Imports – value (1000 US\$)	Exports – qty (Mt)	Exports – value (1000 US\$)
Cereals	106 478 996	16 732 160	38 394 615	7 057 804
Maize	40 913 789	5 076 869	7 013 319	759 271
Wheat	40 238 186	6 760 921	7 906 977	896 782
Barley	10 117 996	1 490 596	436 870	40 048
Rice	9 426 314	2 623 935	19 798 418	4 902 134
Sorghum	2 015 717	233 845	20 587	3061
Rye	381 226	46 196	15 983	2065
Oats	112 604	20 100	5597	701
Buckwheat	104 668	25 227	104 491	20 365
Millet	65 854	15 188	45 735	9099
Triticale	251	74	25 528	5653
Others	3 102 391	439 209	3 021 110	418 625
Soybean	28 045 849	6 025 734	366 951	109 096
Pulses	4 463 201	1 468 603	2 636 496	1 065 219

Tahla 3	Ton ton Asia	n countries of	f arain (caras	I and souhpan)	imports and	exports in 2001

Top ten import	Impo	ort quantity	Top ten	Ехр	ort quantity	Top ten net	95.8 30.1 17.1 13.7 10.4 5.8 5.1 4.3 4.2 3.9 3.6	nport quantity
countries	Mt	Percentage (%)	export countries	Mt	Percentage (%)	import countries		Percentage (%)
Asia, total	134.5	100.0	Asia, total	38.8	100.0	Asia, total	95.8	100.0
1. Japan	31.1	23.1	1. China	9.3	23.9	1. Japan	30.1	31.4
2. China	26.3	19.6	2. Thailand	8.2	21.2	2. China	17.1	17.8
<ol><li>Korea</li></ol>	13.7	10.2	3. India	5.4	14.0	<ol><li>Korea</li></ol>	13.7	14.3
4. Iran	10.4	7.7	4. Vietnam	3.8	9.8	4. Iran	10.4	10.8
<ol><li>Indonesia</li></ol>	5.9	4.4	<ol><li>Kazakhstan</li></ol>	3.6	9.2	<ol><li>Indonesia</li></ol>	5.8	6.1
<ol><li>Saudi Arabia</li></ol>	5.1	3.8	6. Pakistan	3.4	8.6	<ol><li>Saudi Arabia</li></ol>	5.1	5.4
<ol><li>7. Malaysia</li></ol>	4.4	3.3	7. Turkey	1.6	4.0	<ol><li>Malaysia</li></ol>	4.3	4.4
<ol><li>Philippines</li></ol>	4.2	3.1	8. Myanmar	1.0	2.7	<ol><li>Philippines</li></ol>	4.2	4.4
9. Iraq	4.0	2.9	9. Japan	1.0	2.6	9. Iraq	3.9	4.1
10. Israel	3.6	2.6	10. United Arab Emirates	0.5	1.3	10. Israel	3.6	3.7
Others	25.8	19.2	Others	1.1	2.8	Others	-2.3	-2.4



**Figure 5** Change in Asian grain production and harvested area of total cereals by crop over the past decade (1992–2002). (Data from FAOSTAT Database.)

decreased  $\sim 8\%$ . Although this reflects a healthy improvement in yield per unit area, it underscores worrying trends. One is the diversion of agricultural land to noncereal uses, offering higher returns to farmers, and another is the steady loss of high-quality agricultural land. In many places, such as in parts of China, production of grain on small family-based farm units is no longer economically attractive. Other agricultural activities – vegetables, meat production such as ducks, or even flower production may be preferable. In some cases, land may be left idle while the landholder pursues nonagricultural business activities. These shifts are often beneficial in raising the living standards of rural families. But they also show that more or less open markets, keeping grain prices near world levels, are certainly incompatible with full selfsufficiency in grain.

#### The Green Revolution

"India has all the food it needs, but half of it is currently being eaten by rats," according to a western aid official quoted in a Financial Times report of December 2002. In the three years up to 2002, India's reserve of rice and wheat increased from 20 to 60 Mt, relative to an annual production of 220 Mt. Half of Indian children are underweight, and many are malnourished. India has seemingly solved one problem — the technological problem for which the solution began with the Green Revolution — only to be left with a perhaps more intractable problem, that of creating an economic system that distributes food equitably and without undue wastage.

Development of dwarf wheat that could sustain high fertilizer inputs and thus produce high grain yields without lodging began at International Center for the Improvement of Maize and Wheat (CIMMYT) in Mexico in the early 1960s. At that time, India's wheat production was low (~10 Mt per year) and static, showing only random seasonal fluctuations from year to year. Like most low-income, high-population countries, population growth was much more rapid than growth in food production, and a social catastrophe seemed imminent. The dwarf wheat from Mexico was introduced to India, through a system of demonstration fields and a supporting package of technology that emphasized to farmers the uniqueness of the new varieties, and the necessity of growing them with the high inputs of water, fertilizer, and other agricultural chemicals. From the mid-1960s, wheat production in India began to increase rapidly, outstripping growth in population – and leading to the present day problem of distribution of excess production. Over a 20-year period (1961-80), overall food production increased 3.6% per year in Asia as

a whole, from 1981 to 2000 (sometimes referred to as "Late Green Revolution"), it increased 2.1% per year. Thus, we should consider the adoption of high-yielding, high-input wheat varieties as the beginning of an agricultural transformation, and not as an abrupt one-time revolution. Present-day technologies may be seen as contributors to the same continuum of progress. If production increases in the range of 2–3% a year, clearly limiting the population growth to less than this is the key to increased production per person.

Shortly after the dwarf wheat varieties came into use, a parallel development started with dwarf rice varieties, distributed from IRRI Philippines. G. S. Khush, the IRRI plant breeder responsible for much of development of these rice varieties, summarized their biological features:

- reduction in plant height (improved harvest index) and higher biomass,
- photoperiod insensitivity (can grow any time of year) and short growth duration (110 days),
- increased yield stability through disease and insect resistance (biotic stresses),
- tolerance to adverse soil conditions (abiotic stresses),
- · eating quality, and
- comprehensive agronomic knowledge on crop management, e.g., nutrient requirements, water management, mechanization techniques, and equipment.

These technical inputs alone were not enough. As with wheat in India, production conditions had to be improved. The main factors associated with this are:

- Increased provision of irrigation facilities,
- Availability of inorganic fertilizers, and
- Supportive government policies

The Green Revolution is a high-input system. There is no "free lunch" or nonenergy intensive way to produce such dramatic increases in production. Central to this is farmer knowledge and participation in a system with higher risks (e.g., use of credit to finance the required inputs) but better rewards (higher economic returns).

#### The Crisis

#### **Grain Supply in China**

Chinese tradition and the political leadership have a clear understanding of the importance of grain production, from the proverb "Of all things food is the foremost necessity of the people" to the comment by Deng Xiaoping "Without agriculture, there is no stability, without grain, there is chaos." This has an impact even in relation to Hong Kong: "As long as the overall political situation in China is stable and the economy develops further, we will stand by our agreements and principles agreed with Britain" Qiao Shi (quoted 8 January 1995). This was in relation to the agreed transfer of sovereignty of Hong Kong from the United Kingdom to China from 1997, where substantial autonomy would be granted to Hong Kong (as a "Special Administrative Region") for a period of 50 years. Pragmatically speaking, if a government cannot feed its population, it cannot maintain the credibility to stay in power and carry out its normal obligations. This issue is all the more pressing in China with its large population, limited area of arable land, and recent memory of the disastrous famine of the early 1960s, which caused the deaths of over 30 million people.

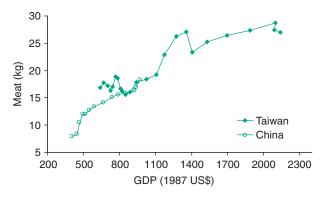
#### Money, Meat, and Grain

The book "Who Will Feed China?" by US environmentalist L. R. Brown, (1995), was perhaps the most influential early study in drawing the attention of western policy-makers to a fairly obvious problem, i.e., grain trade and capacity for global increases in grain production are limited, economic prosperity increases demand, industrialization reduces production, and the huge population of China makes it the key country where these issues converge. Industrialization and urbanization have many effects on agriculture:

- 1. Loss of agricultural land to urbanization takes out the productive farmland, and of course that nearest to the centers of population;
- Increased use of motor vehicles particularly new opportunities for personal ownership of cars, creates need for expanded highway, road, and parking systems, which also absorb agricultural land:
- 3. Competing demands for water reduces that available for agriculture, and increases the relative cost;
- Bringing inferior land under cultivation on the peripheries of existing arable land use results in lower average yields, and increased susceptibility to erosion, salinization, and other environmental problems;
- 5. The movement of people from rural to urban areas in search of better paying jobs reduces agricultural production capacity; and
- 6. Intensive land use requires ever-greater input of agricultural chemicals and other energy-based inputs, simply to maintain production.

Brown's book had pointed out that "the entire world cannot reasonably aspire to the US standard

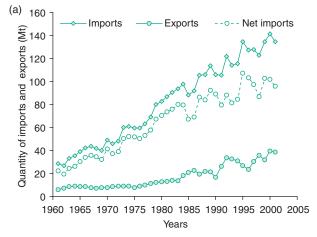
of living" and "we cannot afford to keep adding 90 million people a year indefinitely." In food production terms, the difference between the US and other countries is the total per-person demand for grain. Consumption of animal products – with the animals consuming grain-based feed - makes US per capita grain consumption  $\sim 800 \,\mathrm{kg}$  per year. Even Italy, an affluent country with many cultural affinities with the US, has a consumption of only 400 kg per person (and a healthier diet with less obesity). Prediction of China's future grain consumption – currently  $\sim$ 330 kg per person – is the key to preparing to meet future demand. An earlier study "Grain in China" published by the Department of Foreign Affairs and Trade, Australia (1992), had set out in some detail the technical issues related to this. In general, increased prosperity does not lead to large increases in the direct consumption of grain, assuming basic adequacy in dietary energy intake. However, increases in disposable incomes do lead to marked changes in dietary composition, first in the direction of more diversity, and particularly more meat products (and dairy, eggs, fish, etc.). Further increases in incomes will increase consumption of processed foods, meals prepared outside the home, and other convenience options. Taiwan, with a Chinese population of similar dietary customs, can be used as proxy for studying future dietary changes with increased incomes in the Chinese mainland. As mainland China reaches the purchasingpower-adjusted GDP per capita prevailing in Taiwan in the past, the demand for particular food products should also match Taiwan's at that stage of development. Using data up to 1990, Figure 6 shows that an increase in mainland China's GDP per capita from \$1000 to \$2000 would be expected to increase



**Figure 6** Per capita GDP and meat (pork, beef, and mutton) consumption in China (1977–90) and Taiwan (1952–75). GDP for China is increased threefold to adjust for purchasing power. (Adapted from Garnaut R and Ma G (1992) *Grain in China*, 151pp. Canberra: East Asia Analytical Unit, Department of Foreign Affairs and Trade, Commonwealth of Australia.)

demand for meat by ~10 kg per person per year. Such figures are the basis for concern about China's future needs. An increase in demand from 2003 onwards of a further 10 kg would represent a need for perhaps 40 Mt of grain (depending on feed conversion ratios and the proportion of grain used in the animals' diet). Naturally, one way to proceed is to greatly increase meat imports, with the US and Brazil representing source countries with further potential. However, this, to some extent, just shifts the burden of grain production elsewhere (away from Asia), and would deprive farmers in China of added income if they were simply to produce low-cost commodity grain (at low production efficiency) while imports satisfied needs for higher value products.

For Asia's two biggest countries, we can summarize that China's main driver of demand, for grain, is shifting food consumption patterns (toward meat), whereas India's main driver of demand is increasing population.



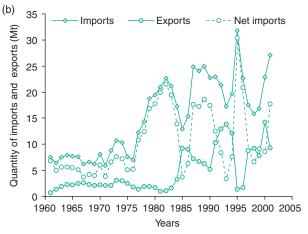


Figure 7 Changes in grain (cereals and soybean) imports and exports in Asia (a) and China (b) from 1961 to 2001.

# "Food Price Rises May Herald a World Crisis"

This November 2003 news item published in the *South China Morning Post*, Hong Kong, quoted Lester Brown "Sudden food price rises on the [Chinese] mainland could be the sign of a coming world food crisis. I view these as the warning tremors before the earthquake." Four consecutive years of world grain harvest decline, and historically low world grain stocks since records were kept, could lead to soaring grain prices worldwide. In 2003, wheat prices in northeastern China rose 32%, maize prices by 100%, and rice by 13%. China faced a 40 Mt shortfall in 2003, and the rest of the world, 56 Mt.

In an era of global grain trade (Figure 7) and heightened expectations of diminishing barriers to trade, all consumers (nations) will increasingly compete — on price — for the basic staples necessary for survival. Virtually all major grain producers still use pricedistorting mechanisms such as farm subsidies (in some countries, payments not to produce grain; in others, artificially high domestic prices, usually coupled with tariffs and quotas to restrict trade).

"More than just a hill of beans" headed a Washington Post article of November 2003 (published in *The Standard*, Hong Kong). This illustrated the complex relationship among farmer needs, urban consumer needs, and development of grain-processing facilities in producing-yet-importing markets (China) and exporting but price-distorting markets (the US). Recent Chinese restrictions on soybean imports have increased domestic prices, benefiting farmers in Heilongjiang, the main soybean-producing province. Limited supplies of soy meal have increased prices of pork, and soybean oil has also become more expensive, affecting urban shoppers across the country. Rising meat consumption in China has led to development of a soybean feed-processing industry employing 250 000 people. Restricted imports, for whatever reason, affect their livelihoods. China's estimated consumption of soybean is 33 Mt, which is projected to rise to 50 Mt within 5 years; in the face of current production of only  $\sim 17 \,\mathrm{Mt}$ . Expectations of free trade under World Trade Organization (WTO) are commonly undermined by nontariff barriers. Clearly Chinese imports of soybean – from the US – should continue and a mutually beneficial trade will result. The reasons for the restrictions (safety standards for genetically modified soybeans, and phytosanitary regulations concerning phytophthora) obviously have a strong policy overtone. However, they may be placed in context of the widespread demands from developing economy nations for US farm subsidies and tariffs to be reduced in the same spirit of free trade.

#### General Discussion

## Is Biotechnology the Answer?

Biotechnology can provide plant varieties that are more stress-resistant, responsive to appropriate inputs, resistant to pests and diseases (removing the necessity for energy-costly treatments), and are suited to specific end uses. Biotechnology cannot change food production from a "water, dirt, and energy" business into an environmentally stress-free self-sustaining system. Massive increases in food production are needed in the next several decades to keep pace with present demographic trends. Hopefully, population growth will slow and the new plateau in food production will prove to be sustainable. In some ways, biotechnology is distracting governments from the true nature of the problem and from their obligations to act on realistic solutions. Biotechnology has served poorly – if at all – in increasing yields relative to energy inputs. Yield increases over the 40 ongoing years of the Green Revolution have been due to development of varieties that can better sustain such inputs. Limits to population and limits to demand are keys to global food security. Food supply has become too politicized for governments to feel secure placing their fundamental food security in the hands of the most efficient producers. Perhaps the time has come to develop an international treaty guaranteeing free and unimpeded trade in basic foods and medicines, no matter what the prevailing conditions of political conflict or even war between nations.

## The Future

Asia is the key to world prosperity, food security, and stability; or the alternative of massive regional famines. Large increases in grain prices will not much affect consumers in the industrialized west. Like oil-price fluctuations, these will be absorbed into the economy. Grain raw material prices are a small percentage of most consumers' monthly food costs in these countries. Most Asian countries will be able to absorb any such structural increases in grain prices – albeit with some economic pain during the readjustment. Most of the poorer Asian countries are more or less self-sufficient in grain, or with increasing prices will have the motivation and capability to become self-sufficient. Rapidly industrializing and developing economies – such as China – will combine continued substantial domestic grain production with the capacity to import additional needs through the strength of their export-focused manufacturing industries. Perhaps long-term grain price increases will enable major producers (in North America and

Europe) to remove farm subsidies and move to more effective free-market production. An additional 100 Mt, for example, of grain entering world trade (at far higher prices than today) will be an attractive prospect to marketing boards and farmers' organizations in the US, Canada, and Australia. But these major exporting countries have to look carefully at the environmental costs of meeting such increased demand. The food supply crisis, although arising from western production constraints and Asian demands, will have its most serious consequences in countries, many in Africa, which have limited production, rapidly rising populations, and few competitive exports. Many of these are the same countries that suffered most in the oil price crises of the past few decades. With grain more than with oil, the impact of the crisis will be more immediate and drastic on the health and survival of the people of these countries.

See also: Grain Production and Consumption: Overview; Africa; Europe; Cereal Grains in North America; Oceania; South America.

# Further Reading

Brown L (1995) Who Will Feed China?, 163pp. New York: W.W. Norton and Company.

Cassman KG and Pingali PL (1995) Extrapolating trends from long-term experiments to farmers' fields: the case of irrigated rice systems in Asia. In: Barnett V, Payne R, and Steiner R (eds.) Agricultural Sustainability: Economic, Environmental and Statistical Considerations, pp. 63–84. London: Wiley.

Garnaut R and Ma G (1992) Grain in China, 151pp. Canberra: East Asia Analytical Unit, Department of Foreign Affairs and Trade, Commonwealth of Australia.

Kaosa-ard MS and Rerkasem B (2000) The Growth and Sustainability of Agriculture in Asia, 303pp. Oxford: Oxford University Press.

Khush GS (1999) Green Revolution: preparing for the 21st century. Genome 42: 646-655.

Khush GS (2001) Green revolution: the way forward. *Nature Reviews: Genetics* 2: 815–822.

Wittwer S, Yu Y, Sun H, and Wang L (1987) Feeding a Billion, 462pp. East Lansing, Michigan: Michigan State University Press.

## **Relevant Websites**

http://apps.fao.org - The FAO database on agriculproduction and trade a comprehensive resource and invaluable to researchers working in this area. It has been extensively used in preparation of this article.

http://www.irri.org - Website of the International Rice Research Institute, Philippines. Information and links on sustainable rice production in Asia.

http://www.worldwatch.org - Website of Worldwatch Institute, for "independent research" for an enviornmentally sustainable and socially just society.

# **Europe**

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## Introduction

In Europe, grain crop production has a long tradition and grains of different plant origin are substantial constituents of the human diet and of animal feed since ancient times.

For the cultivation of grain crops, of cereals especially, Europe has excellent prerequisites because of its climatic and pedological conditions as well as its overall efficient agricultural systems.

Preference for specific grain crops varies very much among European countries and larger geographical regions owing to the wide cultural multiplicity and ethnic diversity in nutritional habits.

Rye may serve as an example. It is not only a cereal very specific to Europe and hardly grown anywhere else in the world, but also within Europe, it is projected as a bread-making cereal as well as animal feed. It is grown almost exclusively in central, northern, and northeastern countries of Europe.

Another point of consideration while recording statistical data is that because the European Union (EU) is politically very diverse, it dominates all the markets including the agricultural one. This is the case, although the EU as vet does not include the accession countries and others like Russia and other countries of the former Soviet Union.

Consequently, data have been prepared and presented with respect to this given political situation but concentrating more on the "mainplayers" to ensure clarity.

## **Grain Production**

#### Cereals

In Europe, cereals are by far the most important agricultural crops (Tables 1-5), and among these, wheat is the principal crop (Table 1). Wheat accounts for nearly half of the total quantity harvested in 2002.

**Table 1** Cereal<sup>a</sup> production in (Mt = millions of metric tons) in Europe in the year 2002

Wheat	213.03
Rice, paddy	3.19
Barley	91.11
Maize	76.49
Rye	19.70
Oats	17.56
Millet	0.42
Sorghum	0.76
Buckwheat	0.67
Triticale	9.54
Canary seed	0.02
Mixed grain	4.57
Total cereals	437.17

<sup>&</sup>lt;sup>a</sup> Including the pseudocereal buckwheat. Source: FAOSTAT Database.

**Table 2** Cereal<sup>a</sup> production in Mt in the European Union (EU-15) in the year 2002

Wheat	104.80
Rice, paddy	2.60
Barley	48.20
Maize	40.62
Rye	4.78
Oats	7.22
Sorghum	0.69
Buckwheat	0.08
Triticale	5.35
Mixed grain	0.67
Total cereals	215.03

<sup>&</sup>lt;sup>a</sup> Including the pseudocereal buckwheat. Source: FAOSTAT Database.

**Table 3** Total cereal production (1000 t) in EU-15 member countries in selected years

Member country	1990–93	1999	2000	2001	2002
Germany	35 070	44 452	45 271	49 538	43 121
France	53 405	64 673	65 524	59 970	68 717
Italy	16 885	17 876	18 303	17 192	17 902
Netherlands	1335	1322	1663	1587	1601
Bel./Luxemb.	2015	2323	2470	2437	2487
Greece	4170	2884	2978	2553	2562
Spain	15 240	16 645	21 414	17 022	20 844
Portugal	1195	1393	1645	1158	1424
Austria	4525	4806	4151	4547	4327
Sweden	5110	4933	5560	5317	5556
Finland	3430	3139	3815	3669	3545
Denmark	7760	8809	8438	9810	8914
UK	20 570	22 329	24 039	18 994	22 660
Ireland	1935	2019	2227	2042	2043
EU-15	1 72 645	1 97 602	2 08 498	1 95 837	2 05 717

Sources: USDA, Washington, EUROSTAT, Luxemburg; Federal Ministry of Consumer Protection, Nutrition and Agriculture Berlin/Bonn.

Barley and grain maize and, to a distinctly less degree, rye and oats are also of significance. Triticale, the result of breeding wheat and rye, is in demand in those cultivation areas where normally rye is grown and where farmers are looking for alternatives.

All other cereals are of minor importance. The pseudocereal buckwheat — a plant belonging botanically to the Polygonaceae family — was grown throughout Europe until the early 1900s. It had mostly been grown in areas with poor soils and served for the production of grits and for specific traditional dishes such as pancakes, crêpes, etc. It has survived as a kind of speciality crop, for example, in northern Germany, Russia, and Austria. In very recent years, there has been a certain rekindling of interest in buckwheat as a functional food in modern nutrition systems because of constituents such as dietary fibers which have been scientifically proven to improve health and avoid diseases in human (animal) metabolism.

Rice is produced only in those parts of Europe which have a suitable climate, e.g., Spain, southern France, Italy, or southern Russia.

On the European continent, the EU, with its 15 member countries, is the most important cereal producer accounting for almost half of the production of all the cereals, wheat, barley, maize, triticale, and rice (Table 2).

Among EU member countries, France is the main cereal producer followed by Germany, UK, Spain, and Italy (Table 3). In comparison with the production during the 1990–93 period, in 2003 nearly all the EU members had significantly increased their cereal production, with Greece being the only exception.

The upcoming enlargement of the EU will further strengthen its role as a leading cereal producer (Table 4). Among the accessing countries are respectable cereal producers like Poland, Hungary, and the Czech Republic with the further possibility of countries such as Romania and Turkey joining the EU.

Growing of cereals is a long-standing tradition in the Russian Federation and in the Ukraine, though both countries are yet to utilize their full agronomic capacity, though it must be stated that in both countries there is a strong tendency towards increased harvest quantities (Table 5).

Also, when studying production data for single cereals of Russia, the Ukraine (Table 5), and of those eight countries going to access the EU in 2004 (Table 6), the role of wheat as most preferred cereal is underlined. Barley is in the second position and with rye and oats being in the next position. Yields for cereals as a whole and for individual cereal

**Table 4** Total cereal production (Mt = millions of metric tons) and yield (tha<sup>-1</sup> = metric tons/hectare) in EU accession countries

Country	Te	otal cereal producti	ion	Yield			
	1999	2000	2001	1999	2000	2001	
Bulgaria	5.13	4.68	5.24	2.92	2.62	2.54	
Cyprus	0.13	0.05	0.13	2.16	0.93	2.44	
Czech Republic	6.94	6.46	7.43	4.35	3.91	4.57	
Estonia	0.40	0.70	0.57	1.25	2.12	1.75	
Hungary	11.42	10.06	14.88	4.70	3.61	4.86	
Latvia	0.78	0.92	0.94	1.89	2.19	2.20	
Lithuania	2.05	2.66	2.29	2.02	2.71	2.70	
Malta	0.01	0.01	0.01	3.96	4.01	4.00	
Poland	25.75	22.34	27.23	2.96	2.54	3.09	
Romania	17.03	10.48	16.55	3.17	1.85	2.68	
Slovakia	2.83	2.20	3.48	3.86	2.71	4.11	
Slovenia	0.47	0.50	0.50	5.11	4.82	4.82	
Turkey	28.88	32.04	25.57	2.20	2.41	1.95	
Accession countries	101.82	93.10	104.82	2.84	2.41	3.01	

**Table 5** Cereal production in Mt in Russia (R) and the Ukraine (U) in selected years

		1999	2000	2001
Wheat	R	31.0	34.4	46.9
	U	13.6	10.2	21.3
Rice, paddy	R	0.4	0.6	0.5
	U	0.06	0.09	0.1
Barley	R	10.6	14.1	19.5
	U	6.4	6.9	10.2
Maize	R	1.1	1.5	0.8
	U	1.7	3.8	3.3
Rye	R	4.8	5.4	6.0
	U	0.9	1.0	1.5
Oats	R	4.4	6.0	8.0
	U	0.8	0.9	1.1
Millet	R	0.9	1.1	1.3
	U	0.2	0.4	0.6
Sorghum	R	0.03	0.22	0.12
	U	0.01	0.01	0.02
Total cereals	R	53.8	64.3	83.6
	U	24.9	23.8	38.8

Source: FAO Production Yearbook, Vol. 55 - 2001.

**Table 6** Total production of wheat, barley, rye, and oats in Mt in selected years in eight countries<sup>a</sup> accessing the EU in 2004

	1995	1998	2000	2001	2002
Wheat	20.13	21.59	19.51	22.71	20.72
Barley	9.12	9.62	7.16	8.80	8.34
Rye	7.18	6.66	4.79	5.63	4.64
Oats	2.08	2.13	1.61	1.89	2.08

<sup>&</sup>lt;sup>a</sup>Czech Republic, Slovakia, Poland, Hungary, Slovenia, Estonia, Latvia, Lithuania

Source: ZMP-Agrarmärkte in Zahlen, 2003.

varieties given reveal a significantly higher cultivation efficiency within the EU-15 when compared to respective data calculated on the whole-Europe basis. The reasons for this efficient agricultural system are multifold. Without doubt, the average EU-farmer is well educated and experienced and can rely on wellestablished governmental and private consulting systems. He has unlimited access to production means like fertilizers, pesticides, high-value varieties, and modern technical equipment. Furthermore, the EU has established a closed market with a couple of subsidies and guaranteed threshold prices within an intervention system minimizing farmers' risks and encouraging them to achieve maximum yields. It should also be mentioned that in large parts of the EU climatic and soil conditions are very favorable for cereal cultivation.

Precipitation is rather high and equally distributed throughout the year, temperatures are moderate, being, on an average, not too low during winter for the growth of winter-type cereals (winter wheat, winter rye, etc.). These cereals have a prolonged vegetation period and are thus higher in yield.

Also among EU member countries, yields of different cereals are varying significantly. Data for wheat yield in 2001 (data not shown) may serve as an example. Average yield ranged from 9.06 t ha<sup>-1</sup> in Ireland to 0.96 t ha<sup>-1</sup> in Portugal. In countries like Belgium, the Netherlands, Germany, France, Ireland, UK, or Denmark yields much higher than the EU average are standard. To a limited degree, there is also a variation in yield within these countries. These phenomena are the result of differences in climate, soil conditions, utilization of production means, and a question of the choice of varieties among which

**Table 7** Average yield in tha<sup>-1</sup> of cereals<sup>a</sup> in Europe in the year 2002

Wheat	3.65
Rice, paddy	5.49
Barley	3.34
Maize	5.66
Rye	2.33
Oats	2.02
Millet	0.33
Sorghum	3.88
Buckwheat	0.41
Triticale	4.14
Canary seed	1.15
Mixed grain	1.10
Total cereals	3.53

<sup>&</sup>lt;sup>a</sup>Including the pseudocereal buckwheat. Source: FAOSTAT Database.

**Table 8** Average yield in  $t ha^{-1}$  of cereals<sup>a</sup> in the European Union (EU-15) in the year 2002

Wheat	5.82
Rice, paddy	6.49
Barley	4.57
Maize	9.13
Rye	4.42
Oats	3.44
Sorghum	6.15
Buckwheat	2.82
Triticale	5.22
Mixed grain	3.59
Total cereals	5.67

<sup>&</sup>lt;sup>a</sup> Including the pseudocereal buckwheat. Source: FAOSTAT Database.

there is a broad range in genetically determined yield-forming potential.

#### Wheat

Additional detailed information on wheat has been provided because of its overall predominance in production and consumption. Data presented in the Tables 1, 2, 5-8 refer to wheat as a single entity. These statistical data do not distinguish between different wheat species. As mentioned above, the so-called soft aestivum wheat (botanical name -Triticum aestivum) is the most important wheat species, in Europe and worldwide. Two types of T. aestivum wheat are cultivated: winter wheat and spring wheat, where winter wheat is by far the most preferred due to its higher yield performance. Spring-type wheats are chosen when they specifically fit into the crop rotation system or in those countries (e.g., Scandinavia) where climate in winter and the growth period are too uncomfortable for

winter-type wheats. In the main wheat-producing countries, the contribution of spring wheat to the total wheat harvest is distinctly less than 1%. *T. aestivum* wheats are covering the wide range of feed and food applications whereas the species *Triticum durum*, known as durum or hard wheat, is specifically grown for pasta production. (This does not mean that pasta production would not be possible from soft wheat, too!)

Durum wheat is more adapted to warmer climatic conditions. Consequently, it is grown in Italy, Spain, France, and Greece where durum wheat cultivation is concentrated within the EU and to a reduced extent in Portugal and Austria (Table 9). In Germany and UK, durum wheat is traditionally not grown but there have been efforts to establish this cereal in regions with suitable climate for reasons of production alternatives for farmers and to supply local durum mills with produce.

In some parts of France, Switzerland, Germany, and Austria, cultivation of spelt wheat (Triticum spelta) has been conserved through the centuries for specific use in certain foods and for brewing a special beer. In recent times, people have become more and more conscious of the important role of a healthy nutrition and it is of benefit for health to consume multifold foods and among them those with functional constituents (such as dietary fiber/ functional food) and in this context Emmer (Triticum dicoccon), small spelt (Triticum monococcum), and Kamuth (Triticum polonicum) are gaining certain renewed recognition. This is more so in the case of organic farming and its niche clientele. However, the production rates are negligibly low. In addition only baked spelt wheat goods, mainly bread, are available in specialized bakeries.

## Rye

Rye is a very important cereal for Europe and — more precisely — for central, east, and northeast European countries and it is hardly grown anywhere else in the world (Tables 1, 2, 5, and 6). This has a historical basis. Rye is assumed to have originated from southwest Asia (Turkey, Iran, Iraq). From there, it is said to have moved to central Europe as a weed along with wheat and barley. But ancient farmers learned to appreciate this "weed" as it turned out to be often more stable and efficient in yield performing than the originally sown cereal, especially on poor sandy soils. Rye has a better drought resistance and efficiency in nutrient uptake from poor substrates.

Consequently, rye was very widely grown in Europe. In some parts, e.g., in UK, it has been replaced again by wheat. But in other parts, it remained of

Member country	2000 (1000 ha)	2001 (1000 ha)	2000 (t ha <sup>-1</sup> )	2001 (t ha <sup>-1</sup> )	2000 (1000 t)	2001 (1000 t)
Germany	9	5	5.0	5.1	43	24
Greece	673	761	2.2	1.9	1450	1429
Spain	868	881	2.2	2.1	1917	1837
France	338	306	5.0	4.4	1676	1354
Italy	1663	1664	2.6	2.3	4310	3769
Austria	16	12	3.0	3.8	44	46
Portugal	139	128	1.2	1.0	173	124
UK	1	1	6.0	6.0	6	6
EU-15	3706	3758	2.6	2.3	9619	8589

Table 9 Cultivation area, yield, and production of durum wheat in EU-member countries in 2000 and 2001

utmost importance in human diet, primarily for bread making.

The most important rye producer is the Russian Federation (Table 5) (the former Soviet Union) followed by Poland (5–6 Mt per year) and Germany (Table 10), which is the most significant rye producer within EU-15. In the Baltic States and in the Nordic countries, rye and rye products are important constituents of human diet and animal feed.

#### **Pulses and Oilseeds**

In comparison with cereals, the production of pulses is of lesser importance in Europe (Table 11). It is mainly the EU, the Russian Federation, the Ukraine, and some other east European countries where the production of specific legumes plays a remarkable role. Table 12 provides details of the highest amounts being reported for peas, broad beans, and for some countries, soybeans. Preference for individual species differs very much between countries, depending strongly on traditional diet as far as human consumption is concerned and on recipes of optimized feed-stuff mixtures in modern animal production.

This statement is also valid for oilseeds (Tables 11 and 13) among which rapeseed and sunflower are of tremendous significance, whereas other species are speciality crops in specific countries. However, for oilseeds it has also to be stated that they are of lesser importance in relation to cereals although they may contribute to a large extent to agricultural production as in Germany or France, for instance.

# **Consumption and Utilization**

Consumption and utilization of grain seeds in Europe is greatly influenced by factors as varied as climate, soil conditions, preference for specific crops based on tradition and ethnic peculiarities, economics, and governmental regulations. These factors are further compounded by the diversity in regions and countries

found on the European continent at large. As it is impossible to discuss every grain species in detail or take into consideration the entire range of diversities, an attempt will be made to focus on major trends and to outline the main fields.

Nearly all grain seeds or components of them serve as food and feed raw materials, and these are by far the most important fields of utilization. Furthermore, these crops or components derived thereof may be used as basic materials for chemical or technical purposes in the nonfood or feed industry. For example, starch is applied for the synthesis of adhesives.

## **Consumption of Cereals**

Taking the EU as an example, it can be shown that this formation as a whole is producing much more cereals than it is able to consume by itself (Table 10). The degree of self supply is more than 100% for soft wheat, rye, and all other cereals. This is specifically valid for the main grain-producing countries like France or Germany. However, there are also countries which lack sufficient production. This means a compensation of surpluses and demands, respectively, within the EU, but it also demonstrates that there is a strong pressure on at least individual countries for export outside the EU or for alternative utilization of cereals.

In 2002, within EU, 193.4 Mt of cereals were consumed, out of which ~120 Mt was used for feed production, and ~45 Mt for food purposes (data not shown). Even after accession of eight new countries in 2004, this general situation will not change very much as they are self-sufficient or even producing surpluses with individual cereals (e.g., rye in Poland). Even if they are not self-sufficient, their consumption market is not very strong due to their restricted number of population.

Countries as Russia, the Ukraine, or Belarus normally do not produce enough cereals to cover their

 Table 10
 Production and consumption of cereals in the EU member countries

			Soft wheat			Rye			Other cereals	
		Production (1000 t)	Consumption (1000 t)	Degree of self supply (%)	Production (1000 t)	Consumption (1000 t)	Degree of self supply (%)	Production (1000 t)	Consumption (1000 t)	Degree of self supply (%)
Germany	2000/01	21 578	15 187	142	4208	2618	161	19 485	18 443	106
	2001/02	22 865	15 590	147	5203	2549	204	21 847	19 291	113
France	2000/01	35 951	19 312	186	146	149	98	30 094	12 709	237
	2001/02	30 642	18 752	163	135	125	108	29 312	13 560	216
Belgium/Luxembourg	2000/01	1683	2537	66	7	21	33	604	1401	43
	2001/02	1830	2537	72	9	21	43	565	1401	40
Netherlands	2000/01	1183	4476	26	35	265	13	421	3185	13
	2001/02	1085	5976	18	23	315	7	628	3535	18
Italy	2000/01	3105	7180	43	11	23	48	16 503	16 909	98
•	2001/02	2898	7400	39	10	23	43	16 119	16 065	100
UK	2000/01	16 694	13 133	127	44	43	102	7247	7088	102
	2001/02	11 954	12 868	93	23	24	96	7605	7271	105
Ireland	2000/01	727	1103	66	0	0	0	1363	1427	96
	2001/02	632	1046	60	0	0	0	1297	1386	94
Denmark	2000/01	4686	3903	120	288	235	123	4458	3134	142
	2001/02	4862	3870	126	315	147	214	4308	3356	128
Greece	2000/01	455	1210	38	29	31	94	3634	3627	100
	2001/02	400	1060	38	30	31	97	3630	3522	103
Spain	2000/01	5416	7231	75	210	209	100	18 167	18 771	97
·	2001/02	3181	6191	51	107	607	18	13 893	18 646	75
Portugal	2000/01	228	1539	15	44	59	75	1319	2303	57
•	2001/02	33	1296	3	28	44	64	1270	2658	48
Finland	2000/01	538	596	90	108	99	109	3398	2477	137
	2001/02	489	575	85	64	98	65	3073	2424	127
Austria	2000/01	1251	810	154	183	198	92	2736	2693	102
	2001/02	1439	830	173	213	200	107	2816	2687	105
Sweden	2000/01	2428	1900	128	184	120	153	3116	2510	124
	2001/02	2362	1835	129	182	160	114	2844	2475	115
EU-15	2000/01	95 923	80 117	120	5497	4070	135	1 12 545	96 677	116
	2001/02	84 672	79 826	106	6342	4344	146	1 09 207	98 277	111

**Table 11** Production (t) and yield (t/ha = metric tons per hectare) of pulses and of selected oil seeds in Europe and in the European Union (EU-15) in 2002

	Europe	е	EU-15		
	Total production	Yield	Total production	Yield	
Total pulses	8 343 476	2.35	4 435 571	2.67	
Beans, dry	565 091	1.49	95 649	1.82	
Broad beans, dry	547 087	2.39	516 587	2.45	
Peas, dry	5 566 185	2.99	2 837 505	3.66	
Chick peas	89 833	0.83	82 013	0.85	
Lentils	40 084	0.86	33 507	0.91	
Linseed	212 652	0.63	113 767	0.84	
Soybeans	1 824 192	1.88	791 505	3.25	
Sunflower seed	12 849 062	1.28	2 800 108	1.69	
Rapeseed	12 034 654	2.53	9 168 515	2.99	
Sesame seed	135	0.94	90	0.77	
Safflower seed	5 750	0.93	250	1.25	

Source: FAOSTAT Database.

Table 12 Production (1000 t) of pulses in selected European countries in 2001

	Total	Dry	Broad	Dry	Lentils	Sovbean
	pulses	beans		peas		,
Albania	32	30				1
Bulgaria	25	9		2	3	4
Czech Rep.	95		5	84		2
Estonia	8	2		6		
Hungary	100	5		90	1	65
Latvia	3			2		
Lithuania	107	5		75		
Poland	323	42		45		
Romania	62	40		20		70
Slovakia	69	3	17	29	2	12
Slovenia	4	3		2		
Switzerland	10			10		2
Yugoslavia	107	34		23		230
Russian Fed.	1278	5		1000	2	262
Ukraine	880	80		695		73
Belarus	227	100		127		

Source: FAO Production Yearbook, Vol. 55 – 2001.

food and feed needs and only in certain years, the Ukraine acts as a wheat exporter on the world market.

As already mentioned, the largest quantities of cereals are used for feedstuff. Food use is dominated by bread production and the production of other baked goods like pastries, cakes, etc. The significance of this market segment is demonstrated in **Table 14** as consumption of flour per capita and year in member countries of the EU and in a few selected non-EU countries.

Flour consumption differs quite a lot between individual countries, within the EU as well as in the

**Table 13** Production (1000 t) of rapeseed and sunflower seed in selected European countries in 2001

	Rapeseed	Sunflower seed
Austria	147	51
Bulgaria		310
Czech Rep.	985	64
Denmark	350	
France	2906	1621
Germany	4168	62
Hungary	225	668
Italy	32	460
Poland	1073	
Romania	75	700
Russian Fed.	112	2700
Slovakia	242	
Spain	36	871
Sweden	106	
UK	1159	
Ukraine	124	2245

Source: FAO Production Yearbook, Vol. 55 - 2001.

others, with the Czech Republic and Romania having extraordinarily high consumption rates.

In the large majority of the European countries the production of bread or comparable foodstuff means production from wheat. This is also true for central, east, and northeastern countries, but here in addition, rye is also used as a raw material for bread making.

The extent to which rye is used is quite different between individual countries. For example, in Germany  $\sim$ 1 Mt of rye are milled for bread production, however this tendency is slowly decreasing.

Bread making does not only mean utilization of pure rye flour. Most breads offered on the market are made of a mixture of rye and wheat flour. In relation to wheat, rye has higher proportions of dietary fibers. Actually, this health benefit is used in arguing in favor of increased rye consumption again.

In countries where rye is traditionally used in human diet, it is also used for feeding of animals. Certain restrictions are related to antinutritive components like pentosans, but this is for young animals only and can be overcome by enzymatic treatment of the raw material.

Rye as well as wheat is used to a certain extent for alcohol production, whereas wheat and maize serve with increasing amounts as raw material for starch production (Tables 15 and 16). These older data from the EU – starch production data are not included in official governmental statistics and therefore hard to obtain – clearly demonstrate the high proportions maize and wheat contribute to total starch manufacturing (Table 15). The reason for increasing utilization of wheat is the production of gluten, which is

**Table 14** Per capita consumption of flour (kg/cap.  $\times$  year) in European countries in selected years; for comparison data from the USA

	1975	1985	1995	1996	1997	1998	1999
France	65.9	64.0	61.6	62.1	63.1	63.6	64.0
Germany <sup>a</sup>	56.9	62.8	62.5	62.7	62.7	64.0	64.1
UK	64.3	63.0	65.0	71.4	75.8	75.8	74.8
Belgium	65.9	79.9	70.0	70.0	55.0	67.0	dna
Luxembourg	46.8	46.1	40.0	40.0	38.0	37.5	dna
Netherlands	59.2	59.1	62.5	62.5	62.5	62.5	dna
Italy	75.4	75.9	70.9	70.9	70.0	71.2	70.7
Spain	58.5	55.5	52.5	52.1	52.1	52.5	53.8
Portugal	dna	60.0	66.0	70.0	70.0	70.0	70.0
Austria	61.0	64.0	61.1	61.1	61.0	64.0	64.0
Sweden	55.8	58.8	63.0	dna	67.8	78.4	78.4
Finland	68.6	67.8	60.3	60.0	61.5	62.2	62.9
Denmark	61.7	75.9	61.9	60.9	59.4	61.8	72.9
Norway	69.6	64.3	66.2	67.5	67.5	dna	dna
Switzerland	51.4	49.6	49.4	50.7	52.6	54.8	54.9
Czech Rep.	dna	dna	101.0	107.0	89.0	90.0	90.0
Romania	dna	114.0	100.0	103.0	109.0	110.0	108.2
USA	49.4	53.4	64.3	67.5	67.8	66.2	65.3

dna = data not available.

**Table 15** Starch production in Europe (EU-15) in selected years and contribution of different raw materials to the total production

	1995	1998
Starch production (Mt)	6.6	7.7
Potato (%)	20	22
Maize (%)	55	49
Wheat (%)	25	29

Source: German Ass. of Starch Industry, 1999.

a by-product very much in demand in the market for technical purposes and as a flour improver in the baking industry. **Table 16** reveals that increasing amounts of starches, i.e., indirectly cereals, are applied for nonfood uses.

In European tradition, maize is of little interest for food use. Minor quantities are used for grits production, as raw material for snacks, while the great majority of grain maize is used in feed.

Because of their  $\beta$ -glucan content with its health benefits, oats are processed to a certain amount to produce special food like flakes, extruded products, etc. But the majority of the production is used for feed.

#### **Consumption of Pulses and Oilseeds**

To a limited extent, pulses are used for human consumption, mainly peas, lentils, or different bean

**Table 16** Utilization of starch and starch derivatives in the European Union (EU-15) in selected years

	1995	1998
Total consumption (Mt)	6.0	7.3
Native starches (%)	29	25
Modified starches (%)	16	18
Saccharification products	55	57
Food uses (%)	55	53
Nonfood uses (%)	45	47

Source: German Ass. of Starch Industry, 1999.

varieties. Mostly they are components of feedstuff mixtures.

Oilseeds are mainly used for oil production and the protein-rich residues are feedstuff ingredients. The oil especially that of rapeseed is of high nutritional value and therefore used in increasing amounts for human nutrition. However, the bulk is used for technical applications, the possibilities of applications are being enlarged by special attempts in science and industry under the term renewable resources.

# Grain Production in an Expanded EU – An Outlook

The expanded EU will remain one of the main players in the world's grain markets. But this is mainly because of its cereal production and, to a less extent, because of its oilseed production which is mainly growing rape. Pulses will remain of minor interest though there is some effort in the EU to support legume production for feed use to replace imported soybean products.

Among cereals produced wheat will remain the dominant crop. Even in those accessing countries where rye still plays a certain role to date, there will be a shift towards wheat production. In the consumers preferences wheat is accepted as the "more modern" cereal, and it may be assumed that the same development will take place in the rye consumer countries such as the Baltic countries or in Poland as it could be observed in Germany during the 1980s: a movement way from "dark breads" towards "white breads" which means "white breads." This development will be supported by the EU's decision to take rye out of the intervention system starting with 2004.

As it already happend in Germany during 2002, farmers in the rye-producing accession countries will also adapt their rye production to the marked needs and they will replace rye by the growth of wheat and tentatively barley. But barley is also one of the overproduction crops, and by special

<sup>&</sup>lt;sup>a</sup>West Germany.

regulations the European Commission will try to bring barley prices within the EU (as cereal prices in general) to world market prices.

Attempts of cereal and nutrition scientists to counteract decreasing rye acceptance among people by outlining its specific nutritional benefits will not be very successful. So it may be assumed that rye will become a speciality crop even in Europe.

For wheat it can be outlined an increasing demand of the wet milling industry because, as already mentioned above, vital wheat gluten will remain a competitive by-product of starch production on EU and world markets.

An outlet for cereals in general may be energy production be it as alcohol to be added in certain amounts to gasoline or be it as burning material for specialized power stations. Both kinds of alternative use will be very much dependent on the framework conditions the EU is setting, for example, by tax reduction.

Burning of cereals for energy production is not very popular among European people at the moment. This is a psychological problem as cereals are understood as bread-making and food raw materials.

Another psychological barrier so far in most of the European countries is the use of genetically modified cereals (or crops in general).

To date it can not be foreseen to which extent this breeding tool will increase total grain production or that of "specialized grain species" for very specific applications, e.g., in the chemical industry.

Grain production in the novel EU member countries will soon achieve the standard of most of the previous members with respect to yield and quality. These countries will contribute to a further increase of the surplus in grain production in spite of the growth of the total European population.

An enormous potential for grain, predominantly cereal production, must be stated for the Ukraine and for Russia. But to what extent this potential will become reality will be very much dependent on the respective political conditions offering farmers the necessary stimulus for efficient production.

See also: Consumer Trends in Consumption. Contaminants of Grain. Grain Crops, Overview. Grain Production and Consumption: Africa; Asia; Cereal Grains in North America; Oceania; South America. Oilseeds, Overview. Pulses, Overview. Rye.

# **Further Reading**

Cornell HJ and Hoveling AW (1998) Wheat-Chemistry and Utilization. Lancaster, Basel: Technomic Publishing.

FAO (Food and Agriculture Organization of the United Nations) Rome: Statistical Yearbooks: Production (www.fao.org).

Schmidt M, Maul A, and Richter M (2003) Statistisches Jahrbuch über Ernährung, Landwirtschaft und Forsten der Bundesrepublik Deutschland, 47. Jhrg. Landwirtschaftsverlag, Münster-Hiltrup.

# **Relevant Websites**

http://apps.fao.org. http://www.verbraucherministerium.de. http://www.zmp.de. http://www.eu-datashop.de.

# Cereal Grains in North America

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# Introduction

The North American continent is a major production area for grain crops and is a primary or secondary producer in the world of many of the grain crops reviewed in this article. Major grain crops grown in North America include barley, maize, oat, rice, sorghum, and wheat. These major grain crops are grown, to greater or lesser extent, in the countries comprising the North American continent (Canada, USA, and Mexico).

Several of the currently grown major grain crops grown in North America were introduced in the last few centuries, e.g., barley, oat, rice, sorghum, and wheat, whereas maize is native to this continent. All of the current major grain crops in North America have grown from small beginnings to their major field crop status today based on successful research and development in cultivar development, production practices, market development, and new uses development.

This article will review the area used annually to produce each of these major grain crops and their annual production in each North American country and on the continent for a recent and continuous 10-year period. This article will also review the 10-year

mean disposition and consumption of each of these major grain crops in each North American country and on the continent. The percentage of world major grain crop production for each North American country and the ranking of the country, if it is in the top five countries of the world, will be noted. In general, all comments regarding crop disposition and consumption refer to averages since the 1990s, unless specifically stated otherwise. Area and production data for all crops relate to crops harvested for dry grain only. For disposition and consumption of commodities, the definitions of the listed elements are taken from the Food and Agriculture Organization of the United Nations (FAO). Values given for production relate to the total domestic production. Imports cover all movements into the country of the commodity. Stock changes indicate changes in stocks at all levels between the production and retail levels. A negative sign for stock quantities denotes a decrease in supply. Exports cover all movements by the crop commodity out of the country. Domestic supply is defined as: production + imports - exports + changes in stocks. Feed is the amount of the commodity and of edible components of the commodity fed to livestock. Seed comprises the amount of commodity used for reproduction. Waste includes the amount of crop commodity lost in processing, storage, and transportation. Food manufacture is the amount of commodity used for manufacture of processed commodities that cannot be converted back to their originating primary commodities. Food comprises the amounts of the commodity not detailed otherwise in the balance sheet, available for human consumption. Other uses comprise quantities of the commodity used for manufacture of nonfood products.

# **Barley**

#### Introduction

Barley (*Hordeum vulgare*) is one of the earliest cultivated field crops. It is easily adapted to a wide range of soil and climatic conditions. It occurs in both spring and winter growth habit forms, but it is the spring form that predominates in North American production. Both 2-row and 6-row barley cultivars are grown in North America.

Barley is used in North America primarily for animal feed. A considerable quantity of barley is also malted and used in the beer-brewing industry, while small quantities of barley are used for food (breads, breakfast cereals, and beverages) and planting seed.

#### **Area Harvested**

Canada has harvested barley from ~4.3 million hectares (Mha) (8.6% of Canadian arable land) annually (Table 1). The area of barley harvested in Canada has remained nearly constant since the 1990s (Table 1). The vast majority of barley production area in Canada is in the Prairie Provinces (Alberta, Saskatchewan, and Manitoba).

The USA has harvested barley from  $\sim$ 2.4 Mha (1.3% of American arable land) annually (**Table 2**). The area of barley harvested in the USA has trended down since the 1990s, beginning at almost 3.0 Mha and ending at  $\sim$ 2.0 Mha (**Table 2**). The vast majority of barley production area in the USA is in the northern and western Plains states.

Mexico is a comparatively small barley producer harvesting barley, from only ~251 000 ha (1.0% of Mexican arable land) annually (**Table 3**). The area of barley harvested in Mexico has varied widely since the 1990s, from a low of 116 000 ha to a high of 311 000 ha (**Table 3**). This variation in harvested area is typical for any small volume crop. The majority of barley production area is in central Mexico.

For the entire North American continent, barley was harvested from an average 7.0 Mha (2.7% of North American arable land) annually (**Table 4**). The area harvested has been fairly consistent every year during 1992–2001 (**Table 4**). Canada's average barley harvested area was 61.6%, the USA 34.8%, and Mexico less than 3.6% of the North American total barley harvested area.

#### **Production**

Canada has produced, on an average, 12.8 million tons (Mt) of barley annually (Table 5). The production of barley in Canada has remained nearly constant since the 1990s (Table 5), paralleling the nearly constant production area for barley harvested in Canada shown in Table 1. The average yield of barley in Canada has been 3.0 t ha<sup>-1</sup>.

The USA has produced, on an average, 7.7 Mt of barley annually (**Table 6**). There has been a noticeable decline in the production of barley in the USA since the 1990s, from almost 10 Mt at the beginning of the decade to less than 6 Mt at the end of the decade (**Table 6**). The average yield of barley in the USA has been 3.2 t ha<sup>-1</sup>, similar to the Canadian average yield.

Mexico has produced, on an average, 528 000 t of barley annually (Table 7). The production of barley in Mexico has varied widely since the 1990s, from a low of 307 000 t to a high of 762 000 t (Table 7). This variation in production is typical

for any small volume crop. The average yield of barley in Mexico has been  $2.1 \, \text{tha}^{-1}$ , a low yield compared to yields for Canada and the USA. Barley is grown in Mexico under water-limiting rain-fed conditions.

For the entire North American continent, average annual barley production was over 21.0 Mt (Table 8). There has been some year-to-year variation in production, but with no clear trend. In terms of North American production proportions, Canada has produced an average 60.8%, the USA 36.7%, and Mexico 2.5% of the barley produced in North America. In global terms, North America has produced, on an average, 14.1% of all the barley produced in the world. The share of world barley production for Canada, USA, and Mexico is 8.6%, 5.2%, and <1.0%, respectively. Canada is in the top five countries of the world for barley production.

## **Disposition and Consumption**

Of the average annual 12.9 Mt of barley produced in Canada and the 24 000 t of barley imported, 9.8 Mt were used domestically and 3.1 Mt were exported (Table 9). Approximately 92% of the barley used domestically in Canada was used for animal feed while small quantities of barley were used for planting seed, food manufacture, food, and other uses (<5%) (Table 9).

Of the average annual 8.2 Mt of barley produced in the USA and the over 902 000 t of barley imported, 7.6 Mt were used domestically and 1.5 Mt were exported (Table 10). Approximately 53% of the barley used domestically in the USA was used for animal feed, while 42% of the barley used domestically was used in food manufacture. Small quantities of barley were used for planting seed and food (<5%) (Table 10).

Of the average annual  $510\,000\,\mathrm{t}$  of barley produced in Mexico and the  $284\,000\,\mathrm{t}$  of barley imported,  $\sim 791\,000\,\mathrm{t}$  were used domestically and  $6\,\mathrm{t}$  were exported (Table 11). Approximately 35% of the barley used domestically in Mexico was used for animal feed, while 59% of the barley used domestically was used in food manufacture. Small quantities of barley were used for planting seed and food (<5%) (Table 11).

Of the average annual 21.5 Mt of barley produced in North America and 1.2 Mt imported, 18.2 Mt were used domestically and 4.6 Mt were exported (Table 12). Over 73% of the barley used domestically in North America was used for animal feed, while 22% was used for food manufacture. Small quantities were used for planting seed, food, and other uses (<5%) (Table 12).

# Maize

#### Introduction

Maize, (Zea mays), is a spring-sown, cross-pollinated annual crop, belonging to the grass family and the tallest of the cereal crops. Early European settlers in North America found maize being cultivated by aboriginal people throughout the continent. Maize is the leading crop in North America and its value is approximately double that of wheat. Maize, along with wheat and rice, is one of the top three cereal crops in the world.

Maize is used in North America primarily for animal feed, while small quantities of maize are used for food manufacture (corn syrup, cornstarch, corn oil) and food (breakfast cereals, tortillas, and alcoholic beverages). Very small quantities of maize are also used for planting seed.

## **Area Harvested**

Canada has harvested maize from ~1.1 Mha (2.2% of Canadian arable land) annually (**Table 1**). The area of maize harvested in Canada has remained nearly constant since the 1990s (**Table 1**). The majority of maize production area in Canada is in southern and western Ontario and southwestern Quebec.

The USA has harvested maize from ~28.4 Mha (15.5% of American arable land) annually (Table 2). The area of maize harvested in the USA has remained fairly constant since the 1990s (Table 2). Maize is grown in nearly every state, but the vast majority of maize production area in the USA is in the Middle-American "corn belt" states of Iowa, Illinois, Nebraska, Minnesota, Indiana, Kansas, Wisconsin, and South Dakota.

Maize is a major crop in Mexico. This is reflected in the significant acreage of harvested maize (**Table 3**). The area of maize harvested in Mexico has remained fairly constant at 7.6 Mha (32.2% of Mexican arable land) annually (**Table 3**). Maize production occurs throughout Mexico with greater concentration in central Mexico.

For the entire North American continent, maize was harvested from an average 37.1 Mha (14.5% of North American arable land) annually (**Table 4**). The area harvested has been fairly consistent every year during 1992–2001 (**Table 4**). Canada's average maize harvested area was 2.9%, the USA 76.6%, and Mexico 20.5% of the North American total maize harvested area.

#### **Production**

Canada has produced on an average 7.4 Mt of maize annually (Table 5). The production of maize in

Canada increased in the early 1990s to its present level (Table 5). The average yield of maize in Canada has been  $7.0 \, \text{t ha}^{-1}$ .

The USA has produced on average 229.4 Mt of maize annually (**Table 6**). The only times in the past decade that production declined below 200 Mt were in 1993 and 1995 (**Table 6**). The average yield of maize in the USA has been 8.1 t ha<sup>-1</sup>, 15% higher than the Canadian average yield.

Mexico has produced on average 18.1 Mt of maize annually (**Table 7**). The production of maize in Mexico has steadily increased since the 1990s from a low of 17 Mt in 1992 to a high of more than 20 Mt in 2001 (**Table 7**). The average yield of maize in Mexico has been 2.4 t ha<sup>-1</sup>. The low average yield for maize in Mexico results from the production of mostly unimproved, open pollinated, populations of maize under water-limiting rain-fed conditions.

For the entire North American continent, average annual maize production was over 254.9 Mt (Table 8). There has been some year-to-year variation in production, with a relatively low production year in 1993. In terms of North American production proportions, Canada has produced an average 2.9%, the USA 90.0%, and Mexico 7.1% of the maize produced in North America. In terms of world maize production, North America has produced on an average, 44.7% of all the maize produced in the world. The share of world maize production for Canada, USA, and Mexico is 1.3%, 40.2%, and 3.2%, respectively. The USA is the top maizeproducing country in the world. Mexico also ranks in the top five countries of the world for maize production.

# **Disposition and Consumption**

Of the average annual 7.3 Mt of maize produced in Canada and the 1.2 Mt imported, 8.0 Mt were used domestically and over 543 000 t were exported (Table 9). Over 78% of the maize used domestically in Canada was used for animal feed, while 10% of maize was used for food manufacture and 7% for other uses (Table 9). Small quantities of maize were used for planting seed and food (<5%).

Of the average annual 224.3 Mt of maize produced in the USA and the 435 000 t of maize imported, 177.8 Mt were used domestically and 46.6 Mt were exported (Table 10). Approximately 76% of the maize used domestically in the USA was used for animal feed, while 19% of the maize was used in food manufacture. Small quantities of maize were used for planting seed, food, and other uses (<5%) (Table 10).

Of the average annual 17.5 Mt of maize produced in Mexico and the 3.4 Mt of maize imported, over 20.2 Mt were used domestically and 122 000 t were exported (Table 11). Approximately 24% of the maize used domestically in Mexico was used for animal feed, while 58% and 5% of the maize was used in food and food manufacture, respectively. Small quantities of maize were used for planting seed and other uses (<5%) (Table 11).

Of the average annual 249.1 Mt of maize produced in North America and the 5.0 Mt imported, 206.0 Mt were used domestically and 47.2 Mt were exported (Table 12). Over 71% of the maize used domestically in North America was used for animal feed, while 17% and 8% was used for food manufacture and food, respectively (Table 12). Small quantities of maize were used for seed and other uses (<5%).

## Oat

#### Introduction

Oat, (Avena sativa), is a member of the grass family that is grown for cereal grain. It is easily adapted to a wide range of soil and climatic conditions. It occurs in both spring and winter growth habit forms, but it is the spring form that predominates in North America.

Oat is used in North America primarily for animal feed while smaller quantities of oat are used in food (breakfast cereals, bakery products, and snack foods). Very small quantities of oat are used for planting seed (<5%).

## **Area Harvested**

Canada has harvested oat from ~1.4 Mha (2.8% of Canadian arable land) annually (**Table 1**). The area of oat harvested in Canada has remained nearly constant since the 1990s (**Table 1**). The vast majority of oat production area in Canada is in the Prairie Provinces (Alberta, Saskatchewan, and Manitoba).

The USA has harvested oat from ~1.2 Mha (0.7% of American arable land) annually (**Table 2**). The area of oat harvested in the USA has declined since the 1990s, beginning the decade at over 1.8 Mha and ending at ~771 000 ha (**Table 2**). The majority of oat production area in the USA is in the north central Plains states (Wisconsin, Minnesota, and the Dakotas).

Mexico is a comparatively small oat producer, harvesting oat from only ~45 000 ha (0.2% of Mexican arable land) annually (Table 3). The area

of oat harvested in Mexico has varied widely since the 1990s, from a low of 20 000 ha to a high of 69 000 ha (Table 3). This variation in harvested area is typical for any small volume crop. The majority of oat production area in Mexico is in the northwestern states.

For the entire North American continent, oat was harvested from an average 2.7 Mha (1.0% of North American arable land) annually (**Table 4**). The area harvested has declined from a high of ~3.0 Mha in the early 1990s to 2.0 Mha in 2001 (**Table 4**). Canada's average oat harvested area was 52.5%, the USA 45.8%, and Mexico 1.7% of the North American total oat harvested area.

#### **Production**

Canada has produced on an average 3.4 Mt of oat annually (Table 5). The production of oat in Canada has remained nearly constant since the 1990s (Table 5). The average yield of oat in Canada has been 2.5 t ha<sup>-1</sup>.

The USA has produced on an average 2.6 Mt of oat annually (Table 6). There has been a noticeable decline in the production of oat in the USA since the 1990s, from more than 4 Mt at the beginning of the decade to less than 2 Mt at the end of the decade (Table 6). The average yield of oat in the USA has been 2.1 t ha<sup>-1</sup>, similar to the Canadian average yield.

Mexico has produced on an average 75 000 t of oat annually (Table 7). The production of oat in Mexico has varied widely since the 1990s, from a low of 32 000 t to a high of 121 000 t (Table 7). This variation in production is typical for any small volume crop. The average yield of oat in Mexico since the 1990s has been 1.7 t ha<sup>-1</sup>, a lower yield compared to that found in Canada and the USA. The lower yield stems from the fact that oat is produced in Mexico under water-limiting rain-fed conditions.

For the entire North American continent, average annual oat production was over 6.1 Mt (Table 8). There has been some year-to-year variation in production, but with no clear trend. In terms of North American production proportions, Canada has produced an average 56.3%, the USA 42.5%, and Mexico 1.2% of the oat produced in North America. In terms of world oat production, North America has produced on an average, 20.5% of the oat produced in the world. The share of world oat production for Canada, USA, and Mexico is 11.5%, 8.7%, and <1.0%, respectively. Canada and the USA are in the top five countries of the world for oat production.

# **Disposition and Consumption**

Of the average annual 3.4 Mt of oat produced in Canada and the over 9000 t of oat imported, 2.2 Mt were used domestically and 1.2 Mt were exported (Table 9). Over 89% of the oat used domestically in Canada was used for animal feed, while small quantities of oat were used for planting seed (7%) and food (<5%) (Table 9).

Of the average annual 2.8 Mt of oat produced in the USA and the over 1.6 Mt of oat imported, 4.3 Mt were used domestically and 79 000 t were exported (Table 10). Approximately 67% of the oat used domestically in the USA was used for animal feed, while 28% of the oat was used in food. Small quantities of oat were used for planting seed (<5%) (Table 10).

Of the average annual  $78\,000\,\mathrm{t}$  of oat produced in Mexico and the  $71\,000\,\mathrm{t}$  of oat imported in each year,  $\sim 149\,000\,\mathrm{t}$  were used domestically and  $43\,\mathrm{t}$  were exported (Table 11). Approximately 82% of the oat used domestically in Mexico was used for animal feed, while 16% of the oat used domestically was used in food. Small quantities of oat were used for planting seed (<5%) (Table 11).

Of the average annual 6.2 Mt of oat produced in North America and 1.7 Mt imported, 6.7 Mt were used domestically and 1.3 Mt were exported (Table 12). Approximately 75% of the oat used domestically in North America was used for animal feed, while 20% was used for food (Table 12). Small quantities were used for planting seed (5%).

## Rice

## Introduction

Rice, (Oryza sativa), is one of the oldest cultivated crops and is the principal food crop of the tropical and subtropical regions of the world. Rice varieties are commonly grouped into three classes according to the shape and length of the grain: long grain, medium grain, and short grain. In North America, only low-land (irrigated) and nonglutinous rice are important commercially.

Rice is used primarily as food with smaller quantities used in food manufacture to produce rice starch and alcoholic beverages. Still smaller quantities of rice are used for seed and other uses (<5%) (Table 10).

## **Area Harvested**

Rice is not grown in Canada. The climate in Canada is unsuitable for rice cultivation.

The USA has harvested rice from  $\sim$ 1.3 Mha (0.7% of American arable land) annually (Table 2).

The area of rice harvested in the USA has remained fairly constant since the 1990s (Table 2). The vast majority of rice production area in the USA is in Arkansas, California, Louisiana, Mississippi, and Texas.

Mexico is a comparatively small rice producer, harvesting rice from only ~84 000 ha (0.4% of arable land) annually (Table 3). The area of rice harvested in Mexico has varied widely since the 1990s, from a low of 53 000 ha to a high of 113 000 ha (Table 3). This variation in harvested area is typical for any small volume crop. Rice is produced in Mexico in the northwestern and central states.

For the entire North American continent, rice was harvested from an average 1.4 Mha (0.5% of North American arable land) annually (Table 4). The area harvested has remained fairly constant (Table 4). The average rice harvest area of the USA and Mexico was 93.8% and 6.2% of the North American total rice harvested area, respectively.

#### **Production**

The USA has produced on average 8.4 Mt of rice annually (**Table 6**). Rice production has been fairly constant (**Table 6**). The average yield of rice in the USA has been 6.6 t ha<sup>-1</sup>.

Mexico has produced on average 372 000 t of rice annually (Table 7). The production of rice in Mexico has remained fairly constant since the 1990s (Table 7). The average yield of rice in Mexico has been 4.4 t ha<sup>-1</sup>, a significantly lower yield compared to that found in the USA. Limited crop inputs for rice grown in Mexico reduce average yields.

For the entire North American continent, average annual rice production was 8.8 Mt (Table 8). In terms of North American production proportions, the USA has produced an average 95.8%, and Mexico 4.2% of the rice produced in North America. In terms of world rice production, North America has produced on average, 1.5% of all the rice produced in the world. The share of world rice production for the USA is 1.5%.

#### **Disposition and Consumption**

Of the average annual 337 000 t of rice imported into Canada, 321 000 t were used domestically and 16 000 t were exported (Table 9). Over 89% of the rice used domestically in Canada was used for food and 8% was used for other uses (Table 9).

Of the average annual 8.2 Mt of rice produced in the USA and the 488 000 t of rice imported, 5.2 Mt

were used domestically and 4.0 Mt were exported (Table 10). Approximately 63% of the rice used domestically in the USA was in food and 14% was used in food manufacture. Small quantities of rice were used for planting seed and other uses (<5%) (Table 10).

Of the average annual 384 000 t of rice produced in Mexico and the 442 000 t of rice imported, ~820 000 t were used domestically and 3 000 t were exported (Table 11). Approximately 85% of the rice used domestically in Mexico was in food and 10% was for food manufacture. Small quantities of rice were used for planting seed and other uses (<5%) (Table 11).

Of the average annual 8.6 Mt of rice produced in North America and the 1.3 Mt imported, 6.3 Mt were used domestically and 4.0 Mt were exported (Table 12). Over 67% of the rice used domestically in North America was used for food and 13% for food manufacture (Table 12). Small quantities of rice were used for planting seed and other uses (<5%). North America is a small rice producer compared to Asia.

# Sorghum

## Introduction

Sorghum (Sorghum bicolor) includes widely diverse types of annual and perennial types. Sorghum grain is the fifth most important cereal in the world. The crop is a heat and drought tolerant, dryland-adapted crop that is generally grown in marginal crop production areas. Sorghum is classified in North America according to its use as a grain sorghum, sweet sorghum, grass sorghum, broomcorn, or special purpose. The statistics presented in this article for sorghum refer only to the grain sorghums.

Sorghum is used in North America primarily as an animal feed. Small quantities are also used for food manufacture (flour) and still smaller quantities of sorghum are used for food (breads, grits, breakfast cereals), other uses (starch), and planting seed.

#### **Area Harvested**

Sorghum is not grown in Canada. As in the case of rice, the climate in Canada is unsuitable for grain sorghum production.

The USA has harvested sorghum from ~3.7 Mha (2.0% of American arable land) annually (Table 2). The area of sorghum harvested in the USA has remained fairly constant since the 1990s, although, there were peak years in 1992 and 1996 (Table 2).

The vast majority of sorghum production area in the USA is the southcentral states of Kansas, Oklahoma, Texas, Mississippi, Arkansas, and Louisiana.

Mexico harvested sorghum from ~1.7 Mha (7.0% of Mexican arable land) annually (Table 3). The area of sorghum harvested in Mexico remained constant since the 1990s, except for 1993, where it declined significantly with only 878 000 ha (Table 3). Sorghum production areas in Mexico include the northwestern, northeastern, and central states.

For the entire North American continent, sorghum was harvested from an average 5.4 Mha (2.1% of North American arable land) annually (Table 4). The area harvested had record highs in 1992 and 1996, when there were over 6.0 Mha (Table 4). The average sorghum harvested area for the USA and Mexico was 69.0% and 31.0% of the North American total sorghum harvested area, respectively.

#### **Production**

The USA has produced on an average 15.3 Mt of sorghum annually (**Table 6**). High production years were 1992 and 1996, coinciding with high area harvested years (**Table 6**). The average yield of sorghum in the USA has been 4.1 t ha<sup>-1</sup>.

Mexico has produced on an average 5.3 Mt of sorghum annually (Table 7). The lowest production was in 1993 that coincided with low area harvested (Table 7). The average yield of sorghum in Mexico has been 3.2 t ha<sup>-1</sup>, a significantly lower yield compared to that found in the USA. Sorghum is grown in Mexico under water-limiting rain-fed conditions.

For the entire North American continent, average annual sorghum production was 20.6 Mt annually (Table 8). There has been a significant decline in production since the 1990s, although, there was high production in 1996. In terms of North American production proportions, the USA has produced 74.4% and Mexico 25.6% of the sorghum produced in North America. In terms of global sorghum production, North America has produced on an average, 33.8% of all the sorghum produced in the world. The share of world sorghum production for USA and Mexico is 25.1% and 8.7%, respectively. The USA is in the top five sorghum producers in the world.

## **Disposition and Consumption**

Of the average annual 3000 t of sorghum imported into Canada, 3000 t were used domestically (Table 9). Of the sorghum used domestically in Canada, 100% was used for animal feed (Table 9).

Of the average annual  $15.5\,\mathrm{Mt}$  of sorghum produced in the USA and the  $440\,\mathrm{t}$  of sorghum imported,  $10.2\,\mathrm{Mt}$  were used domestically and  $5.9\,\mathrm{Mt}$  were exported (Table 10). Approximately 91% of the sorghum used domestically in the USA was used for animal feed, while 5% of the sorghum was used in food manufacture. Small quantities of sorghum were used for food and planting seed (<5%) (Table 10).

Of the average annual 5.1 Mt of sorghum produced in Mexico and the 3.4 Mt of sorghum imported, 8.5 Mt were used domestically and 4000 t were exported (Table 11). Approximately 98% of the sorghum used domestically in Mexico was used for animal feed. Very small quantities of sorghum were used for planting seed (<5%) (Table 11).

Of the average annual 20.6 Mt of sorghum produced in North America and the 3.4 Mt imported, 18.7 Mt were used domestically and 5.9 Mt were exported (Table 12). Over 94% of the sorghum used domestically in North America was used for animal feed (Table 12). Small quantities were used for food manufacture, food, and planting seed (<5%).

## Wheat

## Introduction

Wheat (*Triticum aestivum*, *T. durum*) is a member of the grass family that is grown in spring and winter habit forms for cereal grain. It is the most important cereal in the world in terms of production and use for human food and animal feed.

Wheat used in North America is used primarily as food (bread products, pasta, noodles, pastry, breakfast cereals, and baby foods) and for animal feed. Small quantities (<5%) of wheat are also used for other uses (glues, alcohol, and gluten) and planting seed (Table 10).

#### **Area Harvested**

Canada has harvested wheat from ~11.4 Mha (22.9% of Canadian arable land) annually (Table 1). The area of wheat harvested in Canada has decreased since the 1990s from a high of 13.9 Mha in 1992 to 10.6 Mha in 2001 (Table 1). The vast majority of wheat production area in Canada is in the Prairie Provinces (Alberta, Saskatchewan, and Manitoba) with minor production in southern Ontario and the Maritimes.

The USA has harvested wheat from  $\sim$ 23.8 Mha (13.0% of American arable land) annually (**Table 2**). The area of wheat harvested in the USA

has declined since the 1990s, beginning the decade at almost 25.4 Mha and ending at ~19.7 Mha (Table 2). Wheat production area in the USA varies with the class of wheat. Hard red winter wheat is grown in the central and southern Great Plains and the Pacific northwestern states. Hard red spring is grown in the northern Great Plains, soft red winter in the eastern and southeastern states, white in the northeastern states, and in the Great Plains, and durum is grown in the northern Plains states and California.

Mexico is a comparatively small wheat producer, harvesting wheat from only ~809 000 ha (3.4% of Mexican arable land) annually (Table 3). The area of wheat harvested in Mexico has varied widely since the 1990s, from a low of 652 000 ha to a high of 965 000 ha (Table 3). The vast majority of dryland wheat production area in Mexico is in the central region although there is also irrigated wheat production in the northern states.

For the entire North American continent, wheat was harvested from an average 36.0 Mha (14.1% of North American arable land) annually (**Table 4**). The area harvested has declined from a high of ~40.0 Mha in 1992 to 31.0 Mha in 2001 (**Table 4**). Canada's average wheat harvested area was 31.7%, the USA 66.1%, and Mexico 2.2% of the North American total wheat harvested area.

## **Production**

Canada has produced on an average 25.7 Mt of wheat annually (**Table 5**). The production of wheat in Canada has declined noticeably (31%) since the 1990s from a high of 29.9 Mt in 1992 to 20.6 Mt in 2001 (**Table 5**). The average yield of wheat in Canada has been 2.3 t ha<sup>-1</sup>.

The USA has produced on an average 63.0 Mt of wheat annually (**Table 6**). There has been a decline in the production of wheat in the USA since the 1990s, from more than 67.1 Mt at the beginning of the decade to 53.3 Mt at the end of the decade (**Table 6**). The average yield of wheat in the USA has been 2.6 t ha<sup>-1</sup>, similar to the Canadian average yield.

Mexico has produced on an average 3.5 Mt of wheat annually (Table 7). The production of wheat in Mexico has remained fairly constant since the 1990s (Table 7). The average yield of wheat in Mexico has been 4.3 t ha<sup>-1</sup>, a significantly higher yield compared to that found in Canada and the USA. Mexican wheat production is done under

irrigation, with high inputs used to produce these high-average grain yields.

For the entire North American continent, average annual wheat production was 92.3 Mt annually (Table 8). There has been a significant decline in production (23%) from 1992 to 2001. In terms of North American production proportions, Canada has produced an average 27.9%, the USA 68.3%, and Mexico 3.8% of the wheat produced in North America. In terms of world wheat production, North America has produced on an average, 16.0% of all the wheat produced in the world. The share of world wheat production for Canada, USA, and Mexico is 4.5%, 11.0%, and <1.0%, respectively. The USA is in the top five countries of the world for wheat production.

## **Disposition and Consumption**

Of the average annual 26.9 Mt of wheat produced in Canada and the 399 000 t of wheat imported, 7.9 Mt were used domestically and 19.7 Mt were exported (Table 9). Over 52% of the wheat used domestically in Canada was used for animal feed, while 32% were used for food and 14% was used for planting seed. Small quantities of wheat were used for other uses (<5%) (Table 9).

Of the average annual 63.1 Mt of wheat produced in the USA and the 2.7 Mt of wheat imported, 33.6 Mt were used domestically and 32.1 Mt were exported (**Table 10**). Approximately 70% of the wheat used domestically in the USA was used in food, while 22% of the wheat was used for animal feed and 8% was used for planting seed. Small quantities (<5%) of wheat were used for other uses (**Table 10**).

Of the average annual 3.6 Mt of wheat produced in Mexico and the 1.9 Mt of wheat imported,  $\sim 5.1$  Mt were used domestically and 328 000 t were exported (Table 11). Approximately 73% of the wheat used domestically in Mexico was used in food, while 9% was used for animal feed and 8% was for other uses. Small quantities of wheat were used for planting seed (<5%) (Table 11).

Of the average annual 93.5 Mt of wheat produced in North America and 5.0 Mt imported, 46.6 Mt were used domestically and 52.0 Mt were exported (Table 12). Over 64% of the wheat used domestically was used for food, while 26% was used for animal feed and 8% for planting seed (Table 12). Small quantities were used for other uses (<5%).

# **Data Tables**

Table 1 Area harvested (ha) for major grains grown in Canada 1992-2001

Year	Barley	Maize	Oat	Wheat
1992	3 792 000	857 700	1 242 700	13 830 300
1993	4 159 000	1 012 000	1 343 000	12 374 000
1994	4 092 000	955 000	1 492 000	10 773 000
1995	4 363 000	1 002 500	1 211 000	11 122 700
1996	4 888 000	1 090 000	1 684 000	12 262 000
1997	4 700 000	1 045 100	1 498 500	11 409 900
1998	4 272 000	1 118 300	1 591 600	10 679 700
1999	4 069 300	1 148 800	1 398 400	10 374 800
2000	4 449 900	1 088 300	1 299 000	10 849 600
2001	4 149 500	1 267 500	1 238 400	10 585 300
10-year mean	4 293 470	1 058 520	1 399 860	11 426 130

Source: Food and Agriculture Organization of the United Nations (FAO).

Table 2 Area harvested (ha) for major grains grown in USA 1992-2001

Year	Barley	Maize	Oat	Rice	Sorghum	Wheat
1992	2 948 200	29 168 800	1 819 500	1 267 500	4 876 500	25 398 800
1993	2 732 900	25 468 000	1 539 000	1 146 500	3 608 200	25 378 900
1994	2 698 100	29 345 000	1 623 000	1 341 950	3 594 000	24 997 000
1995	2 541 000	26 389 000	1 195 000	1 251 700	3 340 000	24 685 000
1996	2714000	29 398 000	1 074 000	1 134 751	4 780 000	25 414 000
1997	2 508 000	29 409 000	1 138 000	1 255 753	3 706 000	25 414 000
1998	2 373 000	29 376 000	1 114 900	1 318 075	3 125 000	23 878 000
1999	1 916 000	28 525 000	992 700	1 421 271	3 457 700	21 781 000
2000	2 109 650	29 316 000	942 520	1 229 853	3 126 630	21 502 390
2001	1 735 720	27 845 910	770 930	1 341 143	3 473 860	19 681 290
10-year mean	2 427 657	28 424 071	1 220 955	1 270 850	3 708 789	23 813 038

Source: Food and Agriculture Organization of the United Nations (FAO).

Table 3 Area harvested (ha) for major grains grown in Mexico 1992-2001

Year	Barley	Maize	Oat	Rice	Sorghum	Wheat
1992	289 974	7 219 352	35 238	90 420	1 375 805	915 882
1993	234 156	7 428 220	69 202	58 939	877 663	877 598
1994	115 815	8 193 968	30 928	87 796	1 251 830	964 572
1995	246 407	8 020 392	20 353	78 439	1 372 350	929 331
1996	283 295	8 050 931	64 174	86 778	2 184 720	809 240
1997	243 522	7 406 061	60 761	113 492	1 877 356	772 303
1998	267 548	7 876 819	64 949	101 560	1 953 073	768 844
1999	226 986	7 162 702	50 000	82 583	1 913 109	652 312
2000	290 380	7 131 180	23 026	84 069	1 899 201	707 768
2001	310 702	7 810 850	29 491	53 232	1 942 780	687 248
10-year mean	250 879	7 630 048	44 812	83 731	1 664 789	808 510

Table 4 Area harvested (ha) for major grains grown in North America 1992–2001

Year	Barley	Maize	Oat	Rice	Sorghum	Wheat
1992	7 030 174	37 245 852	3 097 438	1 357 920	6 252 305	40 144 982
1993	7 126 056	33 908 220	2 951 202	1 205 439	4 485 863	38 630 498
1994	6 905 915	38 493 968	3 145 928	1 429 746	4 845 830	36 734 572
1995	7 150 407	35 411 892	2 426 353	1 330 139	4 712 350	36 737 031
1996	7 885 295	38 538 931	2 822 174	1 221 529	6 964 720	38 485 240
1997	7 451 522	37 860 161	2 697 261	1 369 245	5 583 356	37 596 203
1998	6 912 548	38 371 119	2 771 449	1 419 635	5 078 073	35 326 544
1999	6 212 286	36 836 502	2 441 100	1 503 854	5 370 809	32 808 112
2000	6 849 930	37 535 480	2 264 546	1 313 922	5 025 831	33 059 758
2001	6 195 922	36 924 260	2 038 821	1 394 375	5 416 640	30 953 838
10-year mean	6 972 006	37 112 639	2 665 627	1 354 580	5 373 578	36 047 678

Table 5 Production (t) for major grains grown in Canada 1992–2001

Year	Barley	Maize	Oat	Wheat
1992	11 031 500	4 882 600	2 828 500	29 871 300
1993	12 972 100	6 501 200	3 549 100	27 231 500
1994	11 692 000	7 042 900	3 640 000	23 122 100
1995	13 032 500	7 270 900	2 857 500	25 036 500
1996	15 562 000	7 536 400	4 361 000	29 801 400
1997	13 527 000	7 180 000	3 484 700	24 280 300
1998	12 708 700	8 952 400	3 957 500	24 082 300
1999	13 196 000	9 161 300	3 641 300	26 940 800
2000	13 172 000	6 826 700	3 389 400	26 519 200
2001	10 845 600	8 389 200	2 690 700	20 567 600
10-year mean	12 773 940	7 374 360	3 439 970	25 745 300

Source: Food and Agriculture Organization of the United Nations (FAO).

Table 6 Production (t) for major grains grown in USA 1992-2001

Year	Barley	Maize	Oat	Rice	Sorghum	Wheat
1992	9 908 000	240 719 008	4 271 000	8 149 000	22 226 600	67 136 000
1993	8 666 000	160 984 992	3 007 000	7 081 000	13 568 600	65 222 000
1994	8 161 000	255 292 992	3 322 000	8 971 100	16 402 000	63 168 000
1995	7 824 000	187 968 992	2 338 000	7 887 000	11 650 000	59 404 000
1996	8 544 000	234 529 008	2 224 000	7 783 604	20 201 000	61 982 000
1997	7 835 000	233 867 008	2 428 000	8 300 697	16 093 000	67 536 000
1998	7 666 600	247 882 000	2 409 000	8 364 200	13 206 900	69 327 000
1999	6 103 000	239 548 992	2 122 000	9 343 954	15 118 000	62 567 284
2000	6 939 480	251 854 000	2 170 640	8 657 810	11 951 910	60 757 488
2001	5 430 480	241 484 864	1 698 600	9 663 560	13 069 510	53 261 980
10-year mean	7 707 756	229 413 186	2 599 024	8 420 193	15 348 752	63 036 175

Table 7 Production (t) for major grains grown in Mexico 1992–2001

Year	Barley	Maize	Oat	Rice	Sorghum	Wheat
1992	549 966	16 929 344	39 424	394 022	5 353 223	3 620 503
1993	540 529	18 125 264	82 372	287 180	2 581 072	3 582 450
1994	307 266	18 235 826	40 607	373 616	3 701 120	4 150 920
1995	486 636	18 352 856	36 439	367 030	4 169 898	3 468 220
1996	585 754	18 023 626	121 477	394 075	6 809 490	3 375 008
1997	470 671	17 656 258	96 493	469 455	5 711 564	3 656 594
1998	410 766	18 454 710	88 831	458 112	6 474 842	3 235 080
1999	454 133	17 706 376	121 313	394 434	5 720 343	3 020 889
2000	712 619	17 556 900	32 485	351 447	5 842 308	3 493 210
2001	762 156	20 134 300	88 886	226 639	6 566 540	3 275 460
10-year mean	528 050	18 117 546	74 833	371 601	5 293 040	3 487 833

Table 8 Production (t) for major grains grown in North America 1992–2001

Year	Barley	Maize	Oat	Rice	Sorghum	Wheat
1992	21 489 466	262 530 952	7 138 924	8 543 022	27 579 823	100 627 803
1993	22 178 629	185 611 456	6 638 472	7 368 180	16 149 672	96 035 950
1994	20 160 266	280 571 718	7 002 607	9 344 716	20 103 120	90 441 020
1995	21 343 136	213 592 748	5 231 939	8 254 030	15 819 898	87 908 720
1996	24 691 754	260 089 034	6 706 477	8 177 679	27 010 490	95 158 408
1997	21 832 671	258 703 266	6 009 193	8 770 152	21 804 564	95 472 894
1998	20 786 066	275 289 110	6 455 331	8 822 312	19 681 742	96 644 380
1999	19 753 133	266 416 668	5 884 613	9 738 388	20 838 343	92 528 973
2000	20 824 099	276 237 600	5 592 525	9 009 257	17 794 218	90 769 898
2001	17 038 236	270 008 364	4 478 186	9 890 199	19 636 050	77 105 040
10-year mean	21 009 746	254 905 092	6 113 827	8 791 794	20 641 792	92 269 309

Source: Food and Agriculture Organization of the United Nations (FAO).

Table 9 Canada 10-year averages for disposition and consumption (t) 1991-2000

Crop	Barley	Maize	Oat	Rice	Sorghum	Wheat
Production	12 851 080	7 276 740	3 352 590			26 861 160
Imports	23 950	1 161 334	9 015	336 957	3 186	399 034
Stock change	12 970	65 500	4 568	571		249 726
Exports	3 091 387	543 293	1 189 926	16 337	35	19 653 735
Domestic supply	9 796 613	7 960 281	2 176 247	321 192	3 151	7 856 186
Feed	8 988 786	6 224 583	1 940 756		3 151	4 067 000
Seed	403 270	28 810	153 130			1 115 100
Waste	5 260	251 183	7 190	8 794		7 350
Food manufacture	377 991	831 902				
Food	11 712	97 667	75 171	287 454		2 497 134
Other uses	9 593	526 135		24 943		169 601

Table 10 United States 10-year averages for disposition and consumption (t) 1991-2000

Crop	Barley	Maize	Oat	Rice	Sorghum	Wheat
Production	8 175 608	224 251 149	2 783 064	8 176 836	15 527 401	63 098 977
Imports	902 193	435 283	1 635 088	488 013	440	2 671 455
Stock change	-11 460	-337200	9 725	548 056	505 140	-108525
Exports	1 488 728	46 560 232	79 388	4 042 604	5 877 405	32 067 284
Domestic supply	7 577 614	177 789 000	4 348 488	5 170 302	10 155 576	33 594 623
Feed	4 030 000	134 272 497	2 920 300		9 271 300	7 484 702
Seed	236 880	500 310	197 910	181 054	32 370	2 537 869
Waste				769 568		
Food manufacture	3 161 567	33 358 641		704 843	542 284	
Food	149 167	3 672 880	1 230 279	3 270 425	309 622	23 565 666
Other uses		5 984 667		244 412		6 387

Table 11 Mexico 10-year averages for disposition and consumption (t) 1991–2000

Crop	Barley	Maize	Oat	Rice	Sorghum	Wheat
Production	509 854	17 529 267	78 019	383 662	5 067 165	3 566 361
Imports	283 864	3 367 108	70 994	442 113	3 436 623	1 889 012
Stock change	$-3\ 130$	-526000		-2857		-800
Exports	6	121 886	43	3 340	4 232	327 970
Domestic supply	790 582	20 248 489	148 970	819 578	8 499 556	5 126 604
Feed	279 659	4 772 954	121 491		8 298 521	486 829
Seed	14 181	434 450	2 425	5 425	27 959	88 494
Waste	21 552	2 210 783	1 252	34 428	173 076	419 805
Food manufacture	468 268	1 088 320		84 583		
Food	6 923	11 705 722	23 801	694 042		3 721 985
Other uses		36 259		1 232		410 504

Source: Food and Agriculture Organization of the United Nations (FAO).

Table 12 North America 10-year averages for disposition and consumption (t) 1991-2000

Crop	Barley	Maize	Oat	Rice	Sorghum	Wheat
Production	21 536 542	249 057 156	6 213 673	8 560 498	20 594 566	93 526 498
Imports	1 210 007	4 963 725	1 715 097	1 267 083	3 440 249	4 959 501
Stock change	-1 620	-797700	14 293	545 770	505 140	140 401
Exports	4 580 121	47 225 411	1 269 357	4 062 281	5 881 672	52 048 989
Domestic supply	18 164 809	205 997 770	6 673 705	6 311 072	18 658 283	46 577 413
Feed	13 298 445	145 270 034	4 982 547		17 572 972	12 038 531
Seed	654 331	963 570	353 465	186 479	60 329	3 741 463
Waste	26 812	2 461 966	8 442	812 790	173 076	427 155
Food manufacture	4 007 826	35 278 863		789 426	542 284	
Food	167 802	15 476 269	1 329 251	4 251 921	309 622	29 784 785
Other uses	9 593	6 547 061		270 587		586 492

# Acknowledgment

The editing contribution of K. Adams is gratefully acknowledged.

See also: Animal Feed. Barley: Agronomy; Harvesting, Storage, and Transport; Grading and Marketing; Milling and Processing. Chemicals for Grain Production and Protection. Food Safety through the Production Chain. Maize: Dry Milling; Wet Milling; Foods from Maize. Oats. Rice: Overview; Chinese Food Uses. Sorghum: Breeding and Agronomy; Harvest, Storage, and Transport; Utilization. Stored Grain: Handling from Farm to Storage Terminal. Wheat: Agronomy; Harvesting, Transport, and Storage; Grading and Segregation; Dry Milling; Marketing; Wet Milling; Grain Proteins and Flour Quality.

# **Further Reading**

Canadian International Grains Institute (1982) Grains and Oilseeds: Handling, Marketing, Processing, 3rd edn., 1006pp. Winnipeg, Manitoba: Canadian International Grains Institute.

Canada Grains Council (2002) Canada Grains Council Statistical Handbook, 166pp. Winnipeg, Manitoba: Canada Grains Council.

Espenshade EB, Jr (ed.) (2000) Goode's World Atlas, 250pp. Chicago: Rand McNally and Co.

Poehlman JM and Sleper DA (1995) Breeding Field Crops, 4th edn., 494pp. Ames, Iowa: Iowa State University

Slinkard AE and Knott DR (eds.) (1995) Harvest of Gold: The History of Field Crop Breeding in Canada, 367pp. Saskatoon, Saskatchewan: University Extension Press, University of Saskatchewan.

# **Relevant Websites**

http://apps.fao.org - Food and Agriculture Organization of the United Nations databases.

http://cabi.org – CAB International (CABI), Wallingford, UK, Crop Protection Compendium 2002 Edition.

http://cansim2.statcan.ca - Statistics Canada databases.

http://usda.gov - USA Dept. of Agriculture home page, the National Agricultural Statistics Service weblink on the same site provides detailed information.

http://www.fao.org - Website of the Food and Agriculture Organization of the United Nations. http://www.statcan.ca - Website of Statistics Canada.

# Oilseeds in North America

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# Introduction

The North American continent is a major production area for oilseeds and is a primary or secondary producer in the world of many of the oilseeds reviewed in this article. Major oilseeds grown in North America include linseed, rapeseed, soybean, and sunflower. These oilseeds are grown, to greater or lesser extent, in the countries comprising the North American continent (Canada, the USA, and Mexico).

Several of the major oilseeds currently grown in North America were introduced in the last few centuries (e.g., linseed, rapeseed, and soybean) whereas sunflower is native to this continent. All the current major oilseeds in North America have grown from small beginnings to their major field crop status today based on successful research and development in cultivar development, production practices, market development, and new uses development (see Canola: Genetics and Breeding; Agronomy; Harvest, Transport, and Storage; Processing. Soybean: Germplasm, Breeding, and Genetics; Agronomy; Grading and Marketing; Processing; Soymilk, Tofu, and Okara. Sunflower).

This article will review the area used annually to produce each of these major oilseeds and their annual production in each North American country and on the continent for a recent and continuous 10-year period. The 10-year mean disposition and consumption of each of these major oilseeds in each North American country and on the continent will also be reviewed. The percentage of world major oilseed crop production for each North American country and the ranking of the country, if it is in the top five countries of the world, will be noted. In general, all comments regarding crop disposition and consumption refer to averages of the 1990s, unless specifically stated otherwise. Area and production data for all crops relate to crops harvested for dry grain only. For disposition and consumption of commodities, the definitions of the listed elements are taken from the Food and Agriculture Organization (FAO) of the United Nations. Values given for production relate to the total domestic production. Imports cover all movements into the

country of the commodity. Stock changes indicate changes in stocks at all levels between the production and retail levels. A negative sign for stock quantities denotes a decrease in supply. Exports cover all movements by the crop commodity out of the country. Domestic supply is defined as: (production + imports) - (exports + changes in stocks).Feed is the amount of the commodity and of edible components of the commodity fed to livestock. Seed comprises the amount of commodity used for reproduction. Waste includes the amount of crop commodity lost in processing, storage, and transportation. Food manufacture is the amount of commodity used for manufacture of processed commodities that cannot be converted back to their originating primary commodities. Food comprises the amounts of the commodity not detailed otherwise in the balance sheet, available for human consumption. Other uses comprise quantities of the commodity used for manufacture of nonfood products.

# Linseed (Flax)

#### Introduction

Linseed (*Linum usitatissimum*), also known as flax in North America, is one of the earliest cultivated field crops, initially grown for its fiber, but grown, in the last two centuries, for its oil. It is a spring annual adapted to a wide range of soil and climatic conditions in the warm temperate zones of the northern hemisphere.

Linseed in North America is used primarily to produce industrial oil and animal feed meal. Linseed oil is a drying oil used in paints and varnishes and for the manufacture of linoleum flooring. Whole linseed is fed to poultry to produce omega-3 fatty acid enriched eggs. Linseed is also used in food products (breads and breakfast cereals). Small quantities of linseed are used for planting seed.

## **Area Harvested**

Canada has harvested linseed from ~654 000 ha (1.3% of Canadian arable land) annually (**Table 1**). The area of linseed harvested in Canada has varied widely during the 1990s, from a low of ~253 000 ha to a high of 860 000 ha (**Table 1**). The vast majority of linseed production area in Canada is in the Prairie Provinces (Alberta, Saskatchewan, and Manitoba).

The USA has harvested linseed from  $\sim$ 110 000 ha (0.06% of American arable land) annually (Table 2). The area of linseed harvested in the USA has varied widely during the 1990s, from a low of  $\sim$ 37 000 ha to

a high of 234 000 ha (**Table 2**). The vast majority of linseed production area in the USA is in the north-central states of North and South Dakota, Minnesota, and Montana.

Linseed is not grown in Mexico. The climate of Mexico is not suitable for the production of linseed.

For the entire North American continent, linseed was harvested from an average 764 000 ha (0.3% of North American arable land) annually (Table 4). The area harvested has varied widely during the 1990s (Table 4). Canada's average linseed harvested area was 85.6% and the USA 14.4% of the total North American linseed harvested area.

#### **Production**

Canada has produced on an average 829 000 t of linseed annually (Table 5). The production of linseed in Canada has varied widely during the 1990s, from a low of 337 000 t to a high of 1.1 million tons (Mt) (Table 5), paralleling the widely varying production area for linseed harvested in Canada as shown in Table 1. The average yield of linseed in Canada during the 1990s has been 1.3 t ha<sup>-1</sup>.

The USA has produced on an average 134 000 t of linseed annually (Table 6). The production of linseed in the USA has varied widely during the 1990s, from a low of 41 000 t to a high of 291 000 t (Table 6), paralleling the widely varying production area for linseed harvested in the USA shown in Table 2. The average yield of linseed in the USA has been 1.2 t ha<sup>-1</sup>, similar to the Canadian average yield.

For the entire North American continent, average annual linseed production was 963 000 t annually (Table 8). There has been a threefold variation in year-to-year production of linseed, with a trend towards increased production in recent years. In terms of North American production proportions, Canada has produced an average 86.1% and the USA 13.9% of the linseed produced in North America. In terms of global linseed production, North America has produced on an average, 40.4% of all the linseed produced in the world. The share of world linseed production for Canada and the USA is 34.8% and 5.6%, respectively. Canada is the top linseed producer in the world and the USA ranks in the top five countries in the world for linseed production.

## **Disposition and Consumption**

Of the average annual 812 000 t of linseed produced in Canada and the 13 000 t imported, 178 000 t were

used domestically and 675 000 t were exported (Table 9). Of the linseed used domestically in Canada, 74% was used for food manufacture and other uses combined (seed crushed for industrial oil and meal production for feed), and 17% was used for planting seed (Table 9). Small amounts of linseed (<5%) were used for food. Canada is a major exporter of linseed to the world (675 000 t), with significant exports to Europe, the USA, Japan, and South Korea. Canada currently supplies nearly 60% of the linseed used in the USA.

Of the average annual 113 000 t of linseed produced in the USA and the over 170 000 t of linseed imported, 279 000 t were used domestically and 4000 t were exported (Table 10). Approximately 96% of the linseed used domestically in the USA was used for food manufacture and other uses combined. Small quantities of linseed were used for planting seed and food (<5%) (Table 10).

The average 1000 t of linseed imported into Mexico were used domestically (Table 11). All of the domestically used linseed in Mexico was used for food manufacture and other uses combined.

Of the average annual 925 000 t of linseed produced in North America and the 184 000 t imported, 458 000 t were used domestically and 679 000 t were exported (Table 12). Approximately 88% of the linseed used domestically in North America was used for food manufacture and other uses combined (Table 12). Small quantities were used for planting seed (7%) and food (<5%).

## Rapeseed (Canola)

#### Introduction

Rapeseed (Brassica napus and B. rapa) was domesticated as an oilseed crop in Europe in the early Middle Ages. Because of their ability to germinate and grow at low temperatures, the oilseed *Brassicas* are one of the few oil crops that can be grown in the temperate regions of the world. There are both spring and winter growth habit forms, but it is the spring form that predominates in North American production. The oil and meal quality of rapeseed has been dramatically improved in recent decades and a new name for this quality improved form of rapeseed, "canola," has been widely adopted in most of the world. The phrase "canola-quality rapeseed" will be used for this crop.

Canola-quality rapeseed is primarily used to produce edible vegetable oil and meal for animal feed. Small quantities of canola-quality rapeseed are used directly for feed and planting seed.

#### Area Harvested

Canada has harvested canola-quality rapeseed from ~4.6 million hectares (Mha) (9.2% of Canadian arable land) annually (Table 1). The area of canola-quality rapeseed harvested in Canada has remained fairly constant during the 1990s (Table 1). The vast majority of canola-quality rapeseed production area in Canada is in the Prairie Provinces (Alberta, Saskatchewan, and Manitoba).

The USA has harvested canola-quality rapeseed from ~293 000 ha (0.2% of American arable land) annually (Table 2). The area of canola-quality rapeseed harvested in the USA has increased more than tenfold during the 1990s, beginning the decade at 53 000 ha and ending at 590 000 ha (Table 2). The vast majority of canola-quality rapeseed production area in the USA is in the northern Plains States (Minnesota and the Dakotas).

Mexico is a very small canola-quality rapeseed producer harvesting canola-quality rapeseed from only 3000 ha (0.1% of Mexican arable land) annually (Table 3). The area of canola-quality rapeseed harvested in Mexico has varied widely during the 1990s, from a low of 549 ha to a high of 10 000 ha, with a trend towards increased harvested area (Table 3). This variation in harvested area is typical for any small volume crop. The vast majority of canola-quality rapeseed production area in Mexico is in the central area of the country.

For the entire North American continent, canolaquality rapeseed was harvested from an average 4.9 Mha (1.9% of North American arable land) annually during the 1990s (Table 4). The area harvested has varied by a factor of 2 during the 1990s, from a low of 3.1 Mha to a high of 6.0 Mha (Table 4). Canada's average canola-quality rapeseed harvested area was 94.0%, the USA 6.0%, and Mexico <1.0% of the total North American canola-quality rapeseed harvested area.

#### **Production**

Canada has produced on an average 6.3 Mt of canolaquality rapeseed annually (Table 5). The production of canola-quality rapeseed in Canada has varied by a factor of 2 during the 1990s (Table 5), paralleling the variation in harvested area for canola-quality rapeseed in Canada shown in Table 1. The average yield of canola-quality rapeseed in Canada has been  $1.4 \, \text{t ha}^{-1}$ .

The USA has produced on an average 443 000 t of canola-quality rapeseed annually (Table 6). Canolaquality rapeseed production has increased by more than tenfold in the USA, from under 72 000 t at the beginning of the 1990s to over 908 000 t at the end

of the 1990s (**Table 6**). The average yield of canola-quality rapeseed in the USA has been 1.5 t ha<sup>-1</sup>, similar to the Canadian average yield.

Mexico has produced on an average 4000 t of canola-quality rapeseed annually (Table 7). The production of canola-quality rapeseed in Mexico has varied by a factor of almost 30 during the 1990s, from a low of 505 t to a high of 14 000 t (Table 7). This variation in production is typical for any small volume crop. The average yield of canola-quality rapeseed in Mexico has been 1.3 t ha<sup>-1</sup>, similar to yields for Canada and the USA.

For the entire North American continent, average annual canola-quality rapeseed production was 6.8 Mt (Table 8). There has been year-to-year variation in production, with a trend towards increased production, mostly in the USA, in recent years. In terms of North American production proportions, Canada has produced an average 93.4%, the USA 6.6%, and Mexico <1.0% of the canola-quality rapeseed produced in North America. In terms of global canola-quality rapeseed production, North America has produced on an average, 20.1% of all the canola-quality rapeseed produced in the world. The share of world canola-quality rapeseed for Canada, the USA, and Mexico is 18.8%, 1.3%, and <1.0%, respectively. Canada ranks in the top five countries of the world for canola-quality rapeseed production.

## **Disposition and Consumption**

Of the average annual 6.2 Mt of canola-quality rapeseed produced in Canada and the 106 000 t imported, 3.1 Mt was used domestically and 3.2 Mt was exported (Table 9). Over 86% of the canola-quality rapeseed used domestically in Canada was used for food manufacture, 6% for feed, and small amounts were used for planting seed (<5%) (Table 9).

Of the average annual 307 000 t of canola-quality rapeseed produced in the USA and the 292 000 t of canola-quality rapeseed imported in each year of the 1990s, 477 000 t were used domestically and 122 000 t were exported (Table 10). Approximately 88% of the canola-quality rapeseed used domestically in the USA was used for food manufacture, and 8% for other uses (Table 10). Small amounts were used for planting seed (<5%).

Of the average annual 3000 t of canola-quality rapeseed produced in Mexico and the  $583\,000$  t of canola-quality rapeseed imported,  $\sim 586\,000$  t were used domestically and 90 t were exported (Table 11). Approximately 95% of the canola-quality rapeseed used domestically in Mexico was used for food manufacture with amounts used for planting seed (<5%) (Table 11).

Of the average 6.5 Mt of canola-quality rapeseed produced in North America and the 981 000 t imported, 4.1 Mt were used domestically and 3.3 Mt were exported (Table 12). Over 88% of the canola-quality rapeseed used domestically in North America was used for food manufacture (Table 12). Small amounts were used for feed, planting seed, and other uses (<5%).

# Soybean

## Introduction

Soybean, (Glycine max), was domesticated as a forage crop in China  $\sim 2500\,\mathrm{BC}$ . It became a commercially viable oilseed crop in the 1940s due to the development of shatter and disease-resistant new cultivars. There are only spring growth habit forms. Soybeans are grown widely throughout the world for their high-protein meal and oil.

Soybean is used primarily for food manufacture (edible vegetable oil and meal for animal feed) (Table 9). Small quantities of soybeans are also used directly as animal feed, food ("tofu" and soy sauce), and planting seed.

#### **Area Harvested**

Canada has harvested soybean from  $\sim 902\,000\,\text{ha}$  (1.8% of Canadian arable land) annually (**Table 1**). The area of soybean harvested in Canada has varied by a factor of 2 during the 1990s, from  $\sim 623\,000\,\text{ha}$  to over 1.1 Mha (**Table 1**). The majority of soybean production area in Canada is in southwestern Ontario.

The USA has harvested soybean from ~26.7 Mha (14.6% of American arable land) annually (Table 2). The area of soybean harvested in the USA has increased during the 1990s, beginning the decade at 23.6 Mha and ending at ~29.5 Mha (Table 2). The vast majority of soybean production area in the USA is in the Middle American "corn belt" states of Iowa, Illinois, Nebraska, Minnesota, Indiana, Kansas, Wisconsin, and South Dakota and the lower Mississippi Delta.

Mexico is a very small soybean producer, harvesting soybean from only 147 000 ha (0.6% of Mexican arable land) annually (Table 3). The area of soybean harvested in Mexico has varied widely during the 1990s, from a low of 49 000 ha to a high of 323 000 ha, with a trend towards decreased harvested area during the 1990s (Table 3). This variation in harvested area is typical for any small volume crop. Soybean production occurs in central Mexico.

For the entire North American continent, soybean was harvested from an average 27.7 Mha (10.8% of North American arable land) annually (**Table 4**). The area harvested has varied slightly, from a low of 24.0 Mha to a high of 30.7 Mha (**Table 4**). Canada's average soybean harvested area was 3.3%, the USA 96.2%, and Mexico <1.0% of the North American harvested area.

#### **Production**

Canada has produced on an average 2.3 Mt of soybean annually (**Table 5**). The production of soybean in Canada has varied by a factor of 2 during the 1990s (**Table 5**), paralleling the variation in harvested area for soybean in Canada shown in **Table 1**. The average yield of soybean in Canada has been 2.5 t ha<sup>-1</sup>.

The USA has produced on an average 67.7 Mt of soybean annually (**Table 6**). Soybean production has increased by more than 30% in the USA during the 1990s, from 59.6 Mt at the beginning of the 1990s to 78.7 Mt at the end of the 1990s (**Table 6**). The average yield of soybean in the USA has been 2.5 t ha<sup>-1</sup>, similar to the Canadian average yield.

Mexico has produced on an average 255 000 t of soybean annually (Table 7). The production of soybean in Mexico has varied by a factor of 10 during the 1990s, from a low of 56 000 t to a high of 594 000 t (Table 7). This variation in production is typical for any medium volume crop. The average yield of soybean in Mexico during the 1990s has been 1.7 t ha<sup>-1</sup>, lower than the soybean yields seen for Canada and the USA. Soybeans are grown in Mexico under water limiting rain-fed conditions.

For the entire North American continent, average annual soybean production was 70.2 Mt (Table 8). There has been an year-to-year variation in production, with a trend towards increased production, mostly in the USA, in recent years. In terms of North American production proportions, Canada has produced an average 3.2%, the USA 96.4%, and Mexico <1.0% of the soybean produced in North America. In terms of global soybean production, North America has produced on an average, 49.3% of the soybean produced in the world. The share of world soybean production for Canada, the USA, and Mexico is 1.6%, 47.5%, and <1.0%, respectively. The USA is the top soybean producer of the world.

#### **Disposition and Consumption**

Of the average annual 2.2 Mt of soybean produced and 188 000 t imported by Canada, 1.8 Mt was used

domestically and 554 000 t was exported (**Table 9**). Almost 75% of the soybean used domestically in Canada was used for food manufacture (**Table 9**). Another 20% of the soybean domestic supply was used as animal feed. Minor amounts were used for planting seed and food (<5%).

Of the average annual 65.2 Mt of soybean produced in the USA and the 151000 t of soybean imported, 43.1 Mt were used domestically and 22.1 Mt were exported (Table 10). Approximately 88% of the soybean used domestically in the USA was used for food manufacture (Table 10). Small amounts were used for feed, planting seed, and food (<5%).

Of the average annual 315 000 t of soybean produced in Mexico and the 2.9 Mt of soybean imported, ~3.2 Mt were used domestically and 676 t were exported (Table 11). Approximately 77% of the soybean used domestically in Mexico was used for food manufacture (Table 11). Another 14% of the soybean domestic supply was used as animal feed and 6% was used in other uses. Minor amounts were used for planting seed and food (<5%).

Of the average annual 67.8 Mt of soybean produced in North America and the 3.2 Mt imported, 48.1 Mt were used domestically, 22.7 Mt were exported (Table 12). Over 86% of the soybean used domestically in North America was used for food manufacture (Table 12). Small amounts were used for feed, seed, food, and other uses (<5%).

## Sunflower

#### Introduction

Sunflower, (*Helianthus annuus*), was domesticated as a food crop in North America, perhaps as early as 3000 BC. Sunflower was introduced to Europe in the 1600s and was successfully developed as an oil crop in Russia in the early 1800s. Russian plant breeders were able to increase the oil content in sunflower seed from less than 30% to over 50%, the major factor permitting the development of sunflower as an oil crop for the temperate areas of the world.

Sunflower is used primarily for food manufacture (edible vegetable oil and meal for animal feed) (Table 10). Moderate quantities of sunflower are used for animal feed and food (confectionary and bakery products) (Table 10). Small amounts are used for planting seed (<5%).

#### **Area Harvested**

Canada has harvested sunflower from  $\sim$ 62 000 ha (0.1% of Canadian arable land) annually (Table 1).

The area of sunflower harvested in Canada has varied by a factor of 2 during the 1990s, from a low of 35 000 ha to a high of 83 000 ha (**Table 1**). The vast majority of sunflower production area in Canada is in the Prairie Province of Manitoba.

The USA has harvested sunflower from ∼1.2 Mha (0.6% of American arable land) annually (Table 2). The area of sunflower harvested in the USA has remained fairly constant during the 1990s (Table 2). The vast majority of sunflower production area in the USA is in the central Plains States (from Texas to the Dakotas, with North Dakota being the predominant production area State).

Mexico is a minor producer of sunflower, harvesting sunflower from only 968 ha (0.004% of Mexican arable land) annually (Table 3). The area of sunflower harvested in Mexico has varied widely during the 1990s, from a low of 100 ha to a high of 3000 ha, with no apparent trend (Table 3). This variation in harvested area is typical for any small volume crop. The greater part of the sunflower production area is in central Mexico.

For the entire North American continent, sunflower was harvested from an average 1.2 Mha (0.5% of North American land) annually (Table 4). The area harvested has varied by 40% during the 1990s, from a low of 878 000 ha to a high of 1.5 Mha (Table 4). The average sunflower harvested area for Canada, the USA, and Mexico was 5.1%, 94.8%, and <1.0% of the total North American sunflower harvested area, respectively.

#### **Production**

Canada has produced on an average 90 000 t of sunflower annually (Table 5). The production of sunflower in Canada has varied by a factor of 2 during the 1990s (Table 5), paralleling the variation in harvested area for sunflower in Canada shown in Table 1. The average yield of sunflower in Canada has been  $1.4 \,\mathrm{t}\,\mathrm{ha}^{-1}$ .

The USA has produced on an average 1.7 Mt of sunflower annually (Table 6). Sunflower production has varied by a factor of 2 in the USA during the 1990s, from  $\sim$ 1.2 Mt to over 2.4 Mt (Table 6). There is no clear trend of production, however. The average yield of sunflower in the USA has been 1.5 t ha<sup>-1</sup>, similar to the Canadian average yield.

Mexico has produced on an average 933t of sunflower annually (Table 7). The production of sunflower in Mexico has varied by a factor of 35 during the 1990s, from a low of 70 t to a high of over 2000 t (Table 7). This variation in production is typical for any small volume crop. The average yield

of sunflower in Mexico has been 1.0 t ha<sup>-1</sup>, lower than the yields for Canada and the USA. Sunflower is produced in Mexico under water limiting rain-fed conditions.

For the entire North American continent, average annual sunflower production was 1.8 Mt (Table 8). There has been wide year-to-year variations in production, with no clear trend emerging in production. In terms of North American production proportions, Canada has produced an average 5.0%, the USA 94.9%, and Mexico <1.0% of the sunflower produced in North America. In terms of world sunflower production, North America has produced on an average, 7.6%, of all the sunflowers produced in the world. Canada's and Mexico's share of world sunflower production is <1.0% while the USA share of world sunflower production is 7.2%. The USA is in the top five countries in the world for sunflower production.

# **Disposition and Consumption**

Of the average annual 93 000 t of sunflower produced in Canada and the 16 000 t of sunflowers imported, 62 000 t were used domestically and 50 000 t were exported (Table 9). Over 76% of the sunflowers used domestically in Canada was utilized for food manufacture (Table 9). Another 22% was used directly as animal feed and a small amount was used as planting seed (<5%) (Table 9).

Of the average annual 1.7 Mt of sunflower produced in the USA and the 40 000 t of sunflower imported, 1.6 Mt were used domestically and 170 000 t were exported (Table 10). Approximately 62% of the sunflower used domestically in the USA was utilized for food manufacture (Table 10). Another 21% was used directly as animal feed and 16% as food (Table 10). A small amount was used for planting seed (<5%).

Of the average annual 879 t of sunflower produced in Mexico and the 99 000 t of sunflower imported,  $\sim$ 102 000 t were used domestically and 105 t were exported (Table 11). Approximately 96% of the sunflower used domestically in Mexico was used for food manufacture and a small amount was used for food (<5%) (**Table 11**).

Of the average annual 1.8 Mt of sunflower produced in North America and the 155 000 t imported, 1.7 Mt were used domestically and 221 000 t were exported (Table 12). Over 64% of the sunflower used domestically in North America was used for food manufacture (Table 12). Another 20% was used directly as animal feed and 15% as food (Table 12). A small amount was used for planting seed (<5%).

# **Data Tables**

Table 1 Area harvested (ha) for major oilseeds grown in Canada 1992-2001

Year	Linseed	Rapeseed	Soybean	Sunflower
1992	252 900	3 045 300	622 900	51 000
1993	505 800	4 103 500	719 600	77 000
1994	720 300	5 765 600	820 100	83 000
1995	860 000	5 273 000	824 000	44 500
1996	575 000	3 451 000	856 200	35 200
1997	736 500	4 870 000	1 059 600	50 600
1998	857 900	5 428 800	980 100	68 800
1999	776 900	5 564 300	1 004 000	78 900
2000	590 900	4 859 200	1 060 700	68 800
2001	661 700	3 765 000	1 068 900	66 800
10-year mean	653 790	4 612 570	901 610	62 460

Source: Food and Agriculture Organization of the United Nations (FAO).

Table 2 Area harvested (ha) for major oilseeds grown in USA 1992-2001

Year	Linseed	Rapeseed	Soybean	Sunflower
1992	66 770	53 000	23 566 000	826 780
1993	77 300	78 150	23 191 000	1 006 060
1994	69 200	140 300	24 608 000	1 388 090
1995	59 490	174 580	24 906 000	1 363 000
1996	37 230	141 910	25 636 000	1 032 000
1997	59 100	283 080	27 967 000	1 129 900
1998	133 140	437 350	28 506 800	1 413 200
1999	154 190	424 280	29 318 000	1 392 500
2000	209 225	607 810	29 302 790	1 071 210
2001	233 910	590 070	29 532 250	1 033 980
10-year mean	109 956	293 053	26 653 384	1 165 672

Source: Food and Agriculture Organization of the United Nations (FAO).

Table 3 Area harvested (ha) for major oilseeds grown in Mexico 1992-2001

Year	Rapeseed	Soybean	Sunflower
1992	549	322 793	273
1993	621	237 765	2 910
1994	1 801	288 499	585
1995	971	134 396	212
1996	1 135	49 064	344
1997	599	122 548	2 424
1998	2 000	94 065	809
1999	2 000	81 159	1 175
2000	10 000	69 969	100
2001	10 000	73 726	850
10-year mean	2 968	147 398	968

Table 4 Area harvested (ha) for oilseeds grown in North America 1992–2001

Year	Linseed	Rapeseed	Soybean	Sunflower
1992	319 670	3 098 849	24 511 693	878 053
1993	583 100	4 182 271	24 148 365	1 085 970
1994	789 500	5 907 701	25 716 599	1 471 675
1995	919 490	5 448 551	25 864 396	1 407 712
1996	612 230	3 594 045	26 541 264	1 067 544
1997	795 600	5 153 679	29 149 148	1 182 924
1998	991 040	5 868 150	29 580 965	1 482 809
1999	931 090	5 990 580	30 403 159	1 472 575
2000	800 125	5 477 010	30 433 459	1 140 110
2001	895 610	4 365 070	30 674 876	1 101 630
10-year mean	763 746	4 908 591	27 702 392	1 229 100

Table 5 Production (t) for major oilseeds grown in Canada 1992–2001

Year	Linseed	Rapeseed	Soybean	Sunflower
1992	337 000	3 872 400	1 455 300	64 800
1993	627 400	5 479 500	1 851 300	78 500
1994	960 100	7 233 000	2 250 700	117 000
1995	1 105 000	6 436 400	2 293 000	66 200
1996	851 000	5 062 000	2 170 000	54 900
1997	895 400	6 393 100	2 737 700	65 100
1998	1 080 900	7 643 300	2 736 600	111 800
1999	1 022 400	8 798 300	2 780 900	121 900
2000	693 400	7 205 300	2 703 000	119 300
2001	715 000	4 926 300	1 635 200	103 800
10-year mean	828 760	6 304 960	2 261 370	90 330

Source: Food and Agriculture Organization of the United Nations (FAO).

Table 6 Production (t) for major oilseeds grown in USA 1992-2001

Year	Linseed	Rapeseed	Soybean	Sunflower
1992	83 500	71 890	59 612 000	1 163 000
1993	88 400	117 890	50 886 000	1 166 670
1994	74 200	208 670	68 445 000	2 193 660
1995	56 160	250 060	59 174 000	1 818 600
1996	40 690	218 690	64 782 000	1 614 500
1997	61 470	415 640	73 177 000	1 667 800
1998	170 400	709 490	74 599 000	2 392 000
1999	200 150	620 850	72 223 000	1 969 000
2000	272 550	909 030	75 055 288	1 607 730
2001	290 970	908 350	78 671 472	1 550 720
10-year mean	133 849	443 056	67 662 476	1 714 368

 Table 7
 Production (t) for major oilseeds grown in Mexico 1992–2001

Year	Rapeseed	Soybean	Sunflower	
1992	505	593 540	208	
1993	669	497 566	2 423	
1994	2 051	522 583	1 031	
1995	1 006	189 774	376	
1996	1 342	56 074	309	
1997	680	184 526	2 464	
1998	2 000	150 296	615	
1999	3 000	132 824	1 162	
2000	14 000	102 314	70	
2001	14 000	121 671	673	
10-year mean	3 925	255 117	933	

Table 8 Production (t) for major oilseeds grown in North America 1992–2001

Year	Linseed	Rapeseed	Soybean	Sunflower
1992	420 500	3 944 795	61 660 840	1 228 008
1993	715 800	5 598 059	53 234 866	1 247 593
1994	1 034 300	7 443 721	71 218 283	2 311 691
1995	1 161 160	6 687 466	61 656 774	1 885 176
1996	891 690	5 282 032	67 008 074	1 669 709
1997	956 870	6 809 420	76 099 226	1 735 364
1998	1 251 300	8 354 790	77 485 896	2 504 415
1999	1 222 550	9 422 150	75 136 724	2 092 062
2000	965 950	8 128 330	77 860 602	1 727 100
2001	1 005 970	5 848 650	80 428 343	1 655 193
10-year mean	962 609	6 751 941	70 178 963	1 805 631

Source: Food and Agriculture Organization of the United Nations (FAO).

**Table 9** Canada 10-year averages for disposition and consumption (t) 1991–2000

Crop	Linseed	Rapeseed	Soybean	Sunflower
Production	812 020	6 228 952	2 243 850	93 410
Imports	12 780	105 724	188 434	15 529
Stock change	28 000	-140200	-80000	3 070
Exports	674 593	3 176 478	554 292	50 459
Domestic supply	178 207	3 017 997	1 797 993	61 550
Feed		174 681	358 133	13 734
Seed	29 760	37 439	61 240	815
Waste	26 539	186 538	22 380	
Food manufacture	74 220	2 619 339	1 340 900	47 000
Food	1 383		15 339	
Other uses	58 285			

**Table 10** United States 10-year averages for disposition and consumption (t) 1991–2000

Crop	Linseed	Rapeseed	Soybean	Sunflower
Production	113 252	307 288	65 201 830	1 723 196
Imports	169 878	291 628	151 066	40 252
Stock change			-100000	-39 774
Exports	4 200	121 526	22 105 156	169 959
Domestic supply	278 930	477 390	43 147 739	1 553 715
Feed			67 700	331 250
Seed	4 200	2 481	2 087 440	14 565
Waste	4 000	14 164	2 956 405	
Food manufacture	151 000	421 863	38 026 200	957 900
Food	2 100		9 997	250 000
Other uses	117 630	38 882		

Table 11 Mexico 10-year averages for disposition and consumption (t) 1991-2000

Crop	Linseed	Rapeseed	Soybean	Sunflower
Production		2 581	315 447	879
Imports	1 000	583 424	2 849 675	99 381
Stock change			3 000	1 500
Exports		90	676	105
Domestic supply	1 000	585 914	3 167 445	101 655
Feed			459 158	
Seed		30	6 341	
Waste		29 296	96 287	
Food manufacture	544	556 588	2 425 117	98 170
Food			542	3 485
Other uses	456		180 000	

Source: Food and Agriculture Organization of the United Nations (FAO).

Table 12 North America 10-year averages for disposition and consumption (t) 1991-2000

Crop	Linseed	Rapeseed	Soybean	Sunflower
Production	925 272	6 538 821	67 761 127	1 817 485
Imports	183 658	980 776	3 189 175	155 162
Stock change	28 000	-140200	-177 000	-35 204
Exports	678 793	3 298 094	22 660 124	220 523
Domestic supply	458 137	4 081 301	48 113 177	1 716 920
Feed		174 681	884 991	344 984
Seed	33 960	39 950	2 155 021	15 380
Waste	30 539	229 998	3 075 072	
Food manufacture	225 764	3 597 790	41 792 217	1 103 070
Food	3 483		25 878	253 485
Other uses	176 371	38 882	180 000	

# **Acknowledgment**

The editing contribution of K. Adams is gratefully acknowledged.

See also: Canola: Processing. Oil from Rice and Maize. Oilseeds, Overview. Soybean: Processing. Sunflower.

# **Further Reading**

Canadian International Grains Institute (1982) Grains and Oilseeds: Handling, Marketing, Processing, 3rd edn., pp. 1006. Winnipeg, Manitoba: Canadian International Grains Institute.

Canada Grains Council (2002) Canada Grains Council Statistical Handbook, pp. 166. Winnipeg, Manitoba: Canada Grains Council.

Espenshade EB, Jr (ed.) (2000) Goode's World Atlas, pp. 250. Chicago: Rand McNally and Co.

Slinkard AE and Knott DR (eds.) (1995) Harvest of Gold: The History of Field Crop Breeding in Canada, pp. 367. Saskatoon, Saskatchewan: University Extension Press, University of Saskatchewan.

Robbelen G, Downey RK, and Ashri A (eds.) (1989) Oil Crops of the World, pp. 554. Toronto: McGraw-Hill.

Poehlman JM and Sleper DA (1995) *Breeding Field Crops*, 4th edn., pp. 494. Ames, Iowa: Iowa State University Press.

# **Relevant Websites**

http://apps.fao.org — Food and Agriculture Organization of the United Nations databases.

http://www.cabi.org — CAB International (CABI), Wallingford, UK, crop protection compendium 2002 edition.

http://cansim2.statcan.ca — Statistics Canada databases.

http://www.usda.gov - USA Dept. of Agriculture home page.

http://usda.gov — USA, Dept. of Agriculture, National Agricultural Statistics Service.

http://www.canola-council.org - Website of the Canola Council of Canada.

http://www.fao.org — Website of the Food and Agriculture Organization of the United Nations.

http://www.flaxcouncil.ca — Website of the Flax Council of Canada.

http://www.statcan.ca — Website of Statistics Canada.

# **Oceania**

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# Introduction

None of the grains of economic significance are native to the Oceania region (Australia, New Zealand, and the islands of the southern Pacific Ocean). It is only a little over two hundred years since the first European colonists found themselves in eastern Australia (the Sydney of 1788), trying to grow wheat under conditions in the southern hemisphere that were completely different from those of their homeland, England. The first wheat crop, grown at the current site of Sydney's Botanic Gardens, yielded little more than the few bushels that were sown. In contrast to those early days, Australia is now a major exporter of wheat and of many other grains (Table 1).

New Zealand was colonized at a time and under circumstances similar to those of Australia. Grain production in New Zealand is now also significant, but generally not to the extent of being involved significantly in grain export. The many nations of the Pacific islands are not significant producers of grain species. Instead, they are importers of grain and flour products. For these reasons, this article concentrates on grain production in Australia.

## **Historical Perspective**

In its early decades, the Australian grain-growing industry fluctuated from under-production through self-sufficiency to occasional periods of excess (Table 2). Wheat was the main grain grown, being needed for bread making. Farmers had to contest dry conditions, poor soil in some of the areas cultivated initially, attack by the rust pathogen, a reversal of seasons from their previous experience, and varieties that were poorly adapted to the new continent. This last problem was realized in the latter half of the nineteenth century by far-sighted farmers, by staff of "experimental farms," and by an amateur, William Farrer, whose profession as a surveyor took him into the heart of the wheat-growing regions. Whereas farmers attempted to develop improved varieties by selecting seed from plants that looked better than the rest of the crop, Farrer set about the (then) new technique of cross-breeding to increase the genetic diversity from which to select better genotypes.

An example of the selection method is given in **Table 2**; the extension of wheat growing into drier

 Table 1
 Production of the range of grain species in Australia (as 5 year averages of recent years)

Grain species	Production in (kt)	Proportion exported	Domestic and export uses
Cereals			
Wheat	22 400	75%	50% domestic use is food, mainly in bread; 40% domestic use is animal feed, also for starch-gluten manufacture
Barley	6500	70%, including 10% as malt (grain equivalent)	Most is used domestically as animal feed; 7% domestic use for malting and food
Oats	1400	15%	10% domestic use is for human food; 90% domestically for feed
Sorghum	1800	22%	All domestic use is as animal feed
Rice	1400	50%	Domestic and export for food as a range of rice types
	(as milled grain)		
Triticale	760	2%	All domestic use is as animal feed
Maize	360	10%	25% domestically used for food; otherwise feed
Oilseeds			
Canola	1700	77%	Oil is pressed from the grain, and the residue is used for animal feed
Cottonseed	1040	75%	As for canola
Sunflower	120		As for canola
Pulses			
Lupins	1500	56%	Used for feed domestically
Field peas	400	80%	Used overseas for food as splits and flour
Chick peas	200	95%	Food forms overseas include dhal, besan, hummus, and felafel
Faba beans	250		Food uses overseas include snacks and felafel
Lentils	120		Food uses
Mung beans	25		Exports to Asia and Africa used as splits and sprouts

Data from Australian Bureau of Agricultural and Resource Economics, Canberra, Australia. www.abareconomics.com.

regions was attributed to the selection of "Purple Straw" (an earlier-maturing wheat) from a field of "Red Straw" by a South Australian farmer in ~1860, just before the introduction of cross-breeding. The recent use of cross-breeding to produce new wheat varieties is shown in Figure 1. In nearly all cases, crosses have been made, but there are still a few new varieties that have arisen as selections from an already existing variety, such as the selection of "Meering" from the former wheat "Condor," which was presumably released as a mixture of a few genotypes.

Farrer's use of cross-breeding was partly aimed at developing wheats with grain quality suited for export to England by combining the dough quality of Canadian Fife wheats with the drought tolerance of wheats introduced from countries such as India, Palestine, and South Africa. This vision led him to enlist the help of the agricultural chemist, Frederick Guthrie, to evaluate the milling and baking quality of the small samples of his many cross-bred lines. Thus, started the development of small-scale test methods and (probably) the world's first breeder—chemist association, a combination that is now an integral part of the improvement of grain quality for all species. Subsequent years brought the excess

production of wheat that justified Farrer's vision of export to England, largely as a result of the development of new wheat varieties (Table 2). These contributions by Farrer were recognized by the issue of \$2 bank note when Australia changed to decimal currency in 1966 (Figure 2).

However, the greatest expansion in wheat production has occurred in the second half of the twentieth century, with production expanding from ~2 million tons (Mt) in 1940 to a high of almost 25 Mt in 1999/2000. Nevertheless, production has fluctuated on occasions due to drought. As a recent example, the high of 2000 contrasts with the drought year of 2002/3, when the national wheat crop was only a little over 10 Mt, less than half of the crops either side (~24 Mt in both 2001/2 and 2003/4). Similar reductions in production were experienced for all grains in the 2002/3 drought year.

# **Diversification of Markets for Australian Grains**

Further improvements in the quality of wheat varieties and in management practices through the twentieth century extended opportunities for grain

Table 2 Historical developments in the Australian wheat industry

Period of foun	dations 1788–92
1788	Eight acres are sown to wheat at Farm Cove, Sydney.
1790	James Ruse grows "bearded" wheat on a few acres at Rose Hill, Parramatta.
1792	About 200 acres cultivated to wheat in the colony.
. A wheat mark	ket is established 1793–1824
1793	The colony is self-sufficient in wheat and maize.
1803	Rust destroys wheat crops in Sydney's Dundas Valley.
1804	Abundant yields of "Common Brown" wheat in the Sydney district bring self-sufficiency again, but prices are low
1810+	"White Lammas" and "Red Lammas" wheats from England and Scotland are cultivated, mainly in coastal regions  "White Lammas" was late-maturing and poor in baking quality.
1811	James Ruse makes himself a plow; previously most tillage was by hoe.
1822	Various wheats are introduced by the newly established Agricultural Society of New South Wales from England
1022	South Africa, India, and Egypt.
II. Period of ins 1850	ufficient expansion 1825–55  "Red Straw" and "White Essex" (a Lammas type) are introduced.
V Period of de	clining yield 1855–96
1860+	South Australia's climate and geography favor quality-wheat production, and grain is exported to England.
1860	"Purple Straw" (an early-maturing wheat) is selected, probably from a field of "Red Straw" by a South Australiar farmer. This selection starts an extension of wheat growing into drier regions.
1000	
1880	Introduction of Ganz steel roller mills from Hungary alters quality requirements.
1881	"Du Toit's" wheat, early-maturing and rust-resisting, is introduced from South Africa by Dr. Schomburgh, Directo
	of the Adelaide Botanic Gardens. From this introduction, James Ward, of Port Pirie, selects a rust-resisting
	high-yielding wheat named "Ward's Prolific."
1882	Correspondence with "The Australasian" newspaper prompts the English-born surveyor W. J. Farrer to make
	plans for wheat improvement by cross-breeding and selection, thereby going beyond the previous practice o
	selection only for wheat improvement.
1889	Farrer's first crosses are attempted at his property, Lambrigg, near Canberra. The variety "Blount's Lambrigg" is
	selected from cross-bred material provided by A. E. Blount of Colorado, USA.
1890	Disastrous wheat losses, due to rust, prompt the First Rust-In-Wheat Conference to be convened. Farrer's lette
	to the Conference calls for cross-breeding as a means of improving both rust resistance and grain quality.
1893	F. B. Guthrie, recently appointed chemist with the Department of Agriculture of the Colony of NSW, devises small scale tests of milling and baking quality for selecting suitable parents and cross-breds from Farrer's breeding
	program. Improvement of quality is thereby made possible.
1893	Hugh Pye produces "Improved Steinwedel" at Dookie College, Victoria, by making the cross (Steinwedel × Purple
	Straw) × Steinwedel.
1893	A date suggested as the origin of cereal chemistry as a discipline, due to activities in Australia and overseas.
1895	Farrer's "Yandilla" is the first of a new generation of wheats, designed to combine Fife quality with the drought
	resistance and earliness of Indian wheats.
. Period of rap	id expansion 1896–1930
1896	Local millers are forced to modify their machinery to suit imported North American Fife wheats due to local
	shortages. They are thus more ready to accept Farrer's stronger wheats, which they had previously rejected
1898	Farrer is appointed officially as "Wheat Experimentalist" to the NSW Department of Agriculture on an annual
1000	salary of 350.
1900	Release of "Bobs," reportedly obtained by crossing a selection from "Blount's Lambrigg" with "Bald Skinless Barley" by Farrer in 1896. It became popular throughout Australia and represented a breakthrough in the
	production of "strong" wheat.
1901	Export of 25 million bushels of wheat and flour.
1901	Release of "Federation," selected by Farrer from his cross in 1895 between "Purple Straw" and "Yandilla."
1001	Though it fell short of his quality and disease-resistance goals, its yield made it the most popular wheat in
	Australia from 1910 to 1925. It was also widely grown in USA, India, and other countries.
1906	Death of Farrer, and suspension for many years of quality-directed breeding.
1911	NSW "Strong White Wheat" class is established to segregate Farrer's strong wheats. Export of 64 m bushels o
	wheat and flour of all classes. The contrast between this harvest and that of 10 years before is due largely to
	Farrer wheats such as "Federation."
1924	Release of "Ghurka," bred by G. S. Gordon in 1916, becoming a leading Victorian variety in its own right, and also
	a parent of many important soft wheats.
1929	The variety "Bencubbin" commences its phenomenal rise to popularity, not only in Western Australia, where it was bred by E. L. Limbourn, but throughout Australia.

# VI. International marketing expansion 1930–48

The Gepp Royal Commission recommends that breeders should "endeavour to produce new varieties which have the quality of strength."

Table 2 Continued

1945	Availability of "Gabo" wheat saves many farmers from ruin due to rust. It starts Australia's reputation for high-protein baking-quality wheat.
1946	Introduction of "Insignia," selected for tiller survival, permits further extension of the wheat belt into drier areas.
VII. Grain quali	ty targeted to specific market needs 1949–70
1950+	Segregation of specific truck loads of high-protein wheat of selected varieties as Prime Wheat grades by the Prime Wheat Association, in northern New South Wales.
1956	Release of "Dural," the first Australian-bred durum wheat.
1957	Passage of the Wheat Research Act through Federal Parliament, and the consequent formation of the Wheat (Industry) Research Council, later to be replaced by the Grains R&D Corporation with responsibility for research on the wide range of agricultural grains.
1960	Naming of "Gamenya," derived from "Gabo" by I. A. Watson. Though bred in the east, its greatest impact was in Western Australia and as an ideal noodle wheat.
1960+	Growing market awareness, involving an active acknowledgment that about three-quarters of the Australian wheat crop is exported for "exotic" uses, leading to research initiatives on noodles, flat breads, and steamed bread.
1967	"Timgalen," issued by Sydney University, offers excellent quality with high-protein potential, disease resistance, and agronomic qualities that earned it a continuing unrivalled position for prime-wheat production.
1970+	Introduction of near-infrared spectroscopy revolutionizes the analysis of protein and moisture in grains.

exports. Initially these exports were to England, where the grain was used for traditional products, mainly bread. This situation has changed dramatically in the subsequent century. In recent decades, Australia has produced  $\sim 5$  times more than its domestic needs, so that  $\sim 75\%$  of the wheat harvest is exported. Currently, exported wheat goes to a wide range of countries, including Egypt, Indonesia, Iran, Iraq, Japan, and China.

In the early stages of the diversification of export markets (in the 1960s), it was realized that the wheat was being used for the production of a diversity of foods that could then be classed as "exotic," such as "Arabic" flat breads in the Middle East and a range of noodles and steamed breads in Asian countries. Furthermore, in countries such as Indonesia, even the manufacture of leavened bread involved nontraditional methods that required wheat of quality different from the English market.

Accordingly, experts were brought to cereal chemistry laboratories in Australia from the new export markets to demonstrate the production of these exotic products. Figure 3 shows an expert from the Middle East with experimental Arabic "pocket" breads, both from normal and small-scale baking. As a result of seeking this expertise, it was possible for wheat breeders to select new varieties that would suit these quality requirements. The resulting research led to the establishment of specifications for the wide range of wheat-based products made around the world. With the publication of these findings, advantages of the knowledge have flowed to the countries involved and to other exporters.

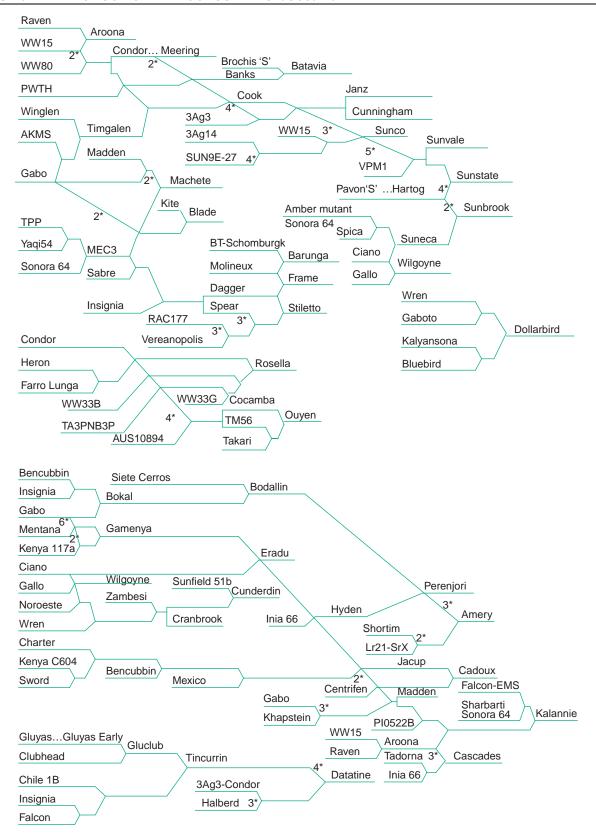
Another form of diversification came with the challenge presented to wheat growers by the sandy soils

of Western Australia, due to the lack of soil nitrogen. An efficient solution to this problem was the introduction of grain-legume crops in agronomic rotation with wheat. The ability of the legumes to fix atmospheric nitrogen led to the provision of a natural source of nitrogen fertilizer, plus the welcome harvest of other grains, particularly lupins and various pea and bean crops (Table 1). These pulses have only emerged as important crops since the 1980s. Expansion of production has depended significantly on the growth of export markets. Recent expansions in wheat and pulse production have warranted the erection of new terminal storage facilities in Perth, Western Australia (Figure 4).

Another form of agricultural diversification accompanied the down-turn in wheat prices during the 1980s. In addition to growing grain legumes, a valuable crop was supplied in the form of canola (rapeseed). Production of canola has continued, leading to the expansion of the oilseed-crushing industry (Table 1). The canola crop has expanded dramatically, production having tripled in the period 1996–99.

#### Wheat

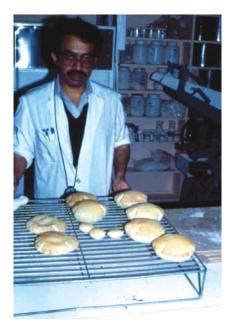
The Australian Wheat Board has been responsible for the marketing of the wheat crop since its formation in 1939. In July, 1999, its status changed from an Australian Government Statutory Marketing Authority to a private company called AWB Limited. The original name ("The Australian Wheat Board") was abbreviated to the initials to build on its reputation, while also reflecting its wider role beyond wheat. It is



**Figure 1** Pedigrees of recent prominent varieties of wheat grown in eastern and western Australia. Pedigrees are read from left to right. For example, Raven was crossed with the line WW15 to produce the variety Aroona. The line WW15 was crossed with WW80 and then crossed again (double backcross, 2\*) to produce the variety Condor. The variety Meering was selected from Condor. Condor was a parent entering into the pedigrees of Banks and Cook. A sister line of Condor was a parent that also entered into the pedigrees of Banks and Cook.



Figure 2 The \$2 bill, introduced with Australia's decimal currency in 1966, featured William Farrer – the wheat breeder responsible, in the period 1890–1910, for providing farmers with new varieties adapted to Australian conditions.



**Figure 3** Arabic "pocket" breads baked experimentally by an expert from the Middle East in the laboratories of the Bread Research Institute of Australia (now BRI Australia) in the 1970s. This type of flat bread comes from the oven puffed into a sphere. On cooling it returns to its flat-bread form. Advice from such experts has assisted Australia in recent decades in providing wheat of the quality suited to specific market requirements.

still the single marketer of bulk Australian wheat internationally, although this is under review.

Wheat varieties registered in Australia are virtually all white grained, in contrast to the red-grained wheats that are common in other wheat-producing countries. This policy direction, taken in the very early days of the industry, has been recognized as a marketing advantage. For many years, only white-wheat varieties have been grown. In addition, the

dry climate of Australia's wheat belt (mainly inland) ensures that the grain produced is very dry. Furthermore, marketers and bulk-handling corporations set rigid standards for the cleanliness of export wheat.

Consequently, Australian wheat has regularly been marketed as "clean, white, and dry." These three characteristics offer millers the triple promise of high flour extraction, namely, the lack of nonmillable material (clean), the white grain permitting a higher extraction rate before flour color is compromised, and dry grain offering less moisture to be paid for at the price of grain (if sold on "as is" weight).

Because of the high proportion of the Australian wheat crop that is exported (Table 1), marketing strategies must take into account the wide range of products for which it is used. Several of these are shown in Figure 5. The main products domestically are the many conventional forms of leavened bread. This type of baked product is in a minority for export uses, which are more likely to include products such as various Arabic flat breads, Chinese steamed breads, many types of noodles (made from hexaploid wheats), and pasta (made from tetraploid durum wheats). Durum wheats make up a small proportion of the crop, but this proportion is increasing.

To meet the wide range of quality specifications for these various products, there are many wheat grades with tight specifications. Varietal identity is an important part of the quality specifications, with a small group of varieties of similar quality type being permitted for each grade. Other specifications cover aspects of the plumpness of the grain, absence of contaminants and of insects, cleanliness, soundness, the absence of defects, and, importantly, protein content. This approach to grading and marketing is in great contrast to the Fair Average Quality (FAQ) era of the earlier part of the twentieth century, when FAQ was



Figure 4 The grain-storage facility in Perth, Western Australia. The very large storage capacity of this recent construction was warranted by the recent increase in grain production in Western Australia. The many storage cells are designed to accommodate the diversity of grain species and grades.



**Figure 5** The range of food products made from Australian wheat. In addition to the many forms of leavened bread, Australian export wheat is used for products such as Chinese steamed bread (white sphere at lower left), Arabic flat breads (lower left), noodles (at right), crisp breads (lower center), and pasta (lower right).

determined as an estimate of quality expectations based on the type of grain that "happened" to be delivered.

The maintenance of grain quality in the current range of grades and classes requires strict systems for assessing grain quality when it is delivered. It is common for grain to be delivered directly from the field to the local silo (grain elevator or storage site), although on-farm storage is becoming more common. At the point of delivery, it is critical for the range of quality attributes to be determined quickly, to allow

for the grain to be dumped into the storage cell appropriate to the grade to which it is allocated. In addition, grain samples are kept to represent the deliveries of each grower and to indicate the composition of the storage cells. These can further be analyzed subsequent to delivery.

#### Rice

Rice production and export is a significant industry for Australia. The annual average rice crop is milled to

produce the equivalent of almost 1.5 Mt of white rice (Table 1). It is grown within the irrigation areas of the Murrumbidgee and Murray river systems in southern NSW, with some further areas of production in Victoria, Western Australia, Queensland, and the Northern Territory. Farming operations are entirely mechanized. In the irrigation area, sowing occurs in September and October into dry seedbeds. Sometimes sowing involves the aerial dispersal of pregerminated seed into shallow water. After ~4 months, water is drained allowing the beds to dry prior to mechanical header harvesting. Yields are high by world standards, averaging over 9 t ha<sup>-1</sup>, with some growers achieving 13 t ha<sup>-1</sup>. Rice is generally delivered at relatively high moisture (20-22%) to avoid cracking of the kernels. Aerated storage is used to further reduce the moisture to  $\sim 15\%$  prior to milling. All deliveries are sampled on delivery and are analyzed for moisture and protein contents by near-infrared transmission spectroscopy. Samples are also checked for variety and for defects.

Rice grown in New South Wales is vested in the Rice Marketing Board of NSW with the Ricegrowers' Co-operative Limited as its authorized buyer, who coordinate the production, storage, processing, and marketing of whole-grain rice and processed rice products. Much of the milled rice is provided for the domestic and export markets in branded retail packs, ready to go onto supermarket shelves. About half of the rice crop is exported, going to more than 40 countries, but Australian rice makes up only 3% of world trade. Papua New Guinea takes ~25% of Australian rice exports. Other significant export markets are the Pacific Islands, Hong Kong, and New Zealand.

Most of the rice grown in Australia is from medium- and long-grain Indica varieties. The long-grain varieties include the fragrant types. The hulls that are removed in the milling process are converted by incineration to high-carbon ash, which is used by the steel industry in Australia and overseas. The ash is used to coat the surface of open-hearth furnaces, thus trapping the heat and improving fuel efficiency. Fire-proof building materials have also been developed using this ash as a starting material.

#### **Coarse Grains**

#### **Barley**

Second in importance, based on volume and quality, is barley — of both domestic and export significance (**Table 1**). Export markets are mainly China, the Middle East, and Japan. Australia is the second most important country in world malting-barley trade,

depending on seasonal fluctuations. The premium use of barley is for malting to produce beer. In addition to the export of malting barley, malt produced in Australia is exported for beer production overseas. Varieties are bred specifically for the purpose of malting, based especially on the production of brewery extract for fermentation purposes after malting. These are all two-row types; Australian six-row barleys are all of feed quality.

Animal feed is the destination of barley that does not meet the stringent requirements of the malting grades, due to failures in meeting specifications for variety, protein content, or absence of defects. Feed barley, together with sorghum and down-graded wheat, are the main grains for the animal-feed industry, their importance being greater because of the low production of maize in Australia.

Barley is also used for human food purposes both domestically and in export markets. Japan has emerged as a major user of food-grade barley. Some of the food uses involve pearling, to remove the bran and husk layers. In other cases, flour is produced and some of this is becoming used to produce high-fiber pasta products, taking advantage on barley's high fiber content in its cell walls ( $\beta$ -glucans and pentosans). The production of distilled beverages is another significant use of food-grade barley.

#### **Oats**

Most of the oat crop is used within Australia. It may serve on-farm as a forage crop to be grazed when plants are young, with the option of allowing the plants to mature and produce harvestable grain. Some varieties are suited to milling and processing for food purposes, but much of the crop is used as animal feed (Table 1).

# Sorghum

Sorghum is a feed grain. Much of the crop is exported for this purpose. The main customers are Japan and Taiwan. Production is mainly confined to the warmer subtropical parts of Australia.

# **Cereal Rye**

Flour produced from rye grain is used in Australia for European-style breads, biscuits, crispbreads, multigrain breakfast cereals, muesli bars, pet food, and special dietary foods that provide higher lysine, pentosan, and  $\beta$ -glucan content than other cereals. Production is minor in volume.

#### **Triticale**

The wheat-rye hybrid triticale has enjoyed reasonable popularity in Australia. Annual production is about

0.75 Mt (**Table 1**), making Australia the world's fourth largest producer of triticale, depending on seasonal fluctuations. Triticale's attraction has mainly been as an alternative to wheat for the stockfeed industry, which is the major domestic end user. However, efforts have been made to develop niche markets for human food.

#### Maize

Maize is a relatively minor crop in Australia, with annual production averaging ~0.33 Mt (Table 1), less than the production figures for all the major cereal grains. About 10% of the maize crop is exported. About one-quarter of the crop is used domestically as human food, mainly for breakfast cereals, sweet corn/vegetable uses, corn-based foods, and industrial manufacture of corn starch. The remainder is used domestically as stock feed. Industrial use of maize is minor compared to northern-hemisphere maize use, because Australian starch production is mainly based on wheat. Maize processing into starch relates mainly to special products such as high-amylose starch.

#### **Pulses**

Combined, the pulses (grain legume species) are the third largest crop in Australia (after wheat and barley) (Table 1). Much of the pulse production is exported. The pea and bean species are largely used overseas for human food purposes, especially for the Indian subcontinent. Australia is the largest producer of lupins; about half of the crop is exported. Australian lupins are largely based on the narrow leafed lupin (*Lupinus angustifolius*). White lupin (*L. albus*) is also grown to a significant extent. The potential of the crop has been enhanced through breeding to reduce the levels of alkaloids, to decrease the risk of grain shattering, and to improve disease resistance.

#### **Oilseeds**

The canola crop has expanded dramatically since the 1990s, production having tripled in the period 1996–99. It was ~2.4 Mt in 1999, rather more than the recent five-year average given in **Table 1**. Cotton-seed production given in **Table 1** (a little over 1 Mt) is the volume of "white seed," after allowing for de-linting and de-hulling. About a quarter of this is crushed domestically for oil production. Production volumes are similar for sunflower and soybean (both 110 000–120 000 t annually). Peanut production is well below these at ~30 000 t. Lesser oilseeds include linseed and safflower. Australia is

a significant exporter of oil crushed from these various oilseeds, especially cottonseed. The residues from oilseed crushing make important contributions to the stockfeed industry.

# Consumption

Australians consume  $\sim 70 \, \mathrm{kg}$  of wheat flour per person per year, mainly in the form of baked goods, but flour also goes into many processed foods. A significant part of this "consumption" (based on statistics) is due to the importance of the starch-gluten industry, which processes dough to separate the starch fraction, using some of the starch for paper manufacture. Wheat starch is also a significant food ingredient. The remaining gluten is dried and used as an additive for bread manufacture (both domestically and as a significant export commodity) and also for incorporation in a range of foods.

Another significant use of grains in Australia is the breakfast-cereal market, which is valued at Aust.\$800 million. These and other grain-based foods represent an important part of Australia's exports of manufactured goods, primarily to the Pacific islands and to Asia.

Goodman Fielder is an important manufacturer of grain-based foods in Australia and New Zealand. Recently, Goodman Fielder had market shares in Australia of about half the packaged bread, cake mix, and snack-food markets,  $\sim\!20\%$  of breakfast cereals,  $\sim\!66\%$  of pastry, and  $\sim\!30\%$  of dessert mixes. In New Zealand, Goodman Fielder had about half of the packaged bread and potato-chip markets.

#### **New Zealand**

Significant volumes of barley and wheat are produced in New Zealand, as is shown in Table 3. Deregulation of the wheat industry some years ago accounted for significant changes in the structure of the industry, increasing competition and the likelihood of importation of grain. Grain yields for both wheat and barley are considerably higher on average than in

**Table 3** Production of wheat and barley in New Zealand for the year 2003

Crop species	Area sown	Production	Grain yield
	(ha)	(kt)	(t ha <sup>-1</sup> )
Wheat	55 000	324	5.9
Barley	82 000	449	5.5

Data from Statistics New Zealand. www.stats.govt.nz.



Figure 6 Wheat field and on-farm storage in the south of the South Island of New Zealand. As late as April (autumn), the standing grain is still waiting to be harvested.

Australia, being  $\sim 6 \, \text{t ha}^{-1}$ . In further contrast to Australia, adequate on-farm storage for the crop is common (Figure 6), so that the grain buying and transport systems are different from those in Australia. Most of the New Zealand harvest is later than in Australia, where early deliveries may start in October. On the other hand, harvest time in the south of the New Zealand South Island may be as late as Easter time (late in the southern-hemisphere autumn).

See also: Barley: Malting. Chickpea: Overview. Gluten and Modified Gluten. Grain Crops, Overview. Lupin: Overview. Milling and Baking, History. Pulses, Overview. Rice: Overview. Triticale. Wheat: Breeding.

# **Relevant Websites**

http://www.abareconomics.com - Australian Bureau of Agricultural and Resource Economics, Canberra, Australia.

http://www.awb.com.au - AWB Ltd, Melbourne, Australia.

http://www.bri.com.au - BRI Australia Ltd.

http://www.pi.csiro.au - CSIRO Plant Industry, Australia.

http://www.crop.cri.nz - New Zealand Institute of Crop and Food Research.

http://www.stats.govt.nz - Statistics New Zealand. http://www.raci.org.au - The Cereal Chemistry Division of the Royal Australian Chemical Institute.

# **Further Reading**

Anonymous (1994) Australian Grains. Victoria, Australia: Morescope Publishing.

O'Brien L and Blakeney AB (eds.) (2000) An Introduction to the Australian Grains Industry. Melbourne: Royal Australian Chemical Institute.

O'Brien L, Morell M, Wrigley C, and Appels R (2001) Genetic pool of Australian wheats. In: Bonjean AP and Angus WJ (eds.) The World Wheat Book, pp. 603–640. Paris: Lavoisier Publishing.

Quail KJ (1996) Arabic Bread Production. St. Paul, MN: American Association of Cereal Chemists.

Wrigley CW and Rathjen A (1981) Wheat breeding in Australia. In: Carr DJ and Carr SGM (eds.) Plants and Man in Australia, pp. 96-135. New York: Academic Press.

# South America

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#### Introduction

South America produces 106 800 000 ton (t) of cereals and 72 300 000 t of the main oilseed crops (Table 1). Forty percent of the world's soybean production and 10% of maize (Figure 1) are harvested

Table 1 Grain production in South American countries. Average 2000-02 (kg per capita per year)

	Wheat	Rice (paddy) Barley	Barley	Maize	Rye	Oats	Millet	Sorghum	Quinoa	Sorghum Quinoa Canary seed Buckwheat Soybean	Buckwheat	Soybean	Sunflower
Argentina	14 882 207	782 590	656 158	15 627 327	113 758	644 023	37 307	2 982 171		19 377		25 714 533	4 364 092
Bolivia	112 606	291 117	64 285	669 643	208	4 678		101 238	23 262			1 077 569	136 400
Brazil	2 708 497	10 591 533	281 405	36 265 769	7 357	292 553		833 037			49 333	37 440 353	94 000
Chile	1 697 752	140 083	67 415	784 909	2 117	336 253							2 088
Colombia	33 127	2 317 656	7 649	1 251 286				221 099				49 174	
Ecuador	18 991	1 370 560	30898	587 834	70	736		10842	983			126 420	240
French Guiana		19 900		29									
Guyana		540 000		3 933									
Paraguay	312842	102 637		792 645				40 586				3 255 703	48 854
Peru	186 923	1 883 377	174 060	1 336 833	96	10 707		283	30 333			2 866	
Suriname		182 357		09								42	
Uruguay	202 667	1 059 596	149 767	192 167		45 000		107 133		2 500		21 133	20 967
Venezuela	220	685 592		1 363 184				453842				5 498	5 174
South America	20 156 163	19 966 998	1 431 637	58875620	123 606	1 333 951	37 307	4 750 231	54 578	21 877	49 333	67 693 292	4 704 815
Data from FAOSTAT data files.	AT data files.												

in South America. The combined share for all cereals is 5.18% (average of the triennium 2000–02).

Brazil and Argentina are important producers of grain and oilseed crops at a world level. Brazil is the main soybean exporter in the world and feeds its population with large harvests of crops such as rice and maize. Argentina is among the top five exporters of wheat, soybean, and maize.

The whole region possesses plenty of arable land and renewable water resources and, although economic uncertainty is endemic, prospects are positive for agricultural development. Improvement (better seed varieties, mechanization, and fertilizer use) in average yields of the major crops and increases in harvested area have boosted the region's production in the last decades.

Grain-based food preferences vary from country to country. Wheat (Argentina, Chile, and Uruguay), maize (Paraguay, Venezuela, Bolivia, and Colombia), and rice (Guyana, Suriname, and Ecuador) are the most important grain raw materials used for food preparation (Table 2).

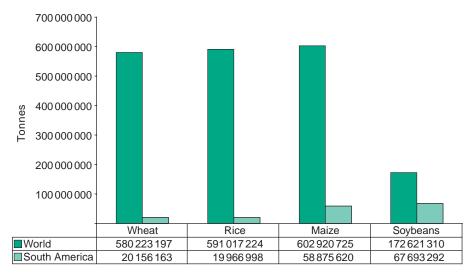
# **Argentina**

The Argentine Republic is the second grain producer and the main exporter in South America (Figures 2 and 3), with an average cultivated area for the last triennium of 27 160 000 ha and average harvest of 67 436 000 t. Since the 1990s, the harvested area increased by 36% and production by 66%. Technological advances, better seed quality, genetic improvement, and better crop management were the major factors in this development.

The Pampas, a rich humid plane in the central eastern part of the country, is the main area of grain production. Wheat was sown during 1999–2001 on an average of 6 000 000 ha with harvest figures of 14 570 000 t. The Buenos Aires province produces 62% of all wheat produced in the country. Argentina is the fifth major wheat exporter, averaging 9 570 000 t in the 2000–03 triennium. Brazil imports most of the Argentine wheat (from 62% to 82% of the total) and the rest primarily goes to Iran, Peru, Bolivia, Chile, and South Africa.

Argentina exports wheat as a commodity but has recently started a small-scale trial to segregate its products based on quality aspects.

Internal wheat consumption is stabilized, with average figures of 4600000t per year. Three-quarters of it is designated to bread production. During 2001, bread consumption was 2160000t (75 kg per capita). Argentines prefer continental bread (70 kg per capita). The country has 12000 bakeries where artisan continental bread is made daily.



**Figure 1** Average production of grain crops in South America and the world in the 2002–03 triennium. (*Source*: FAOSTAT agriculture data files.)

Table 2 Food supply in South American countries. Average 1998–2000 (kg per capita per year)

	Wheat	Rice (milled)	Maize	Total cereal	Potatoes	Cassava	Sweet potato	Beans	Oil crops	Meat
Venezuela	48.7	13.9	48.5	112.7	13.6	12.7				42.5
Uruguay	91.6	13.1	25.9	130.7	37.5		14.5			93.3
Suriname	54.2	69.5		126	14					43.9
Peru	55.1	48.4	13.5	123.5	75	25.3				22
Paraguay	22.2	11.4	51.5	85.5		136	11.8			72.7
Guyana	54.5	89.5		144.3		25.7			28.1	31.6
Ecuador	38.4	50.7	14.2	107.1	34					35.1
Colombia	27	29.6	39.1	97.2	47.3	34.3				33.5
Chile	112.9	6.8	15.4	137.9	50.8					63
Brazil	48.3	39.9	17	106.6	14.4	42.8		16.1	12.8	73.3
Bolivia	45.5	20.5	47.6	117.7	47.6	18.9				48.8
Argentina	119.2		8.7	132.8	62.2					97.1

Data from FAOSTAT data files.

Industrial bread (sliced wrapped white bread) only constitutes a 5% of the total intake of bread. Nevertheless, in recent years sliced bread has shown a steady increase in sales (mainly in supermarkets). Production of cookies has increased tenfold since the 1990s, reaching 285 000 t in 2002. Argentina is the largest consumer of cookies in South America, with an average of 7.5 kg per capita per year.

Durum wheat harvest, used mainly for pasta production, averaged 186 000 t in the 1999–2001 period. Argentina's pasta consumption averaged 6.8 kg per capita in 1999. Argentina exports 400 000 t of flour.

Maize is the second largest cereal crop in the country, in terms of acreage. Almost 3.5 million hectares (Mha) were sown in the 1999–2001 period with an average harvest of 15 225 000 t per year. Two-thirds

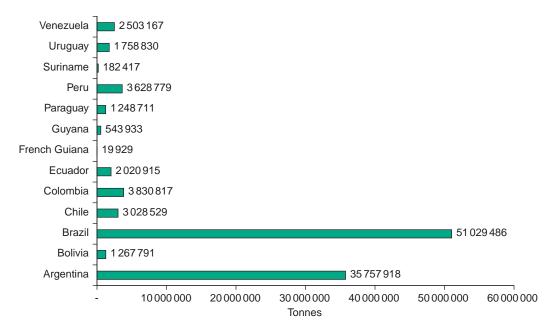
are usually exported (17% of the international market). Spain, Egypt, Chile, Peru, and Brazil are the major buyers of Argentine corn. Argentina is the sixth world producer of maize.

Sorghum figures, for the 1999–2001 triennium, amounted to 3159000t per year and it is mainly destined for feedstuff. Japan is the major importer of Argentine sorghum.

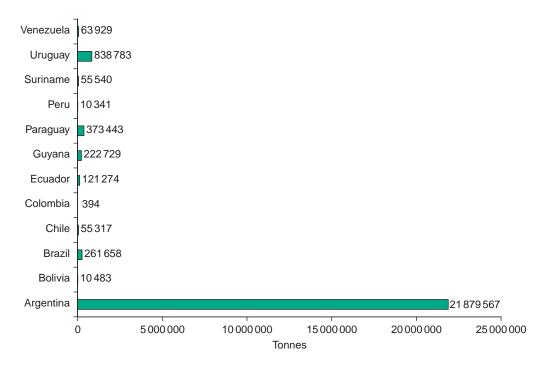
The increasing trend, showed until 1998–99, of rice production has stopped. Average production for the 1999–2001 crops was 1 140 300 t.

Two other cereals with important acreage and production figures (over 500 000 t each) are oats and barley.

Oilseed crop production has been increasing steadily for many years now and altogether, they represent 52% of total grain acreage. The principal



**Figure 2** Average production of cereal crops in South American countries in the 2000–02 triennium. (*Source*: FAOSTAT agriculture data files.)



**Figure 3** Average exports of cereal crops in South American countries in the 1999–2001 triennium. (*Source*: FAOSTAT agriculture data files.)

oilseed grown is soybean, which is also the leading crop in Argentina. Its acreage is 12 600 000 ha and production reached 30 000 000 t in the 2001–02 cycle. Argentina uses GM technology for its soybean cultivars along with nontillage technology. Profits

from the crop production are very high. Nevertheless, there are growing concerns about the sustainability of resources involved in soybean production in the country. Soybean is mainly exported to China and Thailand.

Soybean oil is marginally consumed in the country (10% of all edible oils) and is mainly exported to India, Iran, Bangladesh, Egypt, and Morocco. European countries (Italy, Spain, Holland, and Denmark) import soybean pellets.

The second most important oilseed crop is sunflower, averaging for the 1999–2001 triennium 5 380 000 t. Yields have increased steadily during the last few years. Argentina is the major producer and exporter of sunflower oil, which is the main use for the sunflower seed. For the 1999–2001 period, Argentina produced 1 980 000 t and exported 1 475 000 t of sunflower oil. India, Iran, Egypt, and South Africa are the leading importers of Argentine sunflower oil. Exports of sunflower pellets averaged 1 134 000 t for the triennium 2000–02 and are mainly shipped to European Union countries. Other oilseed crops are groundnut, linseed, and canola.

# **Bolivia**

Located in central South America, Bolivia is the poorest and least developed country in the region. Only 2% of the country's area is arable land and most of the population lives in rural areas. Agriculture employs 50% of the workforce and constitutes 23% of the gross domestic product.

In recent years this Andean country has consolidated a strong growth in oilseed crop production, particularly soybean. The triennium 2000–02 showed average production figures of 1077000 ton per year. The annual growth rate was 16.7% between 1990 and 2000. Santa Cruz state is the major producer, with an average yield of 1900–2000 k per ha without irrigation and fertilizers. The reason for the improvement in soybean production is the availability of new land at accessible prices, the existence of protected markets in the Andean countries, good finance options, national and foreign investments, and upgraded technology. In the 1990s, exports of oilseed crops increased by 747%. Soybean flour accounted for 64% of the exports, 26% of seed, and 10% of oil.

The second most important crop is maize (669 000 ton per year) followed by rice (291 000 ton per year) and wheat. Maize is the leading staple grain in the country. Bolivia also produces quinoa, a high-protein grain native to South America that was the staple food of the Incas.

#### Brazil

In 2000, primary agriculture accounted for 10.1% of Brazil's GDP and  $\sim$ 23% of the total labor force. Agricultural exports accounted for  $\sim$ 4% of the world's total agricultural exports and represented (in the year

2000)  $\sim$ 28% of the country's total exports, whereas imports accounted for only 8.5%.

Brazil is the major grain producer in South America, with an average cultivated area of 31 702 000 ha for the 1999/2001 triennium. The main production area is the South (Parana, Rio Grande do Sul, and Santa Catarina states), which produces almost 50% (48 893 000 t in 2001) of the total harvested grains (98 317 000 t in 2001) (these figures include beans (3 000 000 t in the year 2000)). The South East (12 682 000 t in 2001) and Central Western (28 451 000 t in 2001) regions are also important producers. The latter region has seen an unprecedented increase in the sown area of soybean of 5 000 000 ha since the mid-1990s.

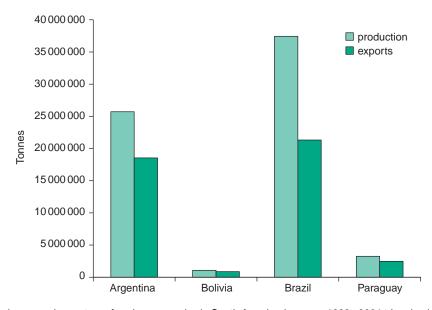
Wheat production (2708000t for the triennium 2000-02) does not satisfy internal demand. Twothirds are imported almost exclusively from Argentina (6789 000 t in 2001) favored by preferential trade terms between Mercosur partners (a common market/customs union formed by Argentina, Brazil, Paraguay, and Uruguay with Chile, Peru, and Bolivia as associates). Other exporters of wheat to Brazil are USA (100 000 t), Paraguay (88 000 t), and Canada (34 000 t). Brazil has been the largest or second largest wheat importer in the world since the 1990s and leads South American imports by an ample margin (Figure 4). Recently, the government has introduced incentives to increase wheat production in order to decrease imports. The principal wheat-producing states in Brazil are Parana (57%) and Rio Grande do Sul (34%).

Maize production for the 2000–02 cycles averaged 36 266 000 t per year, with almost 42 million ton (Mt) produced in 2001. Brazil is the third largest producer in the world, behind USA and China. The harvested area has been fluctuating between 12 and 14 Mha since the 1990s. Parana state is the major producer. The imports are quite variable (506 000 t in 1997, 1728 000 t in 1998, 822 000 t in 1999, and 1770 000 t in 2000) and mainly come from Argentina. Other exporters are Paraguay and USA.

Rice is a staple food in the country, so its consumption is correlated with the population growth. Most of Brazilian rice is cropped in uplands, particularly in the northern and mid-western regions. Rice production in the uplands occupied 2.4 Mha (64% of total area) in 1999, but contributed only 34% of the total grain production. The higher yielding system of rice cultivation, flooded fields, is more common in south and south eastern states. Production for the 1999–2001 seasons averaged 10 666 000 t per year of paddy rice. Rio Grande do Sul is the largest rice-producing state, producing almost half the total harvest. Brazil imported paddy rice 700 000 t – 1 950 000 t

Figure 4 Average imports of cereal crops in South American countries in the 1999–2001 triennium. (Source: FAOSTAT agriculture data files.)

**Tonnes** 



**Figure 5** Major producers and exporters of soybean complex in South America (average 1999–2001 triennium). (*Source*: FAOSTAT agriculture data files.)

in the 1990s mainly from neighboring Uruguay and Argentina.

The soybean complex production (seed, meal, and oil) averaged 32 666 000 t in the 1999–2001 triennium and the harvested area averaged 13 417 000 ha. Mato Grosso, Parana, and Rio Grande do Sul are the main producing states. Brazil exports soybean and soybean meal to The Netherlands, France, Germany, and Spain. Oil is exported mainly to Iran and China (Figure 5).

Other important grains are sorghum, with a production of 896 000 t in 2001, barley (286 900 t in 2001), and oat (333 000 t in 2001).

Brazil's traditional, basic meal consists of rice and beans ("feijoada"). Following recent economic downturn, there was an increase in its consumption as people moved back to less expensive food. Yearly per capita consumption of rice and beans averaged 39.9 and 16.1 kg, respectively, for the 1998–2000 triennium. Beans are one of the main sources of

protein from vegetable origin in Brazil, second only to cereals. Brazilians consume half the total amount of beans destined for food in South America, with a daily intake of 9.7 g of protein per capita (average 1998–2000). Cereals (wheat, rice, and maize together) provide 20.4 g of protein per capita.

#### Chile

The Republic of Chile is a long, narrow country on the western coast of the continent, facing the Pacific Ocean. South-central Chile is where most of the grains are grown. The total grain production for the 2000-02 triennium averaged 3 000 000 t. Wheat (1700000t per year), maize (780000t per year), and oats (330 000 t per year) are the most important crops. Maize (1 100 000 t per year according to 1998-2000 data) and wheat (540 000 t per year, 1998-2000) are also imported to fulfill internal demand. Chile is the main consumer of bread in South America and second in the world (97 kg per capita per year). Chileans are the third major consumers of pasta in the region (8.2 kg per capita in 1999). Chile is one of the three South American countries that fortify basic food (in this case, wheat flour) with micronutrients (iron).

#### Colombia

Situated in the northern part of South America, Colombia's agriculture contributes 14% of GDP, and 20% of the economically active population works in agriculture. In terms of volume, rice  $(2\,318\,000\,\text{t per year},\,2000-02)$  is the second most important crop in the country, behind potato. Most of the rice-producing land is irrigated (84%). Maize production average figures for the 2000-02 cycles were 1 250 000 t per year. Maize is also a primary staple food for the Colombians, who prefer white maize. The third major cereal crop is sorghum, with average figures of 220 000 t per year in the 2000-02 triennium. Colombia is the second largest importer of grains in the region, behind Brazil. On an average, almost 3 000 000 t are imported per year (triennium 1999-2001).

Colombia is the third largest producer of beans in South America (120 000 t per year, average 1998–2000) and rates fourth in consumption with 3.5 kg per capita per annum (average 1998–2000).

# **Ecuador**

Ecuador's economy is predominantly agriculture-based (17% of GDP), and its main grain product is rice. This crop is the source of employment for 22% of

the active population. Its production for the triennium 1999–2001 averaged 1 340 000 t per year.

Maize production exceeds 500 000 t per year and may tend to increase in the future. Colombia buys almost 100% of Ecuador maize exports (72 963 t out of 73 002 t total maize exports in 2002). Maize is grown in small farms with much lower yields than the international average.

Wheat grown in the country (18 670 t per year) satisfies only 4% of the internal consumption (470 000 t per year in the 1999–2001 period). Major exporters to Ecuador are USA and Canada.

In Ecuador, the main edible oil source is palm (75%). Soybean production reached 77 772 t in 2002 and 97 500 t in 2003.

Ecuador has recently approved legislation for iron fortification of wheat flour.

# **French Guiana**

Agriculture is concentrated in coastal areas (9000 ha of arable land), and paddy rice, with less than 20 000 t harvested in the last three seasons (2000–02), accounts for almost 100% of the produced grains.

# Guyana

Annual crops are grown on the narrow coastal strip. Rice is almost the only grain produced, with an average of 540 000 t per year (paddy) in the 2000–02 triennium.

# **Paraguay**

Another country with a strong agricultural and livestock sector (29% of GDP) is Paraguay. La Campina is the major producing area in the country (4 000 000 ha) with a mixture of traditional small farms and well-equipped medium/big farms.

Maize, cotton, and soybean are the most important crops. Paraguay is the third largest South American producer of soybean (3 175 300 ton per year in 1999–2001). Exports averaged 2 Mt in 1999–2001.

Flour production from oilseed for the triennium 1999–2001 averaged 842 000 t and oil production reached 236 000 t in 2001.

Paraguay has the second largest consumption figures for beans (5.5 kg per capita per year, average years 1998–2000) in the region.

#### Peru

The birthplace of the Inca's empire is located on the western coast of the continent, facing the Pacific. Peru has three distinctive environments: the coastal region,

the Andes, and the eastern jungle of the Amazon Basin.

One-third of its population is dedicated to agricultural activities. Nevertheless, Peru is a large importer of grains (third in South America). Almost 2 500 000 t are imported yearly (average 2000–02). Irrigated valleys in the coastal region are the most productive lands. Major crops are rice (1 900 000 t per year) and maize (1 300 000 t per year). Peru, together with Bolivia and Ecuador, accounts for all the world production of quinoa.

Peru is the second largest pasta consumer in the region with 250 000 t per year. On the other hand, bread consumption (38 kg per year) is not high.

# **Suriname**

Most of the agricultural products are cultivated on reclaimed land in the coastal region. The main crop is rice, which accounts for almost all the harvested grain (paddy rice 182 300 t per year in 2000–02).

# **Uruguay**

With only  $\sim 80\%$  of its total area being agricultural land, Uruguay's economy predominantly relies on livestock and, to a lesser extent, on agriculture.

Uruguay produces 1758 829 t per year of cereals (average, 2000–02). The main grain crop is rice (1059 596 t per year of paddy rice for the same period). Other important crops are maize, barley, and wheat (averaging ~200 000 t per year each). Uruguay is the second largest exporter of grains in South America, although figures only reach 4% of the tonnage exported by Argentina. Most of the rice and barley is exported to Brazil. Iran is also an important buyer of Uruguay's rice and recently a small amount was exported to Japan for the first time.

#### Venezuela

Agriculture is concentrated in the "Llanos" regions and does not constitute a major component of the Venezuelan economy. The GDP for the agricultural and livestock sector is 5% of the country's total GDP. Maize, rice, and sorghum are the main crops. Venezuela imported 1 Mt of maize and 1.2 Mt of wheat (average 1999–2001). Maize (white maize) is the most important staple grain in the country. Venezuela is the largest consumer of pasta (13 kg per capita per year in 1999) in South America, second only to Italy (28.5 kg per capita) at the world level. Bean consumption averaged 4.1 kg per capita per year (1998–2000), the third largest in South America, after Brazil and Paraguay.

Venezuela is the South American country that fortifies many of its basic foods. Maize flour is fortified with vitamin A and iron and wheat flour is fortified with iron.

# **Future Prospects**

Brazil and Argentina are likely to maintain their leadership in grain production in the region.

Increments in harvested area are expected, particularly in Brazil (in the virgin lands of "El Cerrado" where 90 000 000 ha could be transformed for agriculture use).

Yields are expected to continue to rise in Argentina, based on technological advances, but the country will have to ponder the benefits/risks of using genetically modified seeds, in terms of market acceptance and competitors' (Brazil does not grow GM crops) policies. Soybean will presumably continue to be the main cash crop. An estimate of 96 300 000 t of soybean will be harvested in South America in the 2003–04 crop year. This may probably be the first time that soybean harvest in South America will surpass US production.

Although Brazil is making great efforts to increase its harvested area of wheat, it is not expected that its role as major importer of this commodity will change in the near future.

Recent political changes in Brazil suggest a stronger collaboration between Mercosur countries that could provide a larger and better offer of agricultural products to the world markets.

See also: Grain Production and Consumption: Overview; Oilseeds in North America.

# **Further Reading**

Hellin J and Hingman S (2003) Feeding the Market, South American Farmers, Trade and Globalization. Kumarian Press.

Mayer E (2001) The Articulated Peasant: Household Economies in the Andes, 320pp. Westview Press.

Morello J and Solbrig O (eds.) (1997) Argentina granero del mundo. Hasta cuando? *Orienta cion Grafica Editora* (In Spanish), 280pp.

Schiøler E (2002) From the rural heart of Latin America: farmers, agricultural research, and livelihoods. In: *Future Harvest*, 80pp. Washington, DC: Danish Royal Ministry of Foreign Affairs (ISBN 87-7964-201-2).

## **Relevant Websites**

http://www.sagpya.mecon.gov.ar — Franco D. Aceite de girasol. Informe de la SAGPYA, Argentina. http://www.sagpya.mecon.gov.ar — Franco D. Aceite de soja. Informe SAGPYA, Argentina (8 pp.).

http://www.agricultura.gov.cl - Gobierno de Chile. Ministerio de Agricultura.

http://www.sagpya.mecon.gov.ar - Pantanelli A. Análisis de la cadena de galletitas. SAGPYA, Dirección de Industria alimentaria.

http://www.sagpya.mecon.gov.ar - Pantanelli A. Análisis de la cadena de productos de panificación. SAGPYA, Dirección de Industria alimentaria.

http://www.sagpya.mecon.gov.ar - Pantanelli A. Galletitas. Informe de la SAGPYA, Dirección de Industria alimentaria.

http://www.sagpya.mecon.gov.ar - Pantanelli A. PASTAS SECAS, Informe de la SAGPYA, Dirección de Industria Alimentaria.

http://www.iica.org.py - Agencia de Cooperación IICA (Paraguay). Situación de la agricultura en el Paraguay.

http://www.sagpya.mecon.gov.ar - Dirección Nacional de alimentación (SAGPYA) Argentina.

http://www.fao.org - Food and Agriculture Organization of the United Nations - statistical database and FAQstat agriculture data are also available on the website.

http://www.agricultura.gov.br - Ministério da Agricultura, Pecuária e Abastecimento (Brasil).

http://www.agrocadenas.gov.co - Ministerio de Agricultura y Desarrollo Rural (Colombia) Observatorio Agrocadenas.

http://www.sica.gov.ec - Ministerio de Agricultura y Ganaderia de Ecuador, Servicio de Información Agropecuaria. Proyecto SICA, Banco Mundial.

http://www.mgap.guv.uy - Republica Oriental del Uruguay. Ministerio de Agricultura, Ganaderia y

http://www.trigoargentino.com.ar - Trigo Argentino, cosecha 2001/2. Informe institucional sobre calidad.

http://www.ciatbo.org - Centro de Investigacion Agricola Tropical (Bolivia).

http://www.indec.mecon.ar - Instituto Nacional de Estadistica y Censos (Argentina).

http://www.odepa.gob.cl - Oficina de Estudios y Politicas agrarias (Chile).

http://www.cia.gov - The World Factbook.

http://www.cimmyt.cgiar.org - Internationl Maize and Wheat Improvement Center.



# LABELING OF GRAIN-BASED FOODS

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#### **Overview**

A well-devised label for all food products and for grain-based foods should help consumers select and purchase food for their needs. In addition to attracting consumers' attention, these labels are legal documents to assist with compliance and consumer protection. Thus, the label must clearly identify the product, comply with any legal standards for that product, e.g., standards of identity, and list the ingredients in descending order of their presence in the product. They must not label the product in any way that is misleading or misrepresents what is in the product. In many countries, products must list nutrient values in the product either on a per serving basis or on a per 100 g basis, the latter being the more common.

The emphasis on health and disease prevention has added another realm to labeling in some countries, the inclusion of health claims. Health claims must be approved by the appropriate regulatory body in a country and must be based on significant scientific

agreement. In addition, there are claims known as nutrient content claims. These can state, for example, that "ACME cereal is a significant source of folic acid." Additionally, they can also contain structure function claims, such as "ACME cereal is a significant source of folate acid, which has been associated with maintaining heart health."

This article will talk about grain labeling in various countries of the world.

#### **Food Labels**

Labels for foods and grain-based foods throughout the world have similar functions. In addition to their obvious function of attracting the consumer's attention, food labels are legal documents in the country where the food is sold. All labels must contain the same basic information. Table 1 gives a detailed listing of information required in the USA. Each piece of information that is required has a very important function. Obviously, the name, style, and form tell the consumer what the product is. The net weight allows price and value comparisons of competing products which are sized differently. The address enables the consumer to contact the manufacturer for additional information, product complaints, and other matters. Probably the piece of information

Table 1 US food and grain label musts

Required item	Extra information needed	Exemptions
Common or usual name of the product	Style and form, if that is important	Standard of identity foods spices, colors (except FD&C #2), and flavors butter, cheese, and ice cream
Net weight	All packages must be labeled with both English and metric units. (If package contains between 1 and 4 pounds, its contents must be stated in terms of total weight in ounces and also weight in ounces and pounds.)	
Name, address, and zip code of manufacturer, distributor, or packer Ingredients in order by weight Statement that product contains artificial color or flavor (if any)	,	

that helps the consumer most is the ingredient statement. This gives the consumer some idea of the relative amounts of the various components in a product, since they must be listed in order by weight from the most to the least prevalent in the final product. Many consumers carefully scrutinize the ingredient statement for foods or additives that must be avoided, either by choice (as in the case of a vegetarian or one with religious food restrictions) or for medical reasons (as in the case of a person with an allergy).

#### The Nutrition Label

Some countries also require nutrition labels. However, the inclusion of nutrition information is voluntary, in other countries. In most countries, the format is also specified even if the labeling is voluntary. Nutrition labeling of all packaged foods including grain-based foods was required in the USA by the Nutrition Labeling and Education Act (NLEA), 1994. Required label formats for NLEA are given in Table 5.

Most grain-based foods are regulated by the US Food and Drug Administration (FDA). Those that include meat such as pepperoni pizza are regulated by the United States Department of Agriculture (USDA). Grain-based foods exempted from labeling are those served by restaurants and delis, those with very small packages (less than 12 square inches of available label space), and those produced by companies with very small annual sales.

The nutrition label is required to include information on total calories and calories from fat and on amounts of total fat, saturated fat, cholesterol, sodium, total carbohydrates, dietary fiber, sugars, protein, vitamin A, vitamin C, calcium, and iron. Other information is voluntary (Table 2). The nutrient content must be given for the food as packaged, but the manufacturer may choose to give nutrition information for the food as prepared or eaten, for example, cereal with 1/2 cup skimmed milk. Percentage of daily intakes are based on US dietary reference values (DRIs) (Table 3).

In North America, the nutrition label must be in legal serving sizes. For example, serving sizes of cakes cannot be made abnormally small so that these foods appear to be lower in fat and sodium than they really are. Serving sizes must be given in common household measures as well as by weight. If an item is packaged

Table 2 Examples of nutritional labels

Shortened format: lal	bel for vegetable	e soup	Simplified format: lab	el for soft drink	
Nutrition facts			Nutrition facts		
Serving size		1 cup (245 g) (240 ml)	Serving size		1 can
Servings per containe	er	2	Amount per serving		
Amount per serving			Calories		145
Calories Calories from Fat		55			% daily value*
		20	Total fat	0 g	0
		% daily value*	Sodium	200 mg	1
Total fat	1 g	2	Total carbohydrate	36 g	12
Sodium	800 mg	33	Sugars	36 g	?
Total carbohydrate	31 g	11	Protein	0 g	0
Dietary fiber	4 g	16			
Sugars	0 g	0	*Percent daily values	are based on a	2000 calorie diet.

Vitamin A 20%, Vitamin C 4%, Iron 2%

2 g

Protein

Not a significant source of saturated fat, cholesterol and calcium \*Percent daily values are based on a 2000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

Calories: 2000 Total fat less than 65 g Sat, fat less than 20 g Cholesterol less than 300 mg Sodium less than 2400 mg Total carbohydrate 300 g Dietary fiber 25 g

Calories per gram:

Fat 9 • Carbohydrate 4 • Protein 4

<sup>&</sup>lt;sup>a</sup> Federal Register (1993).

individually and is considered as a single serving, calories and fat must reflect the single item even though this might be larger than the legal serving size.

The law requires that the nutrient values printed on each package should be obtained from laboratory analysis or from approved databases. The inherent vitamin and mineral content may deviate as much as 20% from the labeled value in an individual sample. Added vitamins and minerals in fortified grain products must be at 100% of the stated amount at the end of the anticipated shelf life of the product. To be in compliance with the law, food manufacturers understate the vitamin and mineral value of the just-manufactured product to be sure of the product's compliance at the end of its shelf life. On the other hand, calories, fat, cholesterol, and sodium must never be labeled as less than the amount in the product.

The Australian-New Zealand Food Authority and most European countries also allow or require nutrition labeling. In these countries, the nutrition label values show the amount of nutrient per 100 g of

**Table 3** Daily values on the nutrition label used for nutritional labeling purposes in the USA. The chart below lists the daily values used to calculate % daily value for the nutrition panel

Food component	Daily value <sup>e</sup>
Total fat	65 g <sup>b</sup>
Saturated fat	20 g <sup>b</sup>
Cholesterol	300 mg
Sodium	2400 mg
Potassium <sup>c</sup>	3500 mg
Total carbohydrate	300 g <sup>b</sup>
Dietary fiber	25 g <sup>ā</sup>
Protein	50 g <sup>b</sup>
Vitamin A	5000 IU
Vitamin C	60 mg
Calcium	1 g
Iron	18 mg
Vitamin D <sup>c</sup>	400 IU
Vitamin E <sup>c</sup>	30 IU
Thiamin <sup>c</sup>	1.5 mg
Riboflavin <sup>c</sup>	1.7 mg
Niacin <sup>c</sup>	20 mg
Vitamin B <sub>6</sub> <sup>c</sup>	2.0 mg
Folate <sup>c</sup>	0.4 mg
Vitamin B <sub>12</sub> <sup>c</sup>	6.0 µg
Biotin <sup>c</sup>	0.3 mg
Pantothenic acid <sup>c</sup>	10 mg
Phosphorus <sup>c</sup>	1 g
lodine <sup>c</sup>	150 µg
Magnesium <sup>c</sup>	400 mg
Zinc <sup>c</sup>	15 mg
Copper <sup>c</sup>	2.0 mg

<sup>&</sup>lt;sup>a</sup>Daily value for adults and children aged 4 or older.

food. This enables easy comparison by the consumer and works well, if consumers have an understanding of the number of grams in a slice of bread or bowl of cereal (Table 4). Since nutrient requirements are set by both national and other food authorities such as those for the EC, the percentage of requirements may also vary by country.

# **Label Descriptors**

Grain-based foods can also be labeled using "nutritional descriptors" such as "light," "low-calorie," or "very low sodium." For example, rice cakes with under 40 calories might be labeled as a "low calorie" food. Table 5 gives a listing of other descriptors allowed in the USA. A reduced-calorie food must have calories reduced by at least one-third and must be labeled to compare it to the food it is replacing. Foods normally low in calories must be labeled so as not to confuse the consumer. For instance, celery may not be labeled as low-calorie celery, as some might think that labeled celery has fewer calories than unlabeled celery. For these foods, the label may read: "celery, naturally low in calories."

# Nutrient Content, Health, and Structure-Function Claims

Nutrient content claims are allowed in the USA provided they are factual and use defined terminology. If a positive claim is made for one nutrient but the food contains another factor such as fat above the disclosure level listed in **Table 6**, then a statement about the level of fat must be made in the same place as the nutrient content claim.

Health claims may also appear on grain-based foods. Europe and Canada have begun to have some health claims. The UK has a health claim for

Table 4 Label of product mg/100 g

	Nutrition in	formation
	Per serving <sup>a</sup> (1 biscuit – 10.4 g)	Per 100 g
Energy	182 kJ (43 cal)	1749 kJ (416 cal)
Protein	0.54 g	5.2 g
Fat	0.95 g	8.1 g
Carbohydrate	_	-
Total	8.11 g	77.9 g
Sugars	3.22 g	30.8 g
Dietary fiber	0.22 g	2.1 g
Sodium	39.0 mg	374 mg
Potassium	15.6 mg	150 mg

<sup>&</sup>lt;sup>a</sup>Servings per package – 24.

<sup>&</sup>lt;sup>b</sup>Daily value based on a 2000 calorie reference diet.

<sup>&</sup>lt;sup>c</sup>Optional on the nutrition label.

<sup>&</sup>lt;sup>d</sup>Daily value based on 11.5 g per 1000 calories.

**Table 5** NLEA nutrition label terminology<sup>a</sup>

Г	O		Alega to the contribute and the Autoria 19	الممام والمام والمراجع المراجع والمرازا والمراز المسما
Free:	Contains no more	a man an amount	mai is mumilionally invial	and unlikely to have a physiological

consequence

Fresh: Can refer only to raw food that has not been processed, frozen, or preserved or to freshly baked bread

High: A serving provides 20% or more of the recommended daily value

Less: Term may be used to describe nutrients if the reduction is at least 25%

Light: Term may be used on foods that have one third fewer calories than a comparable product. Any other

use of "light" must specify whether it refers to the look, taste, or smell; for example, "light in color"

More: Term may be used to show that a food contains at least 10% more of a desirable nutrient, such as fiber

or potassium, than a comparable food

Source of: A serving has 10–19% of the recommended daily intake of the nutrient Fat free: Has less than 0.5 g of fat per reference amount and no added fat or oil

Low fat: Has 3 g or less of fat per reference amount and per 50 g of food if reference amount is small Low in saturated fat: Has 1 g or less of saturated fat per serving and not more than 15% of the food's calories come from

saturated fat

(Percent) fat free: Term may be used only in describing foods that qualify as low fat

Reduced fat: Has at least 25% less fat per reference amount than appropriate comparison food

Sodium free and salt free: Has less than 5 mg of sodium per reference amount

Very low sodium: Has less than 35 mg per reference amount and per 50 g of food if reference amount is small
Low sodium: Has less than 140 mg of sodium per reference amount and per 50 g of food if reference amount is small

Reduced sodium: Has no more than half the sodium of appropriate comparison food

Sugar free: Has less than 0.5 g of sugar per reference amount

Reduced sugar: Has 25% less sugar per reference amount than appropriate comparison food

Calorie free: Has less than 5 calories per reference amount

Low calorie: Has less than 40 calories per reference amount and per 50 g of food if reference amount is small

Reduced calories: Has 25% fewer calories per reference amount than the comparison food

Cholesterol free: Has less than 2 mg of cholesterol per reference amount
Low in cholesterol: Has 20 mg or less cholesterol per serving and per 100 g of food

Reduced cholesterol: Has 25% less cholesterol per reference amount than appropriate comparison food

**Table 6** Levels of ingredients disqualifying health claims on US labels

Fat	11.5 g
Saturated fat	4 g
Cholesterol	60 mg
Sodium	480 mg

whole grains foods. Health claims that might be found on grain-based foods in the USA are given in Table 7.

In the USA, if a food has one or more disqualifying ingredients (**Table 6**), the health claim may be used only if a disclosure statement is included.

Structure-function claims are popular in some countries such as Japan, which allows a number of claims for functional ingredients. These claims are also allowed in the USA under the Dietary Supplement and Health Education Act (DSHEA). Examples of structure-function claims include statements such as "Antioxidants maintain cell integrity" or "Fiber maintains bowel regularity." Neither NLEA nor DSHEA permit claims that "diagnose, mitigate, treat, cure, or prevent a disease or specific class of diseases." The only exception is that of classical nutrient deficiency diseases.

# **Organic Labeling**

In the USA, the organic label for food and grains became official on 21 October 2002. Some consumers wish to select products that are produced by organic farming because of impacts on water quality and soil erosion and preservation of natural and agro-biodiversity. While many consumers use the organic label as a way to avoid genetically modified grains and other aspects that make them feel the food is safer or more nutritious than conventionally produced food and grain, there is little scientific data to support the idea that there is a difference. Nonetheless consumers all over the world want this choice.

Food carrying the organic seal or labeled as organic in the USA must adhere to the following rules. Organic food must be produced without:

- toxic, synthetic pesticides, herbicides, and fertilizers
- genetically modified organisms (GMOs)
- sewage sludge
- irradiation.

Organic grains must come from farms and ranches certified by a USDA accredited state or private agency. Organic grain products must have the name and address of the certifying agent displayed on the

<sup>&</sup>lt;sup>a</sup> From Federal Register (1993).

Table 7 FDA approved health claims typically found on grain-based foods

#### Fiber-containing grain products, fruits, and vegetables and cancer

Sample claim. "Low-fat diets rich in fiber-containing grain products, fruits, and vegetables may reduce the risk of some types of cancer, a disease associated with many factors."

Requirements. Foods must meet criteria for "low fat" and, without fortification, be a "good source" of dietary fiber.

Typical Foods. Whole-grain breads and cereals, fruits, and vegetables.

### Fruits, vegetables, and grain products that contain fiber, particularly soluble fiber, and risk of coronary heart disease

Sample claim. "Diets low in saturated fat and cholesterol and rich in fruits, vegetables, and grain products that contain some types of dietary fiber, particularly soluble fiber, may reduce the risk of heart disease, a disease associated with many factors."

Requirements. Foods must meet criteria for "low saturated fat," "low fat," and "low cholesterol." They must contain, without fortification, at least 0.6 g of soluble fiber per reference amount, and the soluble fiber content must be listed.

Typical Foods. Fruits, vegetables, and whole-grain breads and cereals.

#### Folate and neural tube birth defects

Sample claim. "Healthful diets with adequate folate may reduce a woman's risk of having a child with a brain or spinal cord birth defect."

Requirements. Foods must meet or exceed criteria for "good source" of folate, that is, at least 40 µg of folic acid per serving (at least 10% of the daily value). Folic acid content must be listed on the nutrition facts panel.

Typical foods. Enriched cereal grain products, some legumes (dried beans), peas, fresh leafy green vegetables, oranges, grapefruit, many berries, some dietary supplements, and fortified breakfast cereals.

#### Dietary soluble fiber, such as that found in whole oats and psyllium seed husk, and coronary heart disease

Sample claim. "Diets low in saturated fat and cholesterol that includes 3 g of soluble fiber from whole oats per day may reduce the risk of heart disease. One serving of this whole-oats product provides ? g of this soluble fiber."

Requirements. Foods must meet criteria for "low saturated fat," "low cholesterol," and "low fat." Foods that contain whole oats must contain at least 0.75 g of soluble fiber per serving. Foods that contain psyllium seed husk must contain at least 1.7 g of soluble fiber per serving.

Typical foods. Oatmeal cookies, muffins, breads and other foods made with rolled oats, oat bran, or whole oat flour; hot and cold breakfast cereals containing whole oats or psyllium seed husk; and dietary supplements containing psyllium seed husk.

#### Whole grain and heart disease

Diets rich in whole grain foods and other plant foods and low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and some cancers."

Dietary fiber content at least 1.7 g per 35 g serving.

Typical foods. Breads and cereals.

### Soy protein and heart disease

Sample claim. Diets low in saturated fat and cholesterol that include 25 g of soy protein a day may reduce the risk of heart disease. One serving of (name of food) provides ? grams of soy protein.

Requirements. Scientific studies show that 25 g of soy protein daily in the diet is needed to show a significant cholesterol lowering effect. In order to qualify for this health claim, a food must contain at least 6.25 g of soy protein per serving, the amount that is onefourth of the effective level of 25 g per day.

Typical foods. Soy beverages, tofu, temper, soy-based meat alternatives, and possibly some baked goods.

# Vitamin B<sub>12</sub>, B<sub>6</sub>, and folic acid and reduced risk of heart disease

Sample claim. Foods containing vitamin B<sub>12</sub>, as well as B<sub>6</sub> and folic acid, may reduce the risk of coronary heart disease. Typical foods. Fortified cereal products.

product label. The USDA seal also assures imported **Table 8** The USDA organic label definitions organic food products have met the United States requirements.

There are four possible ways to use the organic label. These are given in Table 8.

# **Labeling of GM Grain**

Grain produced by biotechnology, if allowed to be sold, must be labeled in many countries. There are some exceptions. For example, grains that are sold in bulk would not need to be labeled in Australia. In the USA, labeling of GM grains is not required

100% Organic	Must contain only organically produced ingredients
	Can have organic seal
Organic	At least 95% of the products ingredients must be organic
	Can have organic seal
Made with organic	Must contain at least 70% organic
ingredients	ingredients
	Cannot have organic seal
Some organic	Products <70% organic ingredients
ingredients	Cannot use organic on main package panel
	Individual ingredients listed as "organic" on ingredient panel
	Cannot have organic seal
	Calliot have organic seal

Table 9 Grain labeling terminology for some common grains

Grain	Whole or virtually whole grain	Refined grain
Wheat	Whole wheat flour, whole wheat pastry flour	Wheat flour, white flour, refined flour, cake flour, pastry flour, all purpose flour, bread flour, gluten flour
	Wheat berries, whole grain farro, bulgur, cracked wheat, rolled wheat	Farina
	Whole wheat durum and whole wheat semolina, whole wheat cous cous	Durum and semolina, cous cous
Spelt	Whole grain spelt and whole grain spelt flour	Spelt and spelt flour
Rye	Whole rye or pumpernickel rye Rye berries, rolled rye	Light or white rye often listed as rye
Oats	Steel-cut oats, rolled oats, quick oats, old-fashioned oats, whole oat flour	
Corn	Ground whole cornmeal, whole grain masa harina	De-germed white or yellow cornmeal, hominy, grits
Rice	Brown rice <sup>a</sup> , wehini and other colored rices (wild rice), brown rice flour	White rice, enriched rice
Barley	Whole or naked barley, minimally pearled barley, and their flours	Fully pearled barley and its flour
Quinoa	Whole or crushed	

<sup>&</sup>lt;sup>a</sup> Grain length and variety do not matter – in other words there can be long grain, brown "basamati," and there can be the refined version as white basamati.

as the US FDA views that there is no safety issue associated with these products.

# Labeling Issues Specific to Milled or Processed Grain

Specific terminology is used to describe various grain components from various stages of milling and processing. Consumers are frequently unable to determine if a product is made from whole or refined grain and will incorrectly label a product as whole grain when it is not (Table 9). They often use color as a guide or infer incorrectly from a myriad of label and ingredient terms. For some, whole grain must refer to grain that is in its uncrushed, innate state and reject all but whole kernels (berries) as having the label of whole grain foods. However, this view is not widely held and most think that whole grain can be the crushed parts of that grain so long as all the parts are found in proportions that are present in the intact kernel.

Color is often used by consumers to determine if a product is whole grain. Thus, rye breads are often thought by US consumers to be whole grain because of their dark color. However, many US rye breads are made primarily with rye endosperm and colored with caramel color or molasses. Even those products sold as "pumpernickel bread" in the USA may contain little whole grain rye even though the bread's namesake was originally made with whole grain rye and pumpernickel flour is whole grain rye. Color for wheat breads can be equally deceptive where there

is little or not whole grain but the product is deemed by consumers as dark bread and therefore wholegrain containing. Some whole grain cereals, especially oats and corn, are light in color so consumers mistakenly think the products from these are from refined grains.

Label descriptors are also confusing. Some consumers assume that descriptor words such as organic, natural, or 100% Red River Valley wheat makes the product whole grain. Many do not know that when wheat flour is the first ingredient, it means that the flour is from milled wheat and is a refined product. Some people do not understand that enriched flour means that nutrients have been added to white flour. Depending on the proportions of other ingredients it may or may not provide a significant amount of whole grain to the diet. Products labeled "multigrain," "seven grain," or "nine grain" may contain relatively little whole grain. Even products listed as containing either bran or germ are not technically whole grain and in many products these ingredients are not found in high amounts. Only when products are labeled as made from 100% whole grains or have whole grain as the first listed ingredient can the consumer be certain that 51% of more of the grains in the product are whole grains.

Currently, there is a whole grain health claim in the USA and the UK to help consumers identify products that are whole grain. This needs to be modified as the health claim works well for low-water products such as cereals but works poorly for products such as bread which are high in water or cookies, which, even if

prepared with all whole grain flour, would not have 51% of the total ingredients as whole grain. Perhaps a seal which tells the consumer the portion of whole grain per serving would help make the label more consumer and whole grain friendly.

# **Summary**

Labeling of grain-based foods helps consumers make an informed purchase to meet their individualized nutritional needs. Legally, labeling helps with compliance and consumer protection. Nutritionally labeled products are found in many major markets. With products and the global markets expanding, perhaps a standardized format would be useful to both the processor and the consumer. Organic labeling protocols are found in many parts of the globe and are popular for many consumers as a way to avoid GMOs and for a sense of increased control of their food choices and perhaps some environmental benefits. So far there has been little scientific evidence of the nutritional or food safety benefits of organic grain-based foods over conventional ones.

Terms surrounding grains and their milling treatment such as pumpernickel, pearled, and de-germed continue to confuse consumers and the ability of consumers to make informed choices as to what is whole grain difficult. Agreement as to what is whole grain and how to label it must be sought. A seal or an expanded health claim to help consumers may be important if the goal to increase whole grain consumption is to be met.

See also: Consumer Trends in Consumption. Genetically Modified Grains and the Consumer. Organic Growing of Grains. Plants: Whole-Plant Utilization. Whole-Grain versus Refined Products.

# **Further Reading**

Federal Register (1993) Fed Register 58: 631–691; 2302–2941.

Federal Register (1998g) Food labeling: health claims; whole grains and cardiovascular disease — FDA. *Interim final rule. Fed. Regist.* 22; 63(119): 34107–1.

Federal Register (1998g) Food labeling: health claims; B-complex vitamins, lowered homocysteine levels, and the risk in adults of cardiovascular disease – FDA. *Interim final rule. Fed. Regist.* 22; 63(119): 34097–101.

Federal Register (1999) Food labeling: health claims; soy protein and coronary heart disease. Food and Drug Administration, HHS. Final rule. 26; 64(206): 57700—33 (http://vm.cfsan.fda.gov/~lrd/tpsoypr2.html).

Federal Register: Fed. Regist. September 8, 2000. 65(175): 54685–54739 (http://vm.cfsan.fda.gov/~lrd/tpsterol. html).

Federal Register: December 5, 2000b. (Volume 65, Number 234) Rules and Regulations. pp. 76091–76114. (From the Federal Register Online via GPO Access www.wais.access.gpo.gov visited, Feb. 2001.)

Hutt PB (2001) Guide to US Food Labeling Law. Washington, DC: Thompson Publishing Group. Jones JM (1992) Food Safety. Eagan, MN: Eagan Press. Krebs J (2003) Food Standards Agency Chair, "Is Organic

Food Better for You?" 4 June 2003, speech.

#### **Relevant Websites**

http://www.oznet.ksu.edu — Higgins MH (2002) Healthful Whole Grains! Kansas State University. http://www.fda.gov — US Food and Drug Administration.

www.wheatfoods.org — Wheat Foods Council. www.foodstandards.gov.uk — Food Standards Agency.

www.cspinet.org — Health Canada. www.nin.ca — Canadian National Institute of Nutrition.

# LENTIL

Contents

**Breeding** Agronomy

# **Breeding**

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#### Introduction

Lentil (Lens culinaris Medikus subsp. culinaris) is an important pulse crop that has been traditionally grown in West Asia, South Asia, Ethiopia, North Africa, and, to a lesser extent, in southern Europe. It is also cultivated in South and North America, and in Oceania. Its seed is a rich source of protein and other micronutrients (Fe, Zn,  $\beta$ -Carotene) for human consumption, and the straw is a valued animal feed. Since its domestication, lentil has been grown in rotation with cereals, which provides a sustainable cropping system.

Although lentil was cultivated as early as 8000 BC in West Asia, it remained an under-exploited and underresearched crop until recently. Systematic research for its improvement started in some national institutions and at the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria, in the 1980s. Under the Consultative Group of International Agricultural Research (CGIAR), ICARDA has a world mandate for lentil improvement. The lentil improvement program at ICARDA is closely linked with the national agricultural research systems and with advanced research institutes in the world to address this mission.

The breeding objectives at ICARDA and in national programs are targeted to address the specific needs of different agro-ecological regions. A world collection of wild and cultivated lentil germplasm is maintained at ICARDA and has been instrumental in the development of improved genetic stocks suitable for different environmental niches. Except for a few traits, sufficient variability for important economic characters including stress resistance is present in these germplasms for use in breeding program. Following a bulk-pedigree method, the lentil-breeding program at ICARDA constructs new genotypes to deliver to the national programs. To date, a total of 81 lentil varieties with combination of various desirable traits have been released by 29 national programs emanated from ICARDA-supplied germplasm.

# **Origin and Domestication**

Cultivated lentils originated in the Near East arc. Lens culinaris Medikus ssp. orientalis (Boiss.) Ponert, which closely resembles the cultivated species L. culinaris ssp. culinaris, is widely accepted as the progenitor species. From archeological evidence of carbonized remains it is concluded that the progenitor of cultivated lentil, L. culinaris ssp. Orientalis, originated in the Near East arc. Such carbonized remains appear in early Neolithic settlements which date back to 7000-6000 BC. Ladizinsky reviewed evidence for the center of origin and domestication of lentil.

The domestication of lentil occurred, together with that of emmer and einkorn wheat, barley, pea, chickpea, bitter vetch, and flax, during the Neolithic Agricultural Revolution, which is expected to have taken place in the eastern Mediterranean around the eighth and seventh millennia BC. Lentil culture spread rapidly with that of Neolithic agriculture to the Nile Valley, Europe, and Central Asia. It was part of the Harappan crop assemblage in Indian subcontinent between 2250 and 1750 BC. After AD 1500, the Spanish introduced lentil to South America via Chile. More recently, it has been cultivated in Mexico, Canada, the USA, New Zealand, and Australia. In North America, research on adaptation of lentil started in the late 1970s and it became an important crop of Canada and the USA. According to recent reports, Canada ranks third in area and production, of which  $\sim$ 95% is grown in the province of Saskatchewan. Lentil became an important crop component in the Palouse region of northwestern USA. Lentils were introduced in Australia very recently with only 500 ha under cultivation in 1993. Currently, however, it is grown in 125 000 ha of land with the state of Victoria being the major producer.

# **Phylogeny**

Tournefort was the first to use the word *Lens*, a Latin word to designate a specific genus that describes the seed shape of the cultivated lentil. The genus Lens Miller belongs to the order Rosales, suborder Rosinae, family Leguminosae, and subfamily Papilionaceae and is in the tribe Vicieae. All species in the genus are diploid with 2n = 14 chromosomes and have similar karyotypes.

The taxonomy of *Lens* has undergone numerous changes in recent years. Analyzing previous findings based on origin and spread, morphological, cytological, cytogenetic observation and more recently on the basis of isozyme and molecular studies, Lens taxonomy has been reassessed. The genus now consists of seven taxa split into four species.

- 1. Lens culinaris Medikus;
  - (a) subsp. *culinaris*
  - (b) subsp. orientalis (Boiss.) Ponert
  - (c) subsp. tomentosus (Ladiz.) M. E. Ferguson et al.
  - (d) subsp. odemensis (Ladiz.) M. E. Ferguson et al.
- 2. Lens ervoides (Brign.) Grande;
- 3. Lens nigricans (M. Bieb.) Godr.; and
- 4. Lens lamottei Czefr.

# **Use of Lentil and Its Nutritional Quality**

Lentil is predominantly eaten in South Asia as boiled or fried "dhal." It has a soup-like consistency and is usually eaten with unleavened bread ("roti"). Boiled rice is also served as a staple with lentil dhal. "Khichuri" is made from a mixture of split/de-hulled lentil and cracked wheat or rice. In West Asia and North Africa, "Mujaddarah," made of whole lentil and immature wheat seed, is a popular dish. Of course, lentil soup is popular all over. Also, lentil may be deep-fried and eaten as a snack, or combined with cereal flour in the preparation of foods such as bread and cake.

Numerous lentil-based foods require steaming or boiling the whole seeds, whereas others, including many versions of the popular lentil soup, call for the removal of the fibrous seed testa (de-hulling). Lentil processing includes cleaning, sizing, de-hulling, splitting, and polishing. Only red cotyledon, smallseeded lentil is de-hulled and large-seeded yellow cotyledon lentil is used as whole.

In West Asia, lentil is cultivated for its straw, in addition to its grain. In Syria, for example, a farmer's revenue from straw in a dry year is sometimes greater than from the seed. The straw comes from the traditional threshing process and includes broken branches, pod walls, and leaflets. The protein content of lentil straw varies from 5% to 7% and its

digestible dry matter from 43% to 46%. There is limited genetic variation in straw quality.

The pattern of nutrients in the proximal composition of lentil is similar to that of other grain legumes, but seed protein content (19.5-35.5%), although similar to that of peas (Pisum sativum L.) and Phaseolus vulgaris L., is less than in sovbean (Glycine max L. Merr.). Seeds contain low levels of fat. Fiber concentration is low and is largely within the seed testa, so the fiber in lentil meal can be reduced if it is de-hulled before grinding. In addition to highquality protein, essential amino acids, and major minerals, its seed contains iron up to 505 mg per kg and zinc up to 330 mg per kg on a whole seed basis. Besides, among various vitamins, lentil seed contains 200 mg per kg of  $\beta$ -carotene.

The amino acid composition of the seed, as in other grain legumes, is complementary to that of cereals. The relatively large concentrations of lysine compensate for the minimal concentrations in the cereal grains, while the cereal grains compensate for the minimum concentrations of sulfur-containing amino acids in lentil.

# **Constraints of Production** Addressable by Breeding

Average lentil yields are low because of limited yield potential of landraces, which are also vulnerable to a range of stresses. The major abiotic limiting factors to lentil production are low-moisture availability and high-temperature stress in spring, and, at high elevations, cold temperatures in winter. Among biotic stresses, the diseases rust, vascular wilt, and Ascochyta blight, caused by Uromyces viciae-fabae (Pers.) Schroet., Fusarium oxysporum F. sp. lentis, and Ascochyta fabae Speg. F. sp. lentis, respectively, are globally important key fungal pathogens of lentil. Other diseases such as botrytis blight (Botrytis cinerea Pers.), stemphylium blight (Stemphylium botryosum Wallr.), collar rot (Sclerotium rolfsii Sacc.), root rot (Rhizoctonia solani Kuhn), and stem rot (Sclerotinia sclerotiorum (Lib.) de Bary) are localized problems. Besides, constraints to productivity include agronomic problems of pod dehiscence and lodging.

Adequate variability for all these traits is known to exist within the genetic resources of the crop (wild and cultivated), allowing manipulation through plant breeding. By contrast, several other important traits (such as biomass yield, pod shedding, nitrogen fixation, resistance to pea leaf weevil (Sitona sp.) and aphids, and the parasitic weed broomrape (Orobanche sp.)) are not currently addressable by breeding because of insufficient genetic variation.

# Available Genetic Resources and Variation among Them

The lentil-breeding program at ICARDA is built upon the foundation of the germplasm collections and their efficient use. A large number of germplasm accessions are conserved at ICARDA under FAO auspices, elsewhere at the National Bureau of Plant Genetic Resources, New Delhi, India, Vavilov Institute of Plant Industry, St. Petersburg, Russia, and at United States Department of Agriculture (USDA), USA. The ICARDA collection is by far the largest which comprises 9646 cultivated and 479 wild relatives (Table 1). Among cultivated species conserved at ICARDA, ~11% have been developed at ICARDA through cross-breeding, and dispatched to national programs through the International Nursery Network. The Genetic Resources Unit of ICARDA has

Table 1 Genetic resources conserved at ICARDA

Name of taxa	Accessions	Countries of collection
L. culinaris subsp. culinaris		
Landraces	8748	68
Breeding lines (ICARDA)	898	
L. culinaris subsp. orientalis	225	12
L. culinaris. subsp. tomentosus	9	3
L. culinaris. subsp. odemensis	59	4
L. erviodes	131	11
L. nigricans	46	7
L. lamottei	9	3

characterized 7500 for various morphological and phenological traits to date.

Marked variation among the characters for use in breeding and selection programs have been reported for various morphological characters, response in flowering to temperature and photoperiod, winter-hardiness, iron-deficiency chlorosis, and boron imbalances, drought tolerance and resistance to fungal diseases (Figure 1), and viruses. Wild relatives also have shown considerable variability for morphological traits, winterhardiness, and resistance to drought *Fusarium* wilt, and *Ascochyta* blight resistance.

# **Recent Trends in Lentil Production**

World lentil production tripled since 1970s, from 1.05 million tons (Mt) in 1971 to 3.07 Mt in 2001, through a 119% increase in sown area and a 33.4% increase in average national yield from 611 to 815 kg ha<sup>-1</sup> (Table 2). Three top-ranking countries, viz., India, Canada, and Turkey, increased their productivity and production. Although area under lentil cultivation in Turkey has gone down in the recent

**Table 2** Trend in world lentil production (1971–2001)

Year	Area (Mha)	Production (mt)	Yield (kg ha <sup>-1</sup> )
1971	1.72	1.05	611
1981 1991	2.27 3.26	1.45 2.66	640 814
2001	3.77	3.07	815



Figure 1 Variation in maturity in lentil (drought escape through earliness).

years, it has increased greatly in India, Canada, Australia, and Ethiopia. Production in Asia is concentrated in a band stretching from Turkey in the west, to Bangladesh in the east, accounting for ~74% of world production. Among other Asian producers, China has recently started releasing lentil-related data and Bangladesh increased its productivity through release and cultivation of improved varieties. Iran, Nepal, and Syria have substantially increased production during the 1990s, whereas lentil-cultivated area and production in Pakistan has declined.

Twenty-three percent of lentil production is in North America, where, in addition to Canada, the USA maintains an area of ~80 000 ha and Mexico is a minor producer. In Africa, Ethiopia and Morocco are significant producers and Algeria, Sudan, Egypt, and Tunisia are minor producers. In South America, Argentina and Peru are major producers among six lentil-producing countries in the region. European lentil production is gradually decreasing, with France and Spain being the significant producers. In Oceania, Australia has come up as a significant producer with a production of 180 000 t in 2001.

International trade in small-seeded, red cotyledon lentil is dominated by Australia, Canada, and Turkey, whereas trade in the large-seeded, green lentil is primarily led by Canada and USA. Countries in the Indian subcontinent, and the Middle East are the major importers of red lentil, and southern Europe and South America import large-seeded green lentils.

# Major Agro-Ecology and Breeding Objectives

Knowledge of the patterns of variation in the world germplasm collection is the key to understand factors affecting lentil adaptation to direct lentil breeding. The geographic distribution of variation of landraces in the world lentil collection for morphological characters, responses in flowering to temperature and photoperiod, winterhardiness, iron-deficiency chlorosis, and boron imbalances collectively illustrate the specificity of adaptation in lentil. Additional information on the specificity of adaptation within the crop has come from collaborative yield trials of common entries selected in different locations.

Understanding of genotypes and environmental factors, the local constraints to production, and the various consumer requirements of different geographic areas for seed as food and straw as feed has led the breeding program at ICARDA to develop new genetic materials for a series of separate, but finely geographically targeted streams, linked closely to national breeding programs.

The major agro-ecological regions of production of lentil are: (1) S. Asia, E. Africa, and Yemen; (2) mediterranean low-to-medium elevation; and (3) high-elevation area of West Asia and North Africa. These regions correspond to the maturity groups of early, medium, and late maturity (Table 3). Within each of these major regions, there are specific target areas. Additionally, the lentil improvement activities have recently been extended to Central Asia and Caucasus (CAC) region, where initial thrust has been put to study the adaptation and screening of diverse material suitable in their agro-climatic conditions. For Latin America, large-seed, yellow cotyledon lentils are preferred.

The lentil-breeding program generally uses parents of diverse origin with known traits with the aim to combine gene(s) to contribute to yield and resistance to major biotic and abiotic stresses. Wide crosses

Table 3 Target agro-ecological regions of production of lentil and key breeding aims

Region	Key traits for recombination
Mediterranean low to medium elevation	
300-400 mm ann. rainfall	Biomass (seed + straw), attributes for mechanical harvest and wilt resistance
< 300 mm ann. rainfall	Biomass, drought escape through earliness and wilt resistance
Morocco	Biomass, attributes for mechanical harvest and combined resistance to rust, Ascochyta blight, and wilt
Egypt	Seed yield, response to irrigation, earliness, and wilt resistance
High elevation	
Anatolian highlands	Biomass, winterhardiness, and A. blight resistance
N. African highlands	Seed yield, low level of winterhardiness, and rust resistance
South Asia and E. Africa	
India, Pakistan, Nepal, and Ethiopia	Seed yield, early maturity, resistance to root diseases, rust, and A. blight
Bangladesh	Seed yield, extra-earliness and combined resistance to rust, and Stemphylium blight diseases
Central Asia and Caucasus	Seed yield, large-seed, good standing ability
Latin America	Large-seed, resistant to rust, and A. blight

among cultigens are also done by manipulating planting dates and providing 18 h extended light period to the parents to attain synchrony in flowering. In addition, crosses are made to study inheritance pattern of specific trait(s) and to develop recombinant populations for biotechnological research. More than 200 crosses are made at ICARDA every year.

# **Breeding Methodologies**

In the early stages of lentil varietal development, most of the cultivars released were derived from selection within heterogeneous landraces. Due to increased efforts in lentil breeding at both the national and international levels, new lentil cultivars/genotypes are now being developed through cross-breeding. The methods of breeding lentil are similar to those utilized to breed other self-pollinated crops and include pure line selection or hybridization followed by the bulk method, the pedigree method, the single seed descent, or some modification of these procedures. At ICARDA a bulk-pedigree method is used, where single plant selection is done from F<sub>4</sub> bulks to develop F<sub>5</sub> progeny. A scheme of breeding methodology followed at ICARDA is given below:

- 1. hybridization (parent A × parent B): at Tel Hadya, ICARDA (year 1);
- 2. confirmation of hybridity  $(F_1)$ : at summer nursery, Lebanon (year 1);
- 3. growing F<sub>2</sub> bulks: at Tel Hadya, ICARDA (year 2);

- 4. growing F<sub>3</sub> generation: at summer nursery, Lebanon (year 2);
- 5. growing F<sub>4</sub> generation: single plant selection at Tel Hadya (year 3);
- 6. selection among F<sub>5</sub> progenies for agronomic traits and *Fusarium* wilt reaction (see **Figure 2**): at Tel Hadya (year 4);
- 7. F<sub>6</sub> progenies are evaluated for yield, agronomic traits and *F*. wilt reaction; at Tel Hadya (year 5);
- 8. preliminary yield trial in F<sub>7</sub> at three contrasting locations: Breda, Tel Hadya (Syria) and Terbol (Lebanon);
- advanced yield trial in F<sub>8</sub>: at three locations except those bred for southern latitude countries;
   and
- 10. incorporation into International nurseries in F<sub>9</sub> generation.

The breeding program at ICARDA follows a decentralized approach. For example, in breeding for early and extra-early genotypes for southern latitude countries, targeted segregating populations are sent to the national programs to perform single plant selection in their respective edapho-climatic conditions. Similarly, selection for rust and *Ascochyta* blight resistance is done in hot spots in close collaboration with the national programs.

# **Understanding of Genetic Control**

It is important to know the genetic control of a character before initiating a breeding program for



**Figure 2** Screening against *Fusarium* wilt in lentil at Tel Hadya, ICARDA, Syria. Susceptible check and other test lines affected by the disease (in straw color).

the genetic enhancement of that particular trait. In this endeavor, various authors have studied inheritance patterns of important qualitative and quantitative traits. Information on genetic control has progressed from relatively few characterized genes in the early 1980s to the identification of major genes controlling morphological traits, isozyme loci, and DNA amplification. A list of the traits controlled by major gene(s) with their gene symbols is presented in Table 4.

Estimates of genetic parameters for traits are very useful since they provide information on the inheritance of such traits and help to identify

Table 4 Inheritance of morphological markers

Symbol/ allele	Character	Reference
Fn	Flower number/ inflorescence	Gill and Malhotra (1980)
sn	Early flowering	Sarker <i>et al.</i> (1999)
Gh	Plant growth habit	Ladizinsky (1979)
Gs	Epicotyl color	Ladizinsky (1979)
1	Cotyledon color	Slinkard (1978a)
Yc	Cotyledon color	Slinkard (1978b)
0	Cotyledon color	Singh (1978); Sinha <i>et al.</i> (1987)
Y-B-	Cotyledon color	Sharma and Emami (2000)
Ggc	Gray seedcoat ground color	Vandenberg and Slinkard (1990)
Tgc	Tan seedcoat ground color	Vandenberg and Slinkard (1990)
P	Flower color	Lal and Srivastava (1975)
V	Flower color	Lal and Srivastava (1975); Wilson and Hudson (1978)
W	Flower color	Wilson and Hudson (1978)
Pi	Pod indehiscence	Ladizinsky (1979)
Scp	Seedcoat spotting	Ladizinsky (1979)
Pep	Pubescent peduncle	Sarker <i>et al.</i> (1999)
Glp	Glabrous pod	Vandenberg and Slinkard (1989)
Grp	Green pod color	Vandenberg and Slinkard (1989)
Tnl	Tendril-less leaf	Vandenberg and Slinkard (1989)
Ten	Tendrilled leaf	Sharma and Sharma (1978)
Chl	Chlorina chlorophyll mutant	Vandenberg and Slinkard (1989)
glo	Globe mutant	Gupta et al. (1983)
Sbv	Resistant to PSbMV	Haddad et al. (1978)
Rr	Resistant to rust	Sinha and Yadav (1989); Singh and Singh (1990); Sarker <i>et al.</i> (1990)
Urf1, urf2, urf3	Resistant to rust	Kumar <i>et al.</i> (2001)
Fw	Resistant to Fusarium wilt	Eujayl et al. (1998)
Frt	Radiation frost tolerance	Eujayl <i>et al.</i> (1999)

appropriate breeding methods. Such parameters include subdivision of genetic variance into additive, dominance, and epistatis effects of minor genes. Additionally, parameters such as heritability, expected genetic gain in response to selection, and degree of association between traits are also important to design effective breeding and selection programs. In lentil, a self-pollinated species, genetic variance is expected to be primarily additive; however, the non-additive genetic variance could also be important. Additional information on the components of genetic variance in lentil suggests that additive effects are the major component of the genetic variance for most polygenic traits and that a considerable nonadditive component can be anticipated in early generations.

Again, heritability estimates are indicative of making progress through selection. Additiveness of gene action reflects in high heritability. The heritability estimates for seed yield and its components are moderate to high which indicate that good progress can be expected from effective selection. Heritability estimates for several plant traits such as plant height, lowest plant height, etc. are quite variable. Reproductive traits, such as days from sowing to flowering and to maturity, had moderate to high heritability, indicating that these traits would respond to selection. Seed quality traits such as seed size and seed thickness are strongly heritable.

Correlations between traits must be given careful consideration and interpretation by plant breeders. Correlations between seed yield and number of pods per plant, seeds per pod and secondary branches per plant, and plant height and straw yield have been positive and significant.

# **Evolution of National and International Breeding Programs**

Lentil breeding strategy at national level and at ICARDA has changed with time. In early days, in stage 1, the variation in the germplasm collection was directly exploited. Selection was made among and within locally adapted landraces. These selections were distributed to national programs through the International Nursery Network to test for local adaptation. As a result many cultivars released by national programs are actually selections from landraces in the ICARDA germplasm collection. These stage 1 registrations emphasize: (1) the value of direct exploitation of landraces and (2) the under-exploited nature of lentil germplasm.

The particular combinations of characters required for specific regions were often not found "on the shelf" in the germplasm collection. Consequently, ICARDA started hybridization and selections from segregating populations were made at ICARDA in Syria to produce stage 2 material. Such selections were then distributed after multiplication to the national programs to select in their respective agroclimatic conditions. This has resulted in the release of a number of cultivars in different regions.

However, lentil lines developed from selection at ICARDA in West Asia are mostly limited to adaptation to the home region. As a result, the breeding program has decentralized to work closely with national programs. For the other regions, at stage 3, crosses are agreed upon with national level cooperators and then made at ICARDA, Tel Hadya, Syria, and then country-specific segregating populations shipped to national cooperators for local selection. Selections made by national programs are then fed back into the International Nursery Network for wider distribution.

In stage 4, the national programs directly use ICARDA-derived material in hybridization and selections are made locally.

# **Developments in Biotechnology**

Biochemical and molecular techniques have been used for biodiversity evaluation, assessment of the genetic structure of natural populations, plant systematic and evolution in the genus *Lens* summarized by Ferguson and Robertson, Abo-elwafa, Sharma *et al.* Major investigations were carried out with allozymes, seed protein cDNA and genomic DNA RFLPs, chloroplast DNA RFLPs, and RAPD analysis of genomic DNA. Even though discrimination between lines is possible, seed proteins have not been extensively used for genetic diversity studies.

Establishing linkage maps for agricultural crops is to localize gene(s) for important agronomic traits and to develop tightly linked morphological and molecular and markers to enable indirect selection by marker assisted selection. The earlier linkage maps covered

a small number of markers. With the advancement of classical genetics and molecular research, a more comprehensive linkage map for lentil genome spanning more than 1073 cM has been developed.

Qualitative traits such as epicotyl color, seedcoat pattern or spotting, pod indehiscence, etc. have been localized, and linked DNA markers have been identified. Quantitative traits in recombinant inbred lines of interspecific crosses have been localized with isozyme markers. Detected quantitative trait loci (QTLs) were located in six of the seven chromosomes. Abbo et al. studied the genetics and linkage of seed weight and observed QTLs affecting seed weight were associated with morphological and RAPD-markers, which were distributed over several linkage groups. Recently, one major and two minor QTLs were detected for winter-hardiness.

# **Major Achievements**

#### **Varietal Releases by National Programs**

ICARDA supplies nurseries to national programs comprising a range of genetically fixed materials and segregating populations to select according to their specific needs. On the basis of phenological adaptation, agronomically desirable traits, resistance to prevailing stresses, quality aspects, farmer's and consumer's preference, etc., national scientists identify and select promising lines/single plants for eventual release for commercial cultivation. In this endeavor, to date 77 lentil varieties have been registered by 29 countries for improved yield, disease resistance, and other traits (Table 5).

One of the major achievements is the breaking an ancient bottleneck of narrow genetic base of lentil in South Asia, which produces nearly half of world's lentil. Genetic base was broadened through introgression of genes from ICARDA in the region. Early, high-yielding and disease-resistant varieties have been released in Bangladesh. High-yielding varieties with combined resistance to fungal diseases have been

Table 5 Lentil varieties emanated from ICARDA-supplied materials released by the National Agricultural Research Systems

Region	Country	No. of varieties	Reason
Asia	Bangladesh, India, Nepal, Pakistan, China, Afghanistan, Iran, Iraq, Syria, Lebanon, Jordan, Yemen, Turkey	37	High yield; wilt, rust and <i>Ascochyta</i> blight resistance; good standing ability, resistance, high biomass, early maturity, winterhardiness
Africa	Ethiopia, Egypt, Morocco, Libya, Tunisia, Algeria, Lesotho, Sudan	26	High yield; wilt and rust resistance; early maturity; tolerance to excess moisture
The Americas	Argentina, Canada, Ecuador, USA	6	High yield; rust and A. blight resistance; good standing ability
Oceania	Australia, New Zealand	9	High yield; A. blight resistance; good standing ability
Europe	Portugal	2	High yield
Caucasus	Georgia	1	High yield

released in Pakistan. Extra-early and extra-bold lines have been developed in India to fit in different cropping system niches. Medium maturing cultivar, Shekher (ILL 4404), is being cultivated in mid-hills in Nepal, a new lentil area.

Ethiopia and Morocco released rust resistant varieties, spreading very fast among the farmers. In West Asia, wilt resistant varieties provide momentum in lentil cultivation. All Australian varieties covering an area of 125 000 ha were from direct selection from ICARDA germplasm.

## **Reconstruction of Genotypes Suitable for** Mechanization

To keep lentil in the cropping system by reducing cost of production in West Asia, it is essential that lentil harvest be done mechanically. With more urbanization there has been a tremendous shortage of agricultural labor in this region. This led to a rapid rise in the cost of hand labor, and the total lentil production cost goes up tremendously.

In order to address this constraint and encourage the expansion of the environmentally sustainable legume-cereal rotation in the dry areas, during its first decade ICARDA made a major drive to develop economic machine harvest systems for lentil cultivation. Following the introduction and use by farmers in Syria and Turkey of such systems, a moratorium was placed on further technical research at ICARDA. The Center has developed and promoted a lentil production package that includes mechanization and the use of improved cultivars with good standing ability. Joint ICARDA-NARS breeding programs have produced new varieties suitable for mechanical harvesting such as "Idlib-1" and "Idlib-2" in Syria and "Sayran 96" in Turkey. On-farm trials and demonstrations have verified the value of the mechanization package. On average, mechanical harvesting combined with improved cultivars reduces harvest costs by 17–20%.

# **Future Challenges**

Exciting gains in sustainable production arise from the integration of a change in agronomic practice with a new cultivar. Several such prospects exist for lentil and are given below.

In highlands West Asia, lentil is currently spring sown. However, sowing in late autumn/early winter coupled with the use of a winterhardy cultivar gives yield advantages of  $\sim$ 50%. Although agronomic constraints to winter-sowing lentil in the Highlands require further research, this substantial yield increase can be expected in the future to augment the area and production in this agro-ecological zone.

In the some areas around the Mediterranean, such as in south Syria, lentil production has ceased primarily because of vascular wilt disease. The registration of Fusarium wilt-resistant cultivars Idlib-2' in Syria and "Rachyy" and "Hala" in Lebanon offers the prospect of a return of lentil cultivation to such areas. Further spread of lentil harvest mechanization technology will probably also contribute to increased lentil production in West Asia in the future.

In South Asia, the diseases wilt, rust, and Ascochyta blight are key problems and large areas are left fallow over winter following the harvest of late paddy rice. Farmers need early maturing, disease-resistant varieties with late sowing potential for such situations or when land becomes available for lentil planting after the monsoon floodwaters have subsided. As extra-early maturing cultivars with combined resistance to diseases become available, the prospect will open of a major expansion of lentil production in the rice-based cultivation system in the Indian subcontinent.

Molecular tagging of genes responsible for resistance to biotic and abiotic stresses needs to be done to assist breeding program. Further, research on functional genomics to address some of the key problems should be initiated.

See also: Chickpea: Overview; Agronomy. Oven Technologies. Pea: Agronomy.

# **Further Reading**

Abbo S, Ladizinsky G, and Weeden NF (1992) Genetic analysis and linkage studies of seed weight in lentil. Euphytica 58: 259-266.

Abo-etywafa A, Murai K, and Shimada T (1995) Intra- and inter-specific variations in Lens revealed by RAPD markers. Theoretical and Applied Genetics 90: 335 - 340.

Bayaa B and Erskine W (1998) Diseases of lentil. In: Allen DJ and Lenne JM (eds.) The Pathology of Food and Pasture Legumes, pp. 423–472: CAB International.

Bhatty RS (1988) Composition and quality of lentil (Lens culinaris Medik.): a review. Canadian Institute of Food Science and Technology 21(2): 144–160.

Erskine W and Witcombe JR (1984) Lentil Germplasm Catalog. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

Erskine W, Hussain A, Tahir M, et al. (1994) Field evaluation of a model of photothermal flowering responses in a world lentil collection. Theoretical and Applied Genetics 88: 423-428.

Eujayal I, Baum M, Powel W, Erskine W, and Pehu E (1998) A genetic linkage map of lentil (Lens sp.) based on RAPD and AFLP markers using recombinant inbred lines. Theoretical and Applied Genetics 97: 83-89.

Hansen J and Renfrew JM (1978) Palaeolithic—Neolithic seed remains at Franchthi cave, Greece. *Nature* 271: 349–352.

Ladizinsky G (1979) The origin of lentil and its wild genepool. *Euphytica* 28: 179–187.

Muehlbauer FJ and Slinkard AE (1981) Genetics and breeding methodology. In: Webb C and Hawtin GC (eds.) *Lentils*, pp. 69–90. UK: Farnham Royal, Commonwealth Agricultural Bureau.

Savage GP (1988) The composition and nutritive value of lentils (*Lens culinaris*). *Nutrition Abstracts and Reviews* (*Series A*) 58: 320–343.

Tahir M, Simon CJ, and Muehlbauer FJ (1993) Gene map of lentil: a review. *LENS Newsletter* 20: 3–10.

Zohary D (1972) The wild progenitor and place of origin of the cultivated lentil *Lens culinaris*. *Economic Botany* 26: 326–332.

Zohary D and Hopf M (1993) Domestication of Plants in the Old World. Oxford: Clarendon Press.

# **Agronomy**

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#### Introduction

Lentil (Lens culinaris Medik) is one of the world's oldest crops having been cultivated in southwestern Asia since 7000 BC. The crop is best adapted for production in temperate climates but is now produced in different parts of the world. India is the world's leading consumer of lentil and the second leading producer. In North America, the United States of America (USA) and Canada are the main producers of lentil. Lentil has been grown commercially in the Palouse region of eastern Washington and northern Idaho since the late 1930s. Lentil production in Canada began in 1969, and currently Canada is the leading lentil producer and exporter. Turkey and Australia are major exporters of lentil. Other important lentil-producing countries include Syria, Ethiopia, Argentina, and Chile.

Agronomic requirements for lentil differ from region to region depending on the climatic conditions, cropping system, and variety. In Canada, USA, the

highlands of Turkey, and Iran, lentil is grown in spring on conserved moisture. In the Indian subcontinent, it is grown using soil moisture from the preceding monsoon season supplemented with infrequent winter rainfall. Lentil is also raised in winter in Australia and the lowlands of Turkey and Syria. In each of these growing environments, agronomic practices have been developed to suit climatic and soil conditions, and the predominant lentil varieties.

# **Description of Lentil**

The lentil is an annual herbaceous plant with a slender stem and branches. Plant height ranges from 20 to 75 cm. Lentil plants have indeterminate growth habit, continuing to flower and to produce pods until moisture, nutrient deficiency or temperature stresses occur.

Lentil is classified into two types based on seed size. "Macrosperma" or Chilean lentil has large seeds of  $\sim 6-9$  mm in diameter (mass greater than  $50\,\mathrm{g}/1000$  seeds). "Microsperma" or Persian lentil has small seeds of  $\sim 2-6$  mm in diameter (mass  $40\,\mathrm{g}$  or less/ 1000 seeds). Seeds of both types are generally lensshaped but some microsperma types have rounder seeds. The seedcoat may be red, green, gray, brown, black, or mottled. Cotyledons may be red, yellow, or green (see Lentil: Breeding).

# **Adaptation and Land Requirement**

Lentil is a cool season or temperate crop. The optimum temperature for growth is 18–24°C. The crop has moderate tolerance to drought or high temperatures. In some regions such as southeastern Turkey and Australia, lentil is a winter crop sown in autumn and maturing early in summer. The crop will not tolerate extremely cold, dry winters. Currently, work is under way in USA, Turkey, and the International Center for Agricultural Research in the Dry Areas (ICARDA) in Syria to improve the winterhardiness of the crop. Lentil plants are sensitive to waterlogging and will not tolerate flooding or salinity. Water requirement for optimum lentil yield varies from region to region depending on the rate of evaporation. Wet weather during the reproductive period of crop growth can delay maturity, reduce seed set, and increase susceptibility to foliar diseases.

Lentil is adapted to a wide range of soil types. In India, lentil is grown on different soil types ranging from heavy clay to loamy sands. In the Mediterranean region and in North Africa, fields of varying slope, soil texture and depth are used for lentil production. In southwestern Australia, lentil is grown in well-drained, neutral to alkaline loam, and clay loam

soils. Lentil can be grown from slightly acidic (pH 5.5-6.5) to moderately alkaline soil (pH 7.5-9.0).

# **Land Preparation and Rotations**

Land preparation required for lentil production varies depending on soil type and cropping system. Lentil can be grown under conventional, minimum till or zero till production systems. In the Indian subcontinent and parts of the Mediterranean region, land preparation is usually minimal to conserve moisture. In Africa, land is deep-plowed followed by cultivation with a disc harrow. In USA, autumn-chisel plowing is used to increase water infiltration, decrease erosion, and maximize retention of crop residues in fields intended for lentil production. Tillage in spring is kept minimal. In western Canada, grain yields and net returns for lentil grown on stubble using zero or minimum till are higher than those from conventional tillage systems. Tall stubble reduces soil moisture evaporation resulting in greater water-use efficiency and increased lentil yield. Tall stubble also increased pod clearance making combining or swathing easier and reducing pod shattering.

Throughout the world, the lentil crop is often grown in rotation with cereals. In India, lentil is generally grown in a double-cropping system following a monsoon-season crop of maize, sorghum, jute, or rice. The lentil crop is grown following a fallow where the monsoon rains are low. In eastern India and Nepal relay cropping is practiced with the lentil crop planted in a standing crop of paddy rice, when the rice crop is close to maturity. Multicropping is practiced under the humid subtropical conditions at the foothills of the Himalayas. For example, triple cropping of jute rice—lentil has been found to be more productive than a double cropping of either jute—lentil or rice—lentil.

In the Mediterranean region, North Africa, parts of Ethiopia, Eastern Europe, and North America, lentil is generally a component of a two-to-four course rotation incorporating cereals and forages. Lentil, like other pulses, is an important component of these rotations with advantages extending beyond the year in which the lentil is grown. Pulse crops improve the soil tilth, diversify the rotation, and increase the availability of nitrogen and soil organic matter. Pulse crops break the cereal-disease cycles resulting in low-disease pressure in cereal crops following pulse crops. Table 1 shows the performance of a wheat crop following either lentil or another wheat crop. Seed vield, straw yield, seed N yield, straw N yield, and disease severity were lower in the lentil-wheat rotation than in the wheat-wheat rotation.

# Seeding

Lentil can be seeded with a wide range of seeders ranging from hoe drills to air seeders. Like other pulse crops, lentil seed is very susceptible to damage during seeding; hence, it is important to handle seed carefully especially when using air seeders. Airflow should be kept at low speed and seed moisture should be kept at 14% minimum.

The rates of seed germination, seedling emergence, and growth are sensitive to temperature. Optimum germination for most lentil varieties occurs at 15–25°C. As temperature decreases, the germination process slows down. A seeding depth of 4-5 cm is recommended for good germination and growth.

# **Seeding Rates**

Recommended seeding rates for lentil vary from 40 kg ha<sup>-1</sup> to as high as 160 kg ha<sup>-1</sup>, depending on growing conditions and lentil variety. These rates are based on target population densities for the growing environment and should be adjusted for germination. Seeding rates of  $40 \,\mathrm{kg} \,\mathrm{ha}^{-1}$  are recommended for small-seeded lentil under Indian conditions. In west Asia and the Mediterranean region, seeding rates of 60-80 kg ha<sup>-1</sup> for small-seeded lentil and 120-160 kg ha<sup>-1</sup> for large-seeded lentil are recommended. Studies conducted recently in Mediterranean-like environments of southwestern Australia recommend a target population density of 150 plants m<sup>-2</sup> by using a sowing rate of 90-110 kg ha<sup>-1</sup>. In USA

Table 1 The effect of crop rotation on yield, N yield, and disease severity on the subsequent wheat crop

Crop rotation	Yield (kg ha <sup>-1</sup> )		N Yield (kg ha <sup>-1</sup> )		Diseases scores	
	Seed	Straw	Seed	Straw	Leaf diseases (0–11 scale)	Root diseases (0–4 scale)
Lentil-wheat	1868	2484	57.7	77.7	5.9	0.8
Wheat-wheat	1518	2160	40.7	52.5	8.0	1.4
Standard error	56	85	1.7	2.3	0.2	0.01

Adapted from Mooleki PS (2000) Synchronization of N Availability and Plant N Demand: N and Non-N Effects of Lentil to Subsequent Wheat Crops. PhD thesis, University of Saskatchewan, Saskatoon, Canada.

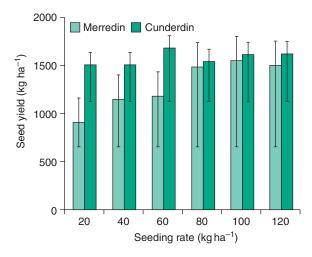


Figure 1 Effect of seeding rate on seed yield of lentil at Merredin and Cunderdin in southwestern Australia in 1996. Vertical lines are standard errors. (Adapted from Siddique KHM, Loss SP, Regan KL, and Pritchard DL (1998) Adaptation of lentil (Lens culinaris Medik) to short season Mediterranean-type environments: response to sowing time. Australian Journal of Agricultural Research 49: 1057-1066.)

recommended seeding rates for Palouse growers are 67-79 kg ha<sup>-1</sup> based on a target plant density of 90 plants m<sup>-2</sup>. In western Canada the target density is 130 plants m<sup>-2</sup> and recommended seed rates range from 45 to 90 kg ha<sup>-1</sup> depending on variety.

Some research shows that lentil yield is stable over a range of population densities while other studies report a general trend towards yield increase with increase in seeding rate. Differences in lentil varieties used, as well as growing conditions the crop is subjected to, can affect the response to seeding rate. For example, Figure 1 shows the effect of the growing environment on lentil seed yield response to seeding rate in southwestern Australia in 1996. In a dry environment like Merredin (mean growing season rainfall = 212 mm) lentil seed yield increased with increase in seeding rate, whereas in a wetter environment like Cunderdin (mean growing season rainfall = 274 mm), seed yield was relatively stable over a wide range of seeding rates. This indicates the limited ability of lentil plants to compensate for low density in dry environments. Compared to some other pulse crops, a lentil plant is small and has a more determinate growth habit. Higher plant population densities are required for early maturing lentil varieties compared to full-season lentil varieties that tend to have larger plant type.

Optimum row spacing depends on lentil variety and cropping system. Differences in lentil performance due to row spacing are more obvious in dry environments than in environments with adequate soil moisture. In narrow row spacings, the crop canopy closes

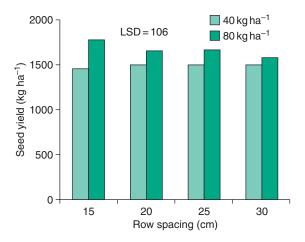


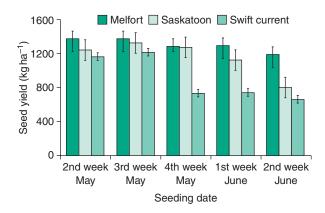
Figure 2 Effect of row spacing and seeding rate on seed yield of lentil line ILL-4401 at Tel Hadia, Syria, 1977-78. (Adapted from Saxena MC (1981) Agronomy of lentils. In: Webb C and Hawtin G (eds.) *Lentils*, pp. 111–129. London: Commonwealth Agricultural Bureau)

faster reducing loss of water by soil evaporation. However, it is the combination of plant density used with a particular row spacing that determines the performance of the lentil crop in a particular environment as shown in Figure 2. In a wet year in Syria, narrower row spacings combined with higher seeding rates gave greater lentil seed yield compared to wider row spacings with low or high seeding rates.

Broad-leaf weed management for the lentil crop is a serious problem wherever lentil is grown. In some environments, high seeding rates have suppressive effects on weeds. Using high plant densities and narrow rows is not advisable in wet environments, the thicker canopy may facilitate rapid spread of foliar diseases.

# Time of Seeding

The optimum date of seeding lentil varies from region to region. In all regions where lentil is grown, seed yield can be optimized by timing sowing such that crop development and growth occurs when climatic variables such as rainfall and temperature are favorable. In USA and Canada, lentil is usually seeded in early spring. Seeding begins as soon as the soil temperature at the seeding depth is  $\sim 5^{\circ}$ C. This usually occurs in late April to early May but wet conditions sometimes delay seeding. In western Canada, the yield advantage due to early seeding is substantial and the decrease in seed yield due to delay in seeding differs from one environment to the next. Figure 3 shows that the decrease in lentil yield with delay in seeding is more drastic at Swift Current (mean annual precipitation = 350 mm) a semiarid environment with lighttextured soils but that yield decrease is more gradual at Melfort (mean annual precipitation = 409 mm)



**Figure 3** Effect of seeding date and location on the seed yield of lentil. Vertical bars are standard errors. (Unpublished data: Nleya T, Gan Y, Vandenberg A, and Kutcher R (2003) Lentils for the Next Century: Diversification of Red Lentil Production Systems for Saskatchewan.)

a cool moist environment with heavier-textured soils. Saskatoon with intermediate conditions in terms of soil moisture and soil type showed a decrease between the two extreme locations. In southwestern Australia, a reduction in seed yield with delayed seeding as high as  $4-29 \,\mathrm{kg} \,\mathrm{ha}^{-1} \,\mathrm{day}^{-1}$  has been reported. A reduction in seed yield due to delay in seeding has been observed throughout most lentil growing regions in the world irrespective of the climatic conditions. This is because early sowing allows for an extended period of vegetative and reproductive growth.

In areas with mild winters such as west Asia and southwestern Australia where lentil is grown as a winter crop, early autumn seeding has advantage over late autumn or spring seeding. In temperate climates like in Canada, severe winter temperatures reduce plant density and yield for the autumn-seeded lentil crops compared to spring-seeded lentil crops (Table 2).

# **Fertilizer Requirements**

Lentil plants have high demand for plant nutrients. Lentil accumulates the maximum amount of nitrogen (N), phosphorus (P), and sulfur (S) in the seed; hence, a considerable amount of each of these nutrients is exported with the seed at harvest. Adequate application of the essential macronutrients including P, potassium (K), and S is necessary for good yields. Nitrogen is supplied via biological N fixation, mineralized soil N, or N fertilizers, or a combination of these sources. The best way to determine nutrient requirements is by soil tests.

Under good conditions for symbiotic association, 70–90% of the N requirement for the lentil crop can be met through biological N fixation, with the

**Table 2** Seed yield for the 2000 autumn-seeded and 2001 spring-seeded lentil at Saskatoon, SK

Lentil genotype	Seed yield (kg ha <sup>-1</sup> )		
	Autumn seeding	Spring seeding	
ILL 323	457	870	
ILL 465	509	914	
ILL 468	246	785	
ILL 632	468	1029	
L21	379	693	
ILL 1918	290	560	
Sazak'91	449	1121	
SPS ILL 669	742	1124	
ICARDA check	125	468	
LC 460053	644	1196	
Indianhead	921	1695	
Milestone	398	1141	
CDC Robin	993	1264	
CDC Sovereign	55	1414	
CDC Redcap	183	1251	
Mean	457	1035	

Unpublished data: Nleya T, Gan Y, Vandenberg A, and Kutcher R (2003) Lentils for the Next Century: Diversification of Red Lentil Production Systems for Saskatchewan.

remainder derived from soil N sources. Thus, a well nodulated lentil crop inoculated with an appropriate lentil rhizobium will generally not respond to the application of inorganic N fertilizer. Lentil growing in soils with low organic matter, in dry environments or seeded early into cool, wet soils may benefit from the application of low rates of N as a "starter N" source. However, because high levels of inorganic N are known to have adverse effects on early nodulation or will delay nodulation leading to poor seedling growth, starter N application rates should be limited to  $10-25 \text{ kg ha}^{-1}$ . Starter N rates greater than these amounts may encourage excessive vegetative growth and prolonged maturity, both of which lead to poor seed set. In wet conditions, too much vegetative growth may create a microclimate suited for disease development.

Adequate P nutrition is essential in lentil production for optimum N fixation, optimum seed yield and for the crop to mature in time. Phosphorus enhances root development and improves the ability of a lentil crop to tolerate stresses such as drought and frost. Although lentil has a relatively high requirement for P, seed yield responses to P fertilizer application are not consistent, even in soils low in available soil P. Moreover, responses to P fertilizer application are related to environmental factors, with greatest responses typically occurring in cool, wet soils.

Lentil seed is very sensitive to seed row applied P fertilizer and a significant reduction in plant stand and seed yield can occur at high P levels.

Small amounts of P ( $\sim$ 20 kg ha<sup>-1</sup> based on a narrow seed row at 15 cm spacing) placed with the seed may not be harmful and the adverse effect on plant stands may be more severe under drier soil conditions. Where P fertilizer application is necessary banding the fertilizer to the side away from the seed is recommended.

Lentil has a high need for K, requiring  $\sim$ 4.7 kg  $\rm K_2O$  for every 100 kg seed produced. However, response to K generally is limited to soils with a low capacity to supply K, such as sandy soils. Like P, K moves by diffusion in the soil; hence, soil temperature and moisture can influence movement and availability to plants.

Sulfur-deficient soils can limit yield of lentil. Plants deficient in S show yellowing from the top downwards, symptoms that are sometimes confused with N deficiency. The distinguishing feature is that with S deficiency top leaves yellow first. To correct S deficiency in the cropping system a sulfate form of S fertilizer must be used. Annual application of elemental S can be used to build S in the soil but will not be available to the crop in the year of application.

Researchers are uncertain of micronutrient requirements for productive lentil crops. Limited research has shown that deficiencies in zinc (Zn), molybdenum (Mo), and boron (B) may occur in lentil production.

Zinc deficiency occurs on alkaline soils and is widespread in soils used for paddy rice production in India, Nepal, and Pakistan where lentil grown after paddy rice has shown response to Zn fertilizer application. Zn deficiency can be corrected by soil application of zinc sulfate or foliar spray of zinc sulfate at the first appearance of deficiency symptoms.

Lentil, like other legume crops, requires Mo for the N fixation process. Mo deficiency is likely to occur in soils with pH lower than 5.6. Low levels of Mo in soils can cause poor growth and limit yield of lentil. Leaves of lentil deficient in Mo are chlorotic and plants are stunted. The Mo-deficiency symptoms are difficult to distinguish from N- and S-deficiency symptoms. Liming and application of P fertilizer can reduce the severity of Mo-deficiency in lentil growing in Mo deficient acid soils. Since Mo concentrations in soils are small and difficult to detect, fertilizer recommendations are often based on cropping history and soil pH. Mo fertilizer can be applied to the lentil crop as a seed treatment or a foliar spray.

Soils which are acidic, coarse-textured, and have low organic matter tend to be deficient in B. Boron deficiency has been observed in lentil fields in northwestern USA and in acidic nutrient-deficient soils in Nepal. Differences in B efficiency have been observed in lentil genotypes and seem to be associated with geographical origin. Boron deficiency in lentil can be corrected by broadcasting B fertilizer. It is

important that B requirements be determined by soil tests and that recommendations be followed when applying B fertilizer. Boron can be toxic to plants if higher concentrations are applied or if applied too close to seedlings. Boron toxicity problems have been reported in some regions of Australia.

# **Weed Management**

Lentil seedlings grow slowly early in the growing season, and at this stage the crop competes poorly with weeds for light, water, and nutrients. Weeds are particularly a problem in temperate climates where the cool weather at seeding time slows down the germination process and weeds can quickly overgrow the crop. Weed control in lentil therefore requires a long-term strategy involving different methods of control and proper crop rotations.

Preventative measures such as diversified crop rotations and tillage are an important part of long-term management strategy. Growing different crops in different years allows for use of different weed management options. Both the date when tillage is done and the kind of tillage have important implications on weed prevention. In temperate climates for example, autumn and spring preseeding tillage is used to control biennial and annual weeds. Preseeding spring tillage should be early enough to allow weed emergence and control before seeding the lentil crop. Where weed seeds are buried deep, shallow tillage can be used to avoid bringing weed seeds close to the surface where they can germinate.

Post-emergence harrowing is generally not recommended because this method can damage seedlings and increase incidence of root and stem diseases. Whenever this method is used it is important that the crop be short, and that the foliage is dry and that operation be conducted on a warm sunny day.

Handweeding with a hoe or hand pulling is a traditional method of weed control and is still practiced in lentil production in countries such as India, North Africa, and West Asia. The number of weedings required depend on climatic conditions and weed infestation level.

Only a limited number of herbicides are registered for use in lentil worldwide. Different herbicides are used to control weeds in lentil in different regions of the world. In USA and Canada Metribuzin<sup>TM</sup> is registered for the control of broad-leaf weeds preemergence and post-emergence. Trifluralin<sup>TM</sup> is applied in the autumn to control both grass and broad-leaf weeds in western Canada. In Australia and New Zealand, successful weed control in lentil has been achieved by using Trifluralin<sup>TM</sup> presowing, Cyanazine<sup>TM</sup> pre-emergence and Metribuzin<sup>TM</sup>

post-emergence. Glyphosate<sup>TM</sup> can be used as a preemergent burn-off treatment to control weed before crop emergence and is also registered for preharvest weed control in lentil. Herbicide users should follow labels for recommended application time, rates, and precautions to be taken.

## **Diseases**

Lentil is affected by many diseases caused by fungi and viruses. Major diseases caused by fungi include the seedling blight/root rot complex, wilt, sclerotinia stem and pod rot, botrytis stem and pod rot, rust, *Ascochyta* blight, anthracnose, and stemphylium blight. The most important seedling blight and root rot diseases are caused by *Pythium*, *Rhizoctonia*, and *Fusarium* species. *Pythium* causes the damping-off of seedlings while *Fusarium* results in root rot. Symptoms include lesions and discoloration at the base of the stem. Diseased plants turn yellow and eventually die. Root rots and seedling blights occur in patches in lentil fields and rarely cause economic losses unless they are associated with other diseases (Table 3).

Wilt caused by *Fusarium oxysporum* f. sp. *Lentis* is a serious disease in most lentil growing areas of the world (Figure 3). The disease may appear as seedling wilt in the early stage of crop growth or as adult plant wilt during reproductive growth. Symptoms are first seen as sudden drooping of leaflets starting at the top of the plant and then progressing downward. This is followed by shriveling of leaves and wilting of the whole plant. Wilt occurring at flowering will lower seed production whereas wilt appearing during the pod filling period will reduce yield drastically.

Sclerotinia caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, causes stem and pod rot. Symptoms include necrotic lesions, which eventually produce white mycelial growth. The fungus overwinters as soil-persistent sclerotia. Botrytis stem and pod rot, caused by *Botrytis cinerea* (Pers.:Fr), is another disease commonly found on lentil crops in temperate

climates. Symptoms include necrotic lesions on stems and pods with gray sporulating mycelial growth. Both these diseases are of major concern when the crop experiences high humidity after full canopy closure and is susceptible to lodging. Including cereal crops in a rotation can reduce the buildup of inoculum of soilborne pathogens.

Rust caused by Uromyces viciae-fabae is an important disease of lentil in areas with mild temperatures and humid conditions. It has been reported in many lentil-growing countries including India, Turkey, Iran, Morocco, and Ethiopia. Rust has not been reported in lentil in North America. The disease affects all aerial parts of the plants and symptoms include yellowish-white pycnidia and aecial cups on the lower surface of leaflets and pods. Uredia then form on either side of leaflets, stems, and pods. Where infection is severe, lentil plants can die before maturity. The disease is spread from inoculum arising from infected plant material usually mixed with seed. Lentil genotypes with some resistance to rust are now used in breeding programs where rust is a serious problem. (see Lentil: Breeding).

Ascochyta blight, caused by Ascochyta lentis (Vassil.) is a serious disease of lentil in many parts of the world, particularly Canada, Australia, and Pakistan. The disease can be seed- or residue-borne. The symptoms include gray to tan lesions on leaves, stems, pods, and seeds. The lesions have dark margins and may have pycnidia in the centers. Under severe infection, leaves turn completely brown and drop and the infected seed is discolored. The fungus can overwinter on lentil stubble in the field. While wind movement of spores from one field to the next is limited, this can happen in adjacent fields. Seed-borne Ascochyta can be treated by using fungicidal seed treatment. The effectiveness of seed treatment depends on the level of infection and on environmental conditions. As low as 1% seed infection can result in severe epidemics in cool, moist conditions, whereas as high as 5% seed infection may not cause severe problems in dry years.

Table 3 Major fungal diseases of lentil

Disease	Causal organism	Favorable climatic conditions
Root rot/seedling rot	Pythium spp.	Wet, cool conditions at seeding
	Rhizoctonia spp.	
	<i>Fusarium</i> spp.	
Wilt	Fusarium oxysporum	Moderately high soil temperatures (20-25°C), sunlight
Stem and pod rot	Sclerotinia sclerotiorum	Wet conditions, dense canopy
	Botrytis cinerea	Late season
Rust	Uromyces viciae-fabae	Mild temperature, humid conditions
Ascochyta blight	Ascochyta lentis	Cool (15-20°C), wet conditions
Anthracnose	Colletotrichum truncatum	Warm (20-24°C), humid conditions
Stemphylium blight	Stemphylium botryosum	Warm, wet conditions

High levels of *Ascochyta* can cause losses in yield and quality of lentil. Foliar fungicides such as chlorothalonil are available for controlling the spread of *Ascochyta* blight in lentil. Genetic sources of resistance are available and are being deployed in breeding programs (*see* Lentil: Breeding).

Anthracnose caused by Colletotrichum truncatum (Schwein.) is a fungal foliar and stem disease of lentil. It has been reported in western Canada where it can cause significant yield losses. Symptoms include gray to cream spots on leaves and stems, yellowing and browning and eventually senescence. The disease spreads rapidly in the field and can be spread on wind-borne chaff and dust during harvest. Anthracnose inoculum can also be carried over in crop residues as sclerotia in the field for several years. The level of seed-to-seedling transmission of anthracnose is low. Treating infected seed with fungicides to control seed-borne anthracnose is not important, but use of disease-free seed can help to minimize the spread of the pathogen in the field. To reduce levels of field inoculum, it is important to ensure that lentil is not planted in the same field for at least 4 years. Foliar fungicides, e.g., chlorothalonil, are available to control anthracnose in lentil. Good timing of application is critical for effectiveness of the fungicides.

Stemphylium blight caused by *Stemphylium botryosum* is a foliar disease of lentil in Canada and Bangladesh. Infected plants suffer leaf drop and premature ripening. The epidemiology of this disease is not well understood. Some sources of genetic resistance are available and they have been used in Bangladesh to control the disease.

Viral diseases of lentil include alfalfa mosaic, bean yellow mosaic, bean (pea) leaf roll viruses (BLRV), pea enation mosaic virus (PEMV), and pea streak. Some of the viruses can be seed-borne while others are transmitted by aphids, white fly, and other insects. The best control method for viral diseases is use of resistant lentil varieties.

#### **Insect Pests**

A wide range of insect pests attack the lentil crop wherever it is grown. Crop losses to insect pests tend to be sporadic and dependent on insect population. Insects that are of economical importance in lentil include aphids (*Aphis* spp.), lygus bugs (*Lygus* spp.), cutworms (*Agrotis* spp.), bruchid beetles (*Bruchus* spp.), lepidopteran pod borers (*Helicoverpa* spp.), and grasshoppers. Cutworms feed on stems and roots of seedlings, whereas aphids and lepidopteran larvae attack leaves stems and flowers. Lygus bugs and bruchid beetles damage pods and seeds. Grasshoppers primarily eat flowers and pods.

Insect pests are controlled by following an integrated pest management system. Awareness of the biology of the insects likely to infest lentil in the region, is the key to knowing when to scout for insects to minimize infestation by agronomic techniques. Natural enemies may be used to keep populations of some insects below economic threshold. Insecticides are available for controlling major pests in lentil. These should be used when necessary and with caution.

# **Harvesting**

In many lentil-producing areas of western Asia, Africa, and India, harvesting is traditionally done by hand. In many countries the chaff and straw are valuable animal feed. Mechanization of lentil harvest has been one of the main objectives of national and international research programs throughout the world. At ICARDA, breeders are developing tall, non-lodging lentil varieties with nonshattering pods to suit mechanical harvesting systems. Mechanical harvesting devices to suit these production systems are also being developed.

Mechanized harvesting is practiced in South America, North America, Australia, New Zealand, and parts of Europe. In these countries combine harvesters are used either to directly combine the crop or to swath the crop and leave it in windrows to dry. The dry plants are then picked and threshed with combines. Rolling of the seedbed to level the soil surface after seeding makes it easier to harvest pods held close to the ground. Rolling also reduces cutterbar damage and speeds up swathing or direct combining. Cutting or swathing is done when ~30% of lower pods have turned tan and the seed rattle. Recommendations are that swathing be done in the morning when humidity is high to minimize pod shattering. Lentil is combined within 2 weeks of swathing.

#### **Acknowledgment**

The authors would like to thank Dr. Godfrey Chongo for reviewing the disease section.

See also: Lentil: Breeding.

# **Further Reading**

Basler F (1981) Weeds and their control. In: Webb C and Hawtin G (eds.) *Lentils*, pp. 143–154. London: Commonwealth Agricultural Bereaux.

Beniwal SPS and Trapero-Casas A (1994) Integrated control of diseases of cool season legumes.

- In: Muehlbauer FJ and Kaiser WJ (eds.) Expanding the Production and Use of Cool Season Food Legumes, pp. 642–665. Dordrecht, The Netherlands: Kluwer Academic.
- Hariri G (1981) Insects and other pests. In: Webb C and Hawtin G (eds.) *Lentils*, pp. 173–189. London: Commonwealth Agricultural Bereaux.
- Khare MN (1981) Diseases of lentils. In: Webb C and Hawtin G (eds.) Lentils, pp. 163–172. London: Commonwealth Agricultural Bereaux.
- Knott CM and Halila HM (1988) Weeds in food legumes: problems, effects and control. In: Summerfield RJ (ed.)
   World Crops: Cool Season Food Legumes, pp. 535–548. Dordrecht, The Netherlands: Kluwer Academic.
- Kraft JM, Haware MP, Jimenez-Diaz RM, Bayaa B, and Harrabi M (1994) Screening techniques and sources of resistance to root rots and wilts in cool season food legumes. In: Muehlbauer FJ and Kaiser WJ (eds.) Expanding the Production and Use of Cool Season Food Legumes, pp. 642–665. Dordrecht, The Netherlands: Kluwer Academic.
- Mahler RL, Saxena MC, and Aeschlimann J (1988) Soil fertility requirement of pea, lentil, chickpea and faba bean. In: Summerfield RJ (ed.) World Crops: Cool Season Food Legumes, pp. 279–289. Dordrecht, The Netherlands: Kluwer Academic.
- Muehlbauer FJ, Summerfield RJ, and Kaiser WJ (1997) Principles and Practices of Lentil Production. US Department of Agriculture, Agriculture Research Services, ARS-141.
- Papendick RI, Chowdhury SL, and Johansen C (1988) Managing systems for increasing productivity of pulses in dryland agriculture. In: Summerfield RJ (ed.) World

- *Crops: Cool Season Food Legumes*, pp. 237–255. Dordrecht, The Netherlands: Kluwer Academic.
- Sakar D, Durutan N, and Meyveci K (1988) Factors which limit the productivity of cool season food legumes in Turkey. In: Summerfield RJ (ed.) World Crops: Cool Season Food Legumes, pp. 137–145. Dordrecht, The Netherlands: Kluwer Academic.
- Saskatchewan Pulse Growers (2000) *Pulse Production Manual*, 2nd edn. Saskatoon, Canada: Saskatchewan Pulse Growers.
- Saxena MC (1981) Agronomy of lentils. In: Webb C and Hawtin G (eds.) *Lentils*, pp. 111–129. London: Commonwealth Agricultural Bereaux.
- Siddique KHM, Loss SP, Regan KL, and Pritchard DL (1998) Adaptation of lentil (*Lens culinaris* Medik) to Mediterranean environments: response to sowing time. *Australian Journal of Agricultural Research* 49: 1057–1066.

#### **Relevant Websites**

http://www.ndpealentil.org — The North Dakota Dry Pea and Lentil Industry website gives information on lentil production, marketing, and cooking and has links to United States Dry Pea and Lentil Council and other related websites.

http://pwa.ars.usda.gov — The US Department of Agriculture Agricultural Research Service website. This website provides a link to the Grain Legume Genetics and Physiology Research Unit at Pullman, Washington with information on lentil production, lentil varieties, and detailed progress reports.

# LIPID CHEMISTRY

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#### Introduction

After protein and carbohydrate, lipids (commonly know as fats or oils) are the third most important macronutrient in human and animal diets. Although lipids are relatively minor constituents in cereal grains, they play a significant part in quality changes during

grain storage, and in most aspects of processing, including milling, brewing, baking, and extrusion. On the other hand, oilseeds are primarily grown for their oil content. The composition of the lipids (or oil) in various oilseeds is therefore an essential consideration in determining the use of the oil, and the lipids are important for the maintenance of health.

This article is intended to give a general overview of lipid composition in cereal grains and oilseeds, their extraction and identification, their importance in cereal technology, and the nutritional values of edible oils.

**Figure 1** Triacylglycerol synthesis: *R*, *R'*, *R''* are usually long-chain alkyl groups and *R*, *R'*, *R''* are typically all different and may also contain one or more carbon—carbon double bonds.

#### **Nomenclature and Classification**

Lipids constitute a wide variety of naturally occurring substances in plant and animal tissues. They are generally insoluble in water; soluble in organic solvents such as ether, chloroform, and alcohols; and contain long-chain hydrocarbon groups in their molecules.

Lipids can be classified based on their physical properties at room temperature — oils are liquid and fats are solid; the polarity-neutral lipids include fatty acids (FAs), glycerides and sterols, and polar lipids include glycerophospholipids (phospholipids) and glyceroglycolipids (glycolipids). The most common classification of lipids is based on their structure — simple or complex. Simple lipids, composed of FAs and alcohol components, include acylglycerols, ether acylglycerols, sterols, and their esters that can be hydrolyzed to two different components — usually an alcohol and an acid. Complex lipids include phospholipids, glycolipids, and sphingolipids, which yield three or more different compounds on hydrolysis.

The fatty acids (FAs), i.e., carboxylic acids, are the main building blocks in lipid structures. Only a small portion of the total lipid fraction consists of free carboxylic acids, or free fatty acids (FFAs). Most of the carboxylic acids in the lipid fraction are found as esters of glycerol, i.e., as triacylglycerols (TAGs), commonly known as triglycerides (Figure 1).

The most common TAGs are those with long-chain carboxylic acids ( $C_{14}$ – $C_{22}$ ). Positions of carboxylic acids in TAGs are very important to their properties and utilization. The saturated forms of FAs are the most stable against oxidation and they also have high melting points in comparison with unsaturated FAs with the same number of carbon atoms. In plant TAGs, unsaturated fatty acids are predominantly located in the 2-positions. Monoacylglycerols (MAGs) contain only one long-chain FA (saturated or unsaturated) at either the 1, or 2, or 3 positions. Similarly

diacylglycerols (DAGs) have two FA chains at any of the two positions. However, the phosphoric acid residue of phosphoglycerides (PLs) and the first glycosidic residue of glycosylglycerides (GLs) are always in position 3. The general classification of principal lipids found in cereal and oilseeds, brief descriptions of their basic structure, and their common abbreviations are listed in Table 1.

# Compositions and Distribution of Lipids in Cereals and Oilseeds

#### **Cereal Grains**

Lipids are minor components of the cereal grains (Cereals: Overview). Wheat, barley, brown rice, rye, and sorghum have lower lipid contents than other cereal crops such as oats, pearl millet, and maize. All cereal grain lipids are richer in nonpolar lipids than in polar lipids. Wheat contains the highest level of glycolipids among all cereal grains, followed by triticale, rye, and barley. Millet, maize, and sorghum lipids contain the lowest glycolipid content. In general, phospholipids also are more abundant in wheat, triticale, and rye lipids and slightly lower in the lipids of barley, maize, oat, sorghum, and rice. Approximate lipid contents and the proportions of nonpolar lipids and polar lipid (separated into glycolipids and phospholipids) in each cereal are listed in Table 2.

TAGs are the major nonpolar lipids in cereal grains, representing from  $\sim 50\%$  of total nonpolar lipids in wheat and barley to as high as 90% in maize. They are deposited in spherosomes (oil droplets) bound by a monolayer membrane and are usually stored by plants in this form. The remainder of the nonpolar lipids are mainly di- and monoglycerides, FFAs, and sterol esters. The major phospholipids in cereal grains are phosphatidylcholine (PC),

Table 1 General classification of lipids and their commonly used abbreviations

Lipid class	Abbreviation
Simple lipids: compounds with two types of structural moiety	
Glyceryl esters: esters of glycerol and fatty acids (see Figure 1)	
Triacylglycerols: 3 × fatty acids	TAGs
Diacylglycerols: 2 × fatty acids at any 2 positions	DAGs
Monoacylglycerols: 1 × fatty acid at any position	MAGs
Sterol esters: esters of sterol and fatty acids	SE
Waxes: esters of long chain alcohols and fatty acids	
Complex lipids: compounds with more than two types of structural moiety	
Phospholipids: glycerol esters of fatty acids, phosphoric acid and other groups containing nitrogen	
Phosphatidic acid: diacylglycerol esterified to phosphoric acid	
Phosphatidylcholine: phosphatidic acid linked to choline	PC
Phosphatidylethanolamine	PE
Phosphatidylserine	PS
Phosphatidylinositol	PI
Lysophosphatidyl-choline, -ethanolamine, -serine, -inositol	LPC, LPE, LPS, LPI
Glycoglycerolipids: 1,2-diacylglycerol joined by a glycosidic linkage	
through position sn-3 to a carbohydrate moiety	
Monogalactosyldiglyceride	MGDG
Digalactosyldiglyceride	DGDG
Monogalactosylmonoglyceride	MGMG
Digalactosylmonoglyceride	DGMG

**Table 2** Approximate lipid contents and the distribution of lipid classes of the total lipids in cereal grains

Cereal grains	Total lipids (% w/w	Lipid class (% w/w of TL)		
	in whole grain)	NL	GL	PL
Barley	2.5-4.7	65-78	7–26	9–26
Maize/corn	5.1-6.0	88-96	3	5
Millet	1.7-11.0	75-94	1-15	1-14
Oats	4.5-10.3	66 - 80	6-10	12-26
Rice (brown)	0.9-3.1	78-87	4-12	8-10
Rye	3.5	63 - 71	10-12	18-25
Sorghum	3.6-6.0	77-86	2-6	11-17
Triticale	2.6-4.6	53-67	10-18	17-29
Wheat	2.1-3.8	60-72	12-22	14-26

 $\mathsf{TL} = \mathsf{total} \ \mathsf{lipids}; \ \mathsf{NL} = \mathsf{nonpolar} \ \mathsf{lipids}; \ \mathsf{GL} = \mathsf{glycolipids}; \ \mathsf{PL} = \mathsf{phospholipids}.$ 

phosphatidylethanolamine (PE), and phosphatidylinositol (PI). Monoacylphosphoglycerides, or lysophospholipids (prefix L added to LP abbreviation, Table 1), are usually regarded as degradation products of PL. The GLs are quantitatively the major components of the glycolipids in whole cereal grains and starchy endosperm. The principal sugar in the GLs is galactose, and glucose is either a minor component or absent. The main galactosylglycerides are monogalactosyldiglyceride (MGDG) and digalactosyldiglyceride (DGDG). Small amounts of galactosylmonoglycerides (monogalactosylmonoglycerides (MGMG) and digalactosylmonoglycerides (DGMG)

are also found in the endosperm of some mature cereal grains. Polar lipids are found in all membranes including the amyloplast membrane. Apart from these major acyl lipids, other lipids in cereals include sterols, and lipid-associated compounds such as carotenoids and tocopherols. Although carotenoids are very minor constituents in cereal grains, color contributed by carotenoids is an important factor in the use of cereal grains in food production, particularly in the use of durum wheat for pasta making.

The lipid content in cereals is influenced by the water content, the stage of maturity, and the variety of the grain crop. Lipids are also unevenly distributed in various parts of cereal grains (Grain, Morphology of Internal Structure). For example, in the wheat grain, the germ comprises only ~4% of the total grain, by weight, but it has the highest lipid content, and the highest proportion of polar lipids (Table 3), whereas the endosperm, the major fraction of wheat grain, has significantly lower lipid content than the other fractions. Lipids in flour (except whole meal) can be subdivided into nonstarch and starch lipids. The nonstarch lipids, which consist of all the endosperm lipids, excluding those inside starch granules, contain more GL than PL, whereas starch lipids are predominantly PL and almost exclusively lyso-PL in which LPC is the main lipid component. The distribution of lipid classes in other cereal grains is qualitatively similar to those in wheat. The germ is the richest source of lipids among all cereal grain

Table 3 Distribution and compositions of lipids in wheat grain fractions

Grains fraction	Grain	Lipid	Total lipids	Lipid class (% w/w of TL)		
	composition (%)	•	in each fraction (% w/w)	NL	GL	PL
Whole grain	100	100	2.1-3.8	60-72	12–22	14–26
Germ	4	30-35	25-35	79-85	0 - 3.5	14-17
Bran	4	2-5	2-4	72-80	6-10	13-18
Endosperm	92	60-70	1.5-2.5			
Nonstarch lipid				33-46	30-38	24-34
Starch lipid				4–6	1-5	90-95

TL = total lipids; NL = nonpolar lipids; GL = glycolipids; PL = phospholipids.

Table 4 Common FA compositions of total lipids in cereal grains

Lipid source	Fatty acid (wt.%)					
	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	
Wheat	17–24	1–2	8–21	55-60	3–5	
Barley	21-24	1–2	9-14	56-59	4-7	
Oats	7–26	1-4	22-48	31-52	1-4	
Rye	12-19	1-2	12-16	57-65	3-12	
Maize/corn	11-37	1-5	11-24	46-63	1-6	
Brown rice	14-26	1–3	36-52	25-39	1-4	
Sorghum	10-17	1–2	21-27	42-58	1-5	
Triticale	16-20	1–2	8-14	57-60	4-6	

fractions, more than one-third of the total lipid is in the germ. In particular, maize has a larger proportion of germ in the grain, 11-25%, by weight, with an exceptionally high lipid content, 39-47% by weight, containing mostly TAGs, with a small amount of PL and traces of GL. Maize germ is, therefore, more suited for edible oil production than any other cereal, after it has been separated from the starch endosperm (see Oil from Rice and Maize).

The FA composition of cereal lipids is generally similar for wheat, barley, triticale, maize, and sorghum (Table 4). All cereal grain lipids are rich in unsaturated FA. Palmitic acid (16:0) is a major saturated FA, and linoleic acid (18:2) is a major unsaturated FA for all cereals expect for brown rice. Rye lipids contain slightly higher levels of linolenic acid (18:3) than those of other cereals. Oat lipids are similar to those of brown rice; both are rich in oleic acid (18:1).

#### **Oilseeds**

Unlike the cereal grains, lipid (oil) is the main constituent of the oilseed crops. Oilseeds (see Oilseeds, Overview) such as canola/rapeseed, safflower, and sunflower are grown for their oil. On the other hand, oil is produced as by-product for other crops such as soybeans (see Soybean: Germplasm, Breeding,

Table 5 Oil contents in oilseeds

Oilseeds	Oil in dry seed (% w/w)
Canola/rapeseed	30-50
Cottonseed	15-25
Linseed	35-45
Peanut	36-56
Safflower	12-47
Soybean	15-22
Sunflower	25-50

and Genetics; Agronomy; Grading and Marketing; Processing), which are grown primarily for their protein-rich content, and cotton (Cottonseed) grown for its fiber. Peanut (Peanuts), a leguminous oilseed, is not only used for edible oil production, but also for direct consumption as various human foods, being a good source of protein as well as oil. Another oilseed crop, linseed, produces oil that is not extensively used for food, but it is nevertheless an important industrial oil.

Lipid contents vary among the oilseed crops, shown as oil contents in Table 5, ranging from about 15% to 25% in soybean and cottonseed, to as high as 50% in sunflower, canola/rapeseed and 56% in peanut. It must be emphasized that both oil content and component FA proportions in an oilseed are subject to

<b>Table 6</b> FA compositions of total lipids in o	oliseeas
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Lipid source						
	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	Others
Canola	3–5	1-1.5	57-60	10-20	10-12	
Rapeseeds	1–3	0.7-1	12-14	11-22	8-10	20-50% erucic (22:1)
Cottonseed	18-25	1-2	17-38	45-55		1-3% myristic (14:0)
Linseed	5-7	3-4	18-20	15-17	52-60	. , ,
Linola			16	72	2	
Peanut	7–12	1-5	33-71	12-46		
Safflower	2-10	1-10	7-42	55-81		
Soybean	6-12	2-5	20-34	46-56	5-12	
Sunflower	5-7	2-6	15-35	60-73		

quite a wide variation. Such variations arise from genetic manipulation of seed varieties, from climatic factors and cultivation practices, and even from latitude of growth.

Lipids in oilseeds are predominantly TAG,  $\sim 90\%$  or more of total lipids, and phospholipids being the main polar lipids. Other minor lipid constituents are FFA, sterols, hydrocarbons, etc. The FA composition of lipids is the main influence in terms of the "quality" and the uses of the oil. Cottonseed oil has high levels of saturated FAs among the oilseeds, 18-25% palmitic acid, and a small amount (1-3%) of myristic acid (7able 6). The main unsaturated FAs are linoleic acid (32-52%) followed by oleic acid (10-32%). The oil has a significant storage stability not only due to its lesser amount of unsaturated FAs content, but also due to the presence of enough tocopherols to reduce lipid peroxidation.

Among the common oilseeds, safflower, sunflower, and peanut oil contain high levels of unsaturated fatty acids. Sunflower oil is characterized by its high concentration of linoleic acid (60-70%) followed by oleic acid (15-35%). The high proportion of polyunsaturated FAs makes sunflower oil a popular source of essential FAs in the diet. Sunflower oil is next to safflower in having high levels of linoleic acid. The FA composition of safflower oil is similar to that of sunflower. The oil of commercial safflower cultivars contains 55-81% linoleic acid and 7-42% oleic acid as major FAs, followed by stearic (1-10%) and palmitic acid (2-10%) as minor FAs. In peanut oil, oleic and linoleic acids constitute 33-71% and 12-46% of the total FAs, respectively. A high proportion of unsaturated FAs in an edible oil, however, does decrease its storage stability and quality, due to oxidation reactions associated with the double bonds in unsaturated FAs.

Unlike other oilseeds, FA composition in rapeseed oil is unusual in that it contains substantial amounts of long-chain FAs, in particular, a significant amount

of erucic acid (22:1) as  $\sim 20-50\%$  of total lipids (Table 6). Major changes in the FA composition of rapeseed oils have arisen as a result of selective breeding for low levels of erucic acid. Because of some indications that erucic acid had anti-nutritional properties, varieties of rapeseed, called "canola," mainly grown in Australia and Canada, have been developed in which the level of erucic acid is essentially zero. Canola varieties are also characterized by markedly increased levels of oleic acid with smaller increases also in linoleic and linolenic acid content compared to those of the former rapeseed varieties due to the reduction of erucic acid content (Table 6). Canola oil has a FA composition similar to peanut with the exception of the lower palmitic and higher linolenic acid contents. Linolenic acid content has also been reduced in some canola varieties in favor of linoleic acid through breeding. A low-linolenic acid content canola variety has less than 2% linolenic acid, and resembles olive oil in terms of its monounsaturated FA content more than the high linolenic acid content oil variety.

Linseed oil is characterized by high levels of linolenic acid (52–60%), which undergoes autoxidation to give thin, cross-linked and tough films, making it unsuitable for edible purposes. It is, however, an important drying oil used to produce oil-based paints. Like canola/rapeseed, through plant breeding, a variety of linseed has been created in Australia, linola or solin, which gives an oil high in linoleic acid and contains less linolenic acid; it can be used for the production of margarine spread instead of sunflower oil. The "linola" oilseed, however, has not been commercialized in Australia, but is in commercial production in Canada.

#### **Extraction and Quantification of Lipids**

Accurate and precise analysis of lipids is important, not only for determining their nutritive values for human health benefit, but also for gaining the knowledge of structural characteristics of lipids and understanding of their functional properties, which may allow development of tailor-made products that are designed for a particular function or application. Analysis of lipids involves several steps which unusually includes preparation of the sample, solvent extraction of lipids, removal of solvent, and separation of lipids into classes for qualitative or quantitative analysis. Sample preparation mainly involves particle-size reduction of plant tissues or seed materials using methods such as grinding. The purpose of this is to increase surface area and facilitate subsequent lipid extraction. In some cases, acid hydrolysis may be required to release lipids, which are covalently bound to proteins and carbohydrates.

The choice of organic solvents for lipid extraction largely depends on both the chemical nature of the sample and the type of lipids to be extracted, i.e., total lipids, starch lipids, polar or nonpolar lipids. Neutral lipids are hydrophobically bound and can be extracted readily by nonpolar solvents, whereas polar lipids, such as glycolipids and phospholipids, which are predominantly bound by electrostatic forces and hydrogen bonding, require polar solvents capable of breaking such bonds. Hexane and diethyl ether are preferred solvents for extracting nonpolar, neutral lipids such as triglycerides. Hexane is widely used for the industrial production of crude oil from oilseeds because of its low cost. However because of concerns about its toxicity, effort has been made to replace it with iso-octane, which is less toxic. However, cereal grains and milled flour contain relatively large amounts of glycolipids and phospholipids, and large amounts of cereal lipids (about half of the total lipids) are bound to polypeptides or polysaccharides. Therefore, more polar solvents such as chloroform, alcohol, or chloroform—methanol (2:1) mixture are needed for the extraction. In particular, water-saturated butanol is very effective in extracting starch lipids, as they are not readily dissociable from the starch by any other solvent system.

Removal of the solvent from lipid extracts is normally conducted under vacuum in a rotary evaporator at or near room temperature. Care must be taken to minimize lipid oxidation during sample preparation, solvent extraction, and storage of lipids. Cereal lipids characteristically contain large proportions of polyunsaturated FAs together with carotenoids and tocols, all of which are highly susceptible to autoxidation. The lipids are only slowly oxidized while the grain tissues are intact, but once the tissues have been disrupted, oxidation must be minimized by keeping lipids in solvents such as chloroform and under a nitrogen atmosphere, and by working at low temperatures if possible.

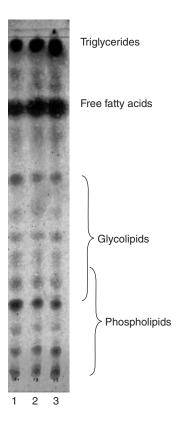
Lipid extracts are complex mixtures of individual classes of compounds and require further separation. Many chromatographic and spectroscopic techniques are in use to separate and quantify individual lipid components. The most commonly used techniques are thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC).

# **Thin Layer Chromatography**

TLC provides separation of a wide variety of compounds with different polarities on a single plate. Silica is still the stationary phase material preferred. Small amounts of lipid material are spotted on a plate. The plate is then developed in a lid-covered glass chamber containing a mixed solvent system, which behaves like a gradient elution during travel on a plate. The separation of lipids is based on relative affinities of the components to the TLC adsorbant. When the solvent front is close to the top of the plate, the plate is removed from the chamber and residual solvent is evaporated under an inert atmosphere. The separated lipid spots can be visualized as brown spots (temporarily) by exposing to iodine vapor or under UV light after spraying with fluorescent agents, or dark spots (permanent) by spraying with sulfuric acid (10-50% in ethanol v/v) and subsequently charring above 160°C. The advantage of using a temporary nondestructive detection is that the lipid spots may be recovered from the silica gel for preparative or analytical proposes. Lipids, after separation on a silica plate, may also be quantitated by densitometric or fluorimetric scanning.

Lipids can be separated into various classes by TLC. As an example, Figure 2 shows the separation of wheat flour lipids extracted by chloroform from different flour mill streams, where more TAGs were detected in the later mill streams (lane 3) as more bran materials were being incorporated into flour. Straightforward unidimensional TLC is adequate for separation of nonpolar lipids and some polar lipids. In most cases, glycolipids overlap phospholipids in TLC systems that separate phospholipids. More complex GL or PL are preferentially separated by two-dimensional TLC, using a neutral/basic solvent such as in the first dimension, and an acidic solvent in the second dimension.

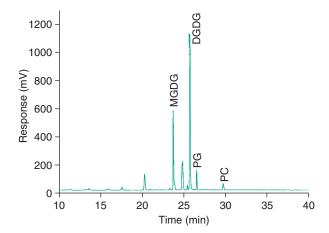
TLC offers a much higher sample throughput due to the possibility of performing separations simultaneously, and it can handle "not-so-clean" samples since the separation medium is used only once. TLC is a cost-effective, simple, and easy chromatographic technique.



**Figure 2** Separation of wheat flour lipids by TLC on a silica gel G plate  $(20 \text{ cm} \times 20 \text{ cm})$  developed in chloroform: methanol: water (90:20:2 v/v/v). Wheat flour was collected from different mill streams (1-3: early-late), where more TAGs were detected in the later mill streams as progressively more bran materials were being incorporated into flour.

#### **High-Performance Liquid Chromatography**

In principle, separations by HPLC are carried out by essentially the same separation principle as TLC. Using HPLC, it is easier to generate large numbers of "theoretical plates" (a measure of resolution efficiency); HPLC is simpler to automate than TLC. However, HPLC is much more expensive than TLC in terms of both equipment and running costs. HPLC separation occurs in a stainless steel column packed with a very uniform, finely divided, microspherical adsorbant material of controlled porosity and degree of hydration. A high-pressure pump ensures adequate and constant flow of solvent, the "mobile phase," through the column, and a flow-through detector continuously monitors the column eluate. Adsorption chromatography with columns of silica gel is commonly used for the separation of lipids. Elution of the column may be carried out either with a solvent mixture of constant composition or by gradient elution, in which the solvent composition is varied linearly or in a stepwise fashion with both binary and ternary solvent systems. The choice of the elution

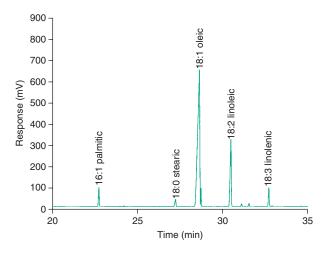


**Figure 3** HPLC analysis of wheat flour polar lipids extracted by chloroform. Separation was carried out on a PVA-Sil threaded guard column ( $23 \times 4.0 \,\mathrm{mm}$  i.d.) with gradient elution at  $0.5 \,\mathrm{ml}\,\mathrm{min}^{-1}$  and detected by a ELSD. Four steps of gradient were used for the separation  $-0-10 \,\mathrm{min}$ : 100% eluent A (2,2,4-trimethylpentane); 10–20 min: 90% eluent A + 10% eluent B (tert-butylmethylether); 20–35 min: 100% eluent B; and 35–40 min: 100% eluent C (methanol/n-ethylmorpholine/glacial acetic acid (500: 4.2: 1.5 v/v)). For abbreviation of lipids see **Table 1**.

systems is sometimes restricted by the use of the detection system. For example, a solvent mixture of constant composition with low UV absorbance may be essential for a UV detector. Although gradient solvent systems can be used with an evaporative lightscattering detector (ELSD), the choice of solvents is constrained by the need for sufficient volatility for evaporation in the detector under conditions that do not cause evaporation of the solute. Complicated programs (up to eight steps) are sometimes required to achieve the desired separations. However, ELSD has presently become the detection method used most often in the separation of lipids by HPLC. HPLC for analysis of all simple lipid classes in one chromatographic run, has not yet been used widely, probably because TAG (major components in all cereal grains and oilseeds) dominate the system. HPLC methods have been devised more for specific analysis of individual components. The use of HPLC for the separation of complex lipids has increased. Separation of glycolipids MGDG and DGDG, along with several phospholipids from wheat flour lipid extracts at analytical scale (Figure 3), as well as preparative scale, has been achieved.

#### Gas chromatography

GC is the most useful tool for the analysis of the FA composition in lipids. Derivatization of FAs is required to increase their volatility. Fatty acid methyl esters (FAMEs) may be prepared by different transmethylation techniques and then separated on



**Figure 4** GC analysis of a canola oil. FAs methyl esters separated using a capillary GC column (SP2560, Supelco) and detected by an FID at 240°C.

GC columns and detected by a flame ionization detector (FID). The gas phase for GC is usually nitrogen or helium for packed columns and helium or hydrogen for capillary columns. Since the principal FAMEs in cereal lipids are 16:0, 16:1, 18:0, 18:1, 18:2, and 18:3, heptadecanoic acid (17:0) can be used as an internal standard. Identification and quantification of individual FAs can be achieved by comparing and calibrating with relatively cheap FAME standards. Figure 4 shows a typical GC profile of FA compositions in canola oil. FA compositions in lipid classes can also be obtained by GC analysis of lipids in each class separated or prepared by TLC or other column chromatography techniques.

Lastly, mass spectrometric detection is being used increasingly for the detection and analysis of complex lipids. The technique can be used with direct inlet injection or linked to HPLC, or GC systems where the mass spectrometer serves both to detect lipids and to obtain information on their molecular structure.

# The Role of Lipids in Cereal Technology

Apart from being used directly as a source of human food and animal feed, a large proportion of cereal grains is milled to flour, which is then used for producing various food products. Wheat has been the predominant cereal grain used for baked goods, largely due to its ability to form a dough when flour is mixed with water. Rye ranks next in importance while other cereals assume a role in specialized products and in the preparation of composite flours.

The rheological properties of doughs made from wheat flour are principally determined by the gluten protein (Gluten and Modified Gluten), and lipids (naturally occurred in cereal grain, excluding added fat) appear to make little contribution. When protein is removed from a flour, dough and bread-making properties are lost. In contrast, good dough properties and bread-making capacity are retained after removal of the nonstarch lipids. However, flour lipids do affect the loaf volume and crumb texture of bread. Fractions of polar and nonpolar lipids have opposite effects on baking performance.

Incremental addition of the extracted lipid to a defatted flour produces an unusual effect on the loaf volume and texture in a rapid baking test in which exogenous lipid is omitted. Loaf volume decreases to a minimum at a lipid content intermediate between that of the defatted and untreated flour, thereafter increases. At high lipid contents (above the inherent lipid content of the flour), the loaf volume lipid curve tends to plateau. The changes in volume are paralleled by changes in crumb grain which, at first, also deteriorate and then improve. Fractions of polar and nonpolar lipid affect loaf volume and crumb grain in opposite ways. The polar lipid fraction has generally beneficial effects, whereas the nonpolar fraction has deleterious effects. Thus, if the ratio of polar to nonpolar lipid is increased, the minimum of the loaf volume-lipid content curve is shifted to lower lipid contents. If the percentage of polar to nonpolar lipid in a flour is varied from 0 to 100 at a constant lipid level, test-bake loaf volume increases approximately in a linear manner. Addition of different lipids to test their effects on dough has shown that the unsaturated FAs such as linoleic acid are the components that mainly contribute to the deleterious effects of the nonpolar fraction. Higher loaf volume and better crumb texture in bread is favored by a high ratio of polar/nonpolar lipid and a high content of flour lipid. However, the variation in loaf volume that can be attributed to lipid is relatively small, and by far the greatest variation is imposed by the effect of gluten protein quality.

The effects of lipids have also been studied for Arabic bread, steamed bread, cakes, and biscuits. Despite differences in processing, the general features with respect to lipids have much in common. It is the crumb texture which is influenced most and, like bread, differences in lipids have not been found to account for major variations in quality. The role of the (polar) lipid appears to act as a complex mixture of components with surface activities in stabilizing or destabilizing the gas bubble structure during expansion of the loaf and thus the final texture.

In addition to their effects in influencing volume and texture of baked products, lipids also play a role in staling mechanisms. Generally, the presence of lipid reduces bread staling and enhances shelf life. This is largely due to the interaction between lipids and starch. When monoacyl lipid monomers are present to form the lipid—starch complex, an insoluble amylose-lipid film is likely to be formed on the surface of the starch granules. Such a film is capable of acting as a barrier against water transport involved in the staling process.

The contribution of the lipid component to flour quality should not be ignored when considering parameters to be used as a basis for selection in plant breeding programs even though protein composition appears to be the major factor that determines flour quality. The "quality factors" in flour lipid, as presently known, are a high nonstarch lipid content, a high ratio of polar to nonpolar lipid, and a low free-fatty-acid content.

#### **Oils and Nutrition**

Lipids are the richest source of energy on a weight basis. They also play a significant role as barriers, such as for skin and in stabilizing biological membranes. Appropriate intake of lipids is essential for health maintenance. However, a high consumption of fat, especially saturated FAs, may be connected with several chronic diseases, such as heart disease and obesity. Of the lipid components of a normal diet, the most important FAs are linoleic (n-6 PUFA) and  $\alpha$ -linolenic (n-3 PUFA) acids, the two primary essential FAs. They play a role in stabilizing biological membranes by creating physical properties that are optimal for the transport of substances across the membrane and for the biochemical reactions occurring in the membrane. Through metabolism, they are converted into a whole range of oxygenated compounds, which exert a range of profound physiological activities involving lowering plasma cholesterol, aggregating red blood cells, and smoothing muscle performance, all attributes that are required for good health. Since the human body is unable to synthesize them, they must be obtained from dietary sources.

The important source of the essential FAs is oilseeds. Although cereal grains contain high amounts of linoleic acid, due to the generally low total lipid content of the grain, it is unlikely to have much contribution to the essential FAs intake in the human diet, except when the lipids are extracted from the lipidrich germ and produced as oil, such as maize germ oil (Oil from Rice and Maize). Oils extracted from oilseeds, however, have a sufficient influence in total fat intake of the human diet since they are used directly for frying foods, as cooking ingredients and as spreads.

See also: Cereals: Overview. Oilseeds, Overview. Oil from Rice and Maize.

#### **Further Reading**

Barnes PJ (1983) Lipids in Cereal Technology. London: Academic Press.

Chung OK and Ohm J-B (2000) Cereal lipids. In: Karel K and Ponte JR, Jr (ed.) *Handbook of Cereal Science and Technology*, 2nd edn. NY: Marcel Dekker.

Christie WW (2003) Lipid Analysis – Isolation, Separation, Identification and Structural Analysis of Lipids, 3rd edn. Bridgwater, UK: The Oily Press.

Gunstone FD (1996) Fatty Acid and Lipid Chemistry. Glasgow, UK: Chapman and Hall.

Morrison WR (1978) Cereal lipids. In: Pomeranz Y (ed.) *Advances in Cereal Science and Technology*, vol. II, pp. 221–348. St. Paul, MN: American Association of Cereal Chemists.

Salunkhe DK, Chavan JK, Adsule RN, and Kadam SS (1992) World Oilseeds – Chemistry, Technology, and Utilization. New York: Van Nostrand.

#### **Relevant Websites**

http://www.aocs.org – American Oil Chemists' Society (AOCS).

http://www.issfal.org.uk — International Society for the Study of Fatty Acids and Lipids (ISSFAL).

http://www.iterg.com - ITERG French Institute for Fats and Oils.

http://www.plantlipids.org – National Plant Lipid Cooperative (NPLC).

http://www.oilworld.de - Oil World.

http://www.lipid.co.uk – Scottish Crop Research Institute/The Lipid Analysis Unit.

http://www.eurofedlipid.org — The European Federation for the Science and Technology of Lipids (Euro Fed Lipid).

# LUPIN

Contents

Overview **Breeding Agronomy** 

# Overview

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### **Definition and Origins of Lupins**

The seed or grains of domesticated *Lupinus* species are generically called lupins. The same term is used widely in the community in referring to wild and domesticated plants and seed/grain of all Lupinus species. The genus *Lupinus* belongs to the Fabaceae, a family of legumes. There are 12 lupin species, all largeseeded, native to Europe and the Mediterranean regions. Three of these are now fully domesticated. Lupinus albus, the European white lupin or Albus lupin has a white flower. Wild types of L. angustifolius, the narrow-leafed lupin, have a blue flower: however modern cultivars bred for low alkaloid content in Australia have a white flower and are known widely as the Australian sweet lupin (ASL). Lupinus luteus, the yellow lupin (YL), has a yellow flower. Their grain is widely used in the animal feeds industry and to a lesser extent as a food (or food ingredient). There are  $\sim 100$ lupin species native to the Americas. They are mostly small-seeded and only L. mutabilis, the pearl or Andean lupin, has been used as a food source. Worldwide, several species are being domesticated for possible use in agriculture (see Lupin: Breeding). The main value of lupin crops to farmers are their replenishment of soil nitrogen, providing a disease-break for cereals in crop rotation systems, and a marketable grain. Lupins tend to grow relatively better on poorer soils than most other crop species (see Lupin: Agronomy).

Lupins are also well known for their spectacular flowers. The Russel lupin, a hybrid derived from L. polyphyllus, is widely used as an ornamental flower, particularly in Europe. Parts of the southern highlands on New Zealand are a blaze of color from the alpine lupin, a variant of the Russel lupin, in springtime and early summer. The (bluebonnet) flower of *L. texensis* is the floral emblem of Texas.

It is widely accepted that *Lupinus* evolved from the tropical and subtropical Sophorea, a primitive tribe of the subfamily Papilionoideae (pea flowering plants), 40 million years ago. Nowadays, wild lupin species cover almost all climatic zones; sub-Arctic Alaska and Iceland, Mediterranean and semidesert regions, the highlands of East Africa, Mexico and the Andes, and the subtropical lowlands of the Eastern Americas.

# **Grain Morphology and Composition**

Lupins have a typical dicotyledon structure (see Grain, Morphology of Internal Structure). Their thick seedcoat (hull or testa) comprises ~30% of the seed weight for L. luteus, 25% for L. angustifolius, 15% for L. albus, and 12% for L. mutabilis. This is considerably higher than for most domesticated grain species. Within the cotyledons (kernels), energy is mostly stored in the form of thickened cell-wall material ( $\sim$ 25% of the cotyledons) and oil bodies  $(\sim 6-14\%)$ . There is virtually no starch in any of the lupin species. This is in marked contrast to crops such as field peas and chickpeas, which can have 50-70% of the cotyledon weight as starch and have low protein and oil content, and the soybean with 15-20% oil, some starch and a high protein content. Their crude protein content ranges from  $\sim$ 28% to 42%. Proximate analyses for whole grain of the major domesticated species, and the Andean lupin, are shown in Table 1. There are some wild and partly domesticated Lupinus lines containing up to 45% crude protein and up to 21% oil, others have as little as 20% crude protein and 3% oil.

The thick seedcoat, which is mostly cellulose and hemicellulose, of *Lupinus* species means that it is important to also consider the composition and nutritional value of their cotyledons: these data are in Table 2.

**Table 1** Chemical composition of the major lupin species (g per kg as received)<sup>a</sup>

	L. albus (Albus lupin, white lupin)	L. angustifolius (Australian sweet lupin, narrow leaf lupin)	L. luteus (yellow lupin)	L. mutabilis (pearl lupin, Andean lupin)
Moisture	86	85	94	71
Protein (N × 6.25)	361	322	414	460
Ash	33	28	37	40
Crude fat	91	58	57	110
Crude fiber	104	150	127	110
ADF <sup>b</sup>	152	197	195	
$NDF^c$	171	227	230	
$TDF^d$	390	284	320	
Lignin	15	7	5	
Starch <sup>e</sup>	<10	<10	<10	
Gross energy (MJ)	19.0	18.6	18.6	

<sup>&</sup>lt;sup>a</sup>Average of worldwide data.

**Table 2** Chemical composition of the kernels of major lupin species (g per kg as received)<sup>a</sup>

L. albus	L. angustifolius	L. luteus
75	100	85
401	400	530
36	27	45
114	66	73
150	88	195
62	71	220
140	77	300
440	380	420
30	7	
20.4	18.9	19.7
	75 401 36 114 150 62 140 440 30	75 100 401 400 36 27 114 66 150 88 62 71 140 77 440 380 30 7

<sup>&</sup>lt;sup>a</sup>Average of worldwide data.

The major lupin proteins are a group of globulins, called conglutins. The three major ones are conglutin  $\alpha$  (which sediments at  $\sim$ 11–12S and is analogous to legumin of peas and glycinin of soybeans), conglutin  $\beta$ (which sediments at  $\sim$ 7S and is analogous to vicilin of peas and conglycinins  $\alpha$  and  $\beta$  of soybeans), and conglutin  $\gamma$  (a sulfur-rich protein which sediments at  $\sim$ 2S and is analogous to conglycinin of soybeans). They comprise ~85% of the total protein and have similar size and physical properties to the storage proteins of other grain legume species. The remaining 15% of proteins are albumins, which are soluble at pH 5 and vary in size from  $\sim$ 6000 to 117000 Da. There is evidence for the 2S globulin actually being an albumin: depending on the species, this fraction consists of 4 (L. luteus), 5 (L. albus), or 6 (L. angustifolius) proteins. For more details on lupin and other legume proteins, see Cereals: Protein Chemistry.

**Table 3** Essential amino acid profile for major lupin species (% in grain)<sup>a</sup>

Amino acid	L. albus	L. angustifolius	L. luteus	L. mutabilis
Arginine	4.7	3.6	4.4	4.6
Cystine	0.5	0.5	0.7	0.7
Histidine	0.6	0.8	1.4	1.3
Isoleucine	1.4	1.2	1.5	2.0
Leucine	2.3	2.1	2.8	2.9
Lysine	1.6	1.4	2.1	2.5
Methionine	0.2	0.2	0.4	0.3
Phenylalanine	1.2	1.1	1.8	1.7
Threonine	1.2	1.0	1.5	1.8
Tryptophan	0.4	0.3	8.0	0.3
Tyrosine	1.7	1.1	1.2	1.4
Valine	1.4	1.2	1.3	1.7

<sup>&</sup>lt;sup>a</sup>Data are averaged from values obtained worldwide.

Typical amino acid profiles for the domesticated lupin species, compared to FAO standards for infants and others, are shown in Table 3. The deficiencies in lysine and methionine are common to most grain legumes.

The seed hulls and cotyledons contain different types of carbohydrate. Lupin hulls are predominantly composed of structural polysaccharides: cellulose, hemicelluloses, and pectins. The main carbohydrate reserves of the cotyledons are the nonstructural polysaccharides of the cell walls, with the main components being galactose, arabinose, and uronic acid. These complex compounds are referred to as nonstarch polysaccharides (NSP). Their structure, based on a rhamnogalacturonans backbone, is not yet fully defined. The water-soluble portion,  $\sim 5\%$  of the seed, is considered to have an antinutritional effect due to its viscous nature and effect on intestinal transit time.

<sup>&</sup>lt;sup>b</sup>Acid detergent fiber.

<sup>&</sup>lt;sup>c</sup>Neutral detergent fiber.

<sup>&</sup>lt;sup>d</sup>Total dietary fiber.

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<sup>&</sup>lt;sup>c</sup>Neutral detergent fiber.

<sup>&</sup>lt;sup>d</sup>Total dietary fiber.

The insoluble NSP,  $\sim$ 30% of the seed, has a minimal effect on nutrient utilization by monogastric species. An important attribute of insoluble NSP is their ability to hold large quantities of water, about eightfold by weight for lupins, and maintain normal gut motility. The profiles for various species are shown in Table 4. The oil content of lupins varies from  $\sim$ 15% in the pearl lupin down to 4% in the YL. There are differences in individual fatty acid profiles, they are typically high in oleic and/or linoleic acids and contain a total of  $\sim$ 75% unsaturated fatty acids (cf.  $\sim$ 85% in olive oil). The oil is heat-stable and has a high antioxidant capacity.

Variation in the content of major essential minerals in lupins, up to  $\sim 30\%$  of the mean, is typical of legume species, probably reflecting both genotype and environmental factors (Table 5). The essential trace mineral content of lupin species is influenced by genotype but also tends to reflect the soil types on which they are grown. For example, the selenium content of lupin seed grown in Western Australia varies according to species and rainfall zone. Where the species were grown together, the selenium content of L. albus > L. luteus > L. angustifolius. In all cases, the higher the rainfall, the lower the selenium content. Similar variations apply to the content of copper, cobalt, and zinc in lupins. The accumulation of manganese by L. albus is well documented with most of the low values coming from crops grown on sandy soils and high values coming from crops grown on heavy red clays. In contrast, the manganese content of L. angustifolius is low, from 9% to 30% the amount in L. albus grown at the same site (Table 5).

A survey of the cadmium and lead content of *L. angustifolius* (178 samples) and *L. albus* (12 samples) grown in Australia found no samples exceeded the Codex Alimentarius limits of 0.1 and 2.0 mg per kg, respectively. There can be some accumulation of cadmium by *L. luteus*, which may be a consequence

**Table 4** Nonstarch polysaccharide components for major lupin species (g per kg in grain)<sup>a</sup>

	L. albus (Albus lupin, white lupin)	L. angustifolius (Australian sweet Iupin, narrow Ieaf Iupin)	L. luteus (yellow lupin)
Rhamnose	13	10	10
Arabinose	67	50	70
Xylose	22	10	21
Mannose	1	1	1
Galactose	202	170	133
Glucose	23	13	42
Uronic acids	50	29	43
NSP, total	390	284	320

<sup>&</sup>lt;sup>a</sup>Data are averaged from values obtained worldwide.

of the different root architecture in this species and the soils in which it is grown.

Legume grains contain a range of compounds, traditionally known as antinutritional factors (ANF) or antinutrients, with apparent untoward effects on species ingesting them. These are also known as bioactive compounds. These compounds had a protective role in evolution by protecting against predators and may have other important functional roles. Plant breeders have mostly reduced their content to a point of balance between lowering the content of ANF, to enhance the nutritional value of the grain, and retaining the defensive or, otherwise, functional role. While some ANF have a negative effect on feed utilization by farmed livestock, they can also have beneficial effects in humans, such as preventing the development of some forms of cancer and of osteoporosis. A summary of data for the ANF content of lupins is shown in Table 6.

The content of proteinaceous ANF in domesticated lupin species is very low. Typically, trypsin inhibitor activity is <0.3 mg per kg, and chymotrypsin inhibitor activity <0.6 mg per kg. Lectin activity is virtually nonexistent in all lupin species. So, unlike most legume grains, lupins do not need to be heated to denature the proteinaceous ANF and make them safe for consumption by humans or animals.

Phytate (inositol hexaphosphate and lower substituted homologues and their salts) contributes about one-half of the total phosphorus content in lupins. This is rapidly mobilized upon germination. Phytate can form insoluble complexes with divalent cations, particularly Ca<sup>2+</sup> and Zn<sup>2+</sup>, thus making them less

**Table 5** Minerals content of major lupin species (range of datasets)

	L. albus	L. angustifolius	L. luteus				
Mineral content	Mineral content (g per kg)						
Calcium	1.2-3.3	1.5-3.1	1.8 - 3.0				
Magnesium	0.9 - 1.6	1.1-2.0	2.2 - 3.2				
Phosphorus	2.5 - 9.0	2.1 - 4.3	3.4 - 7.3				
Potassium	2.8-11.1	6.6-109.1	8.8-11.6				
Sulfur	2.1 - 2.7	1.5-2.9	4.0 - 4.9				
Sodium	< 0.1 – 1.1	0.3-1.1	< 0.1				
Mineral content	(mg per kg)						
Copper	3.1-8.1	2.5-6.8	5.9-12.0				
Iron	21-44	31-150	52-87				
Manganese	23-3772	6.7-76	25-59				
Molybdenum	0.8-3.1	0.7 - 2.9					
Zinc	22-38	24-45	39-82				
Mineral content	Mineral content (μg per kg)						
Cobalt	10-430	10-260	na				
Selenium	20-360	18-240	na				

available for absorption and utilization. The net effect of phytate in the diet depends on the overall composition of the food particularly the amount and types of protein content and the total mineral content. Human studies have shown similar rates of absorption of phosphorus from lupin-based foods and comparable soy products. Germination lowers the phytate content by  $\sim\!60\%$  and fermentation by  $\sim\!80\%$ .

Tannins are compounds of plant origin with molecular weights ranging from 500 to 2000 Da, and with one to two phenolic hydroxyl groups per 100 Da. This enables them to form cross-linkages between proteins and other macromolecules. There are two subgroups of tannins. Hydrolysable tannins typically have a central glucose core with the hydroxyl groups being wholly or partly esterified with gallic acid or hexahydrodiphenic acid. Condensed, nonhydrolysable tannins are higher oligomers of flavan-3-ols with varying degrees of substitution. Their astringent taste and ability to precipitate proteins, resulting especially in the inactivation of gut enzymes, give the tannins their antinutritional role. In lupins, as with other grain legumes, the tanning are concentrated in the seedcoats (hulls) and the simple act of de-hulling will minimize any adverse effects. The concentration of condensed tannins, those most responsible for protein binding, is so low in lupins that it is unlikely to affect human or animal nutrition.

Saponins are plant glycosides in which the nonsugar moiety is a steroid or a triterpenoid compound. Their bitter taste acts as a feed deterrent, and they have a secondary antinutritional effect by increasing the permeability of small intestinal mucosa cells. This causes a loss of essential electrochemical concentration gradients, facilitating the uptake of materials to which the gut would not normally be permeable. Only traces of saponins are present in *L. albus*, while concentrations in *L. angustifolius* and, *L. luteus* range from 55 to 730 mg per kg. Saponins are

**Table 6** Antinutritional factors in the major lupin species<sup>a</sup>

Botanical name	L. albus	L. angustifolius	L. luteus
Total alkaloids (mg per kg)	<200	<200	200-500
Oligosaccharides (%)	7.5	5.2	12.3
Saponins (mg per kg)	<1	570	55
Condensed tannins (%)	0.01	< 0.01	0.02
Lectins (activity <sup>b</sup> )	trace	trace	trace
Trypsin inhibitors (mg per g)	0.13	0.14	0.29
Phytate (%)	0.79	0.58	0.96

<sup>&</sup>lt;sup>a</sup>Data are averaged across worldwide figures.

generally harmless to humans: some are claimed to be beneficial in lowering blood cholesterol levels and protecting against coronary heart disease. The concentrations in lupins are lower than in many other legume species.

Lupin oligosaccharides are higher α-galactosides of sucrose. Raffinose has one galactose moiety linked to sucrose through an  $\alpha$  1,4 bond, while stachyose has two, verbascose three and ajugose four. These compounds cannot be metabolized by humans and other nonruminant species, and they undergo bacterial fermentation in the colon to produce carbon dioxide, methane and hydrogen. This causes abdominal discomfort and cramps and leads to flatulence, which seems to be a major reason for the low interest in consuming grain legumes in many societies. The oligosaccharides are a rich source of nutrients for bifidobacteria in the colon. This can have a beneficial effect, as the bifidobacteria counter the activity of putrefying bacteria and reduce their production of harmful, and possibly carcinogenic, fermentation products. One further source of confusion about the significance of oligosaccharides is the inclusion by some authors of sucrose in their total for oligosaccharides. This is nutritionally confusing as sucrose can be absorbed from the stomach and upper digestive tract of humans and nonruminant animals.

Isoflavones have generally been regarded as ANF because of their negative effects on fertility in ruminants grazing pasture medics and subterranean clovers. However, they are now recognized as preventative agents against some forms of cancer and osteoporosis in women. Isoflavones are present in green-leaf material and in the hypocotyls of germinating seeds (sprouts). There is conflicting evidence as to their presence in mature grain.

The lupin alkaloids are usually bicyclic, tricyclic, or tetracyclic derivatives of quinolizidine (Figure 1). The individual alkaloids and their concentrations vary widely between, and sometimes within, species (Table 7). Grain of modern domesticated lupin cultivars typically contains less than 200 mg per kg alkaloids. In contrast, grain from "bitter" wild types that still exist in many countries may contain from 5000 to  $40\,000$  mg per kg alkaloids. There have been human deaths associated with the consumption of grain from bitter wild types of lupin. One would need to eat  $\sim 10\,\mathrm{kg}$  of the modern varieties of lupin



Figure 1 Structure of quinolizidine.

<sup>&</sup>lt;sup>b</sup>Lectin activity is usually measured as units of agglutinating activity against red-cell preparations from blood of various mammalian species. Several authors argue that there is no significant activity in *Lupinus* species, some have detected activity against specially sensitised cells or against species not considered significant for human concern.

**Table 7** Quinolizidine alkaloids in the major lupin species (% in alkaloid fraction)<sup>a</sup>

	L. albus	L. angustifolius	L. luteus	L. mutabilis
Albine	15			
Lupanine	70	70		70
Multiflorine	3			
13-Hydroxylupanine	8	12		2
4-Hydroxylupanine	1			7
Angustifoline		10		<1
Lupinine			60	
Sparteine			30	20

<sup>&</sup>lt;sup>a</sup> Data are for modern cultivars or developing lines of these species. Up to 10% gramine, an indole alkaloid, is present in L. luteus.

grain in a very short time to absorb sufficient alkaloids to be at risk. The quinolizidine alkaloids have a short half-life in humans and are excreted largely unchanged in the urine of >90% of humans. Traces of gramine, an indole alkaloid, occur in some lines of L. luteus but the significance of these levels is not known.

The only mycotoxin associated with lupins to date is the family of phomopsins, a group of linear hexapeptides with an antimicrotubule effect which is most pronounced in the liver and kidney. They are capable of disrupting mitosis and of reducing the activity of motile cells and organisms, rendering their host prone to secondary infections. There is no relationship between lupinosis and lupin-alkaloid poisoning that can occur when sheep graze bitter lupins and their stubble, or graze the perennial lupins of North America. Grains that contain phomopsins are smaller and less dense than normal-colored, nontoxin containing grain and can be easily removed from the rest of the harvested grain by grading, using screens, gravity tables, and aspirators. A rapid enzyme-linked immuno-sorbent assay for the phomopsins is readily available to the food industry. The most recent cultivars of L. angustifolius are resistant to invasion by the responsible fungus (*Diaporthe toxica*), so that the risk of phomopsins ingestion is now extremely low, and likely to be even less in the future.

#### **Grain Processing**

#### **De-Hulling**

The simplest processing of lupin grain is repetitive abrasion of the testa in a ripple flow mill, tangentially abrasive de-hulling device, or similar device. (The thickness and hardness of the testa make it harder to de-hull lupins than most grains.) Alternatively, the grain may be conditioned by the addition of water, to swell the grain, followed by a mild heat

treatment to help break the bonding between testa and cotyledons. It is then possible to de-hull using the same roller mills as used for milling wheat and other cereals. The major components are separated by air aspiration and grading across gravity tables. The germ fraction usually separates with the hulls, and is mostly lost.

The main reason for de-hulling is that the cotyledons are much more nutritious than whole grain for nonruminant animals such as pigs, poultry, and fish. It is then possible to mill the cotyledons to make flour, or to separate it into protein-rich and fiber-rich fractions for the feed, human food, and cosmetic industries.

#### Milling

Hammer milling of whole grain to make a coarse meal is common in the animal feed industry, simply to make the constituents more available to ruminant animals by increasing the surface area of material for digestion, and in some cases where it may be uneconomic to de-hull for nonruminant feeds. Hammer cutting and roller milling of the splits are used to make a meal or fine flour for nonruminant feeds. The particle size range can be from 10 to 800  $\mu$ m, which is far greater than for cereal, pea, and soy flours.

#### **Fractionation**

Several reports in the scientific literature and patents describe the separation of protein, fiber, and oil fractions from cotyledons. Some processes cater for recovery of alkaloids, for use as biological agents against insect pests and some microorganisms, and low-molecular-weight carbohydrates for use in the chemicals industry. These mostly involve wet grinding of the cotyledons and separation of fractions according to solubility at different pH or in different solvents. There may be an initial dry milling and separation of particles by air classification.

#### **Extrusion**

Extrusion (*see* Extrusion Technologies) can improve the quality of lupin-based formulated feeds. There is no expansion of the pellets as with starch-enriched feeds, but there are improvements in feed digestibility, which makes the process cost-effective.

#### **Food Uses**

#### **Traditional Uses**

Lupins have been used as a food for as much as 6000 years in the Andean highlands, and over 3000 years around the Mediterranean. The pearl lupin of the Andean Highlands of South America known locally as "tarwi" or "choco," was extensively cultivated, and consumers would soak the seed in running water to remove most of the bitter alkaloids before toasting or boiling and drying to make "kirku." Andean people have long recognized the benefits of consuming lupins, associating their use with religious festivals. In South America, the traditional use of tarwi as staple food continues in many Andean communities. Around the Mediterranean lupini beans, large-seeded bitter grain of L. albus, have been extensively used as a snack food, and as a bean substitute in times of drought. They are still commonly used as a bar snack. The beans (grains) are substantially debittered before being pickled and bottled or canned for use as a snack food. In the Middle East, the grain of L. albus is used to make snack foods, most commonly at the time of Ramadan (several thousand tons are imported from Australia each year for this). They are usually boiled whole and spiced, with the testa being removed before eating. Another use is to make a paste similar to the traditional "falafel" from faba beans.

#### Bean/Sprout

Immature seeds do not yet contain any alkaloids and have a similar taste and nutritional value to immature (green) peas. They can be used as an alternative to soybeans to make "edamame." Lupins can be germinated to make a sprout suitable for vegetable or salad use. Germination lowers the content of alkaloids, phytate, and oligosaccharides on a dry-weight basis, but also the overall content of the protein fraction. The lupin sprout compares favorably with soy and mung sprouts for taste and texture, although it has a slight beany and bitter aftertaste. Unlike most species, there is no vitamin C production on germination. There is, however, considerable production of the isoflavones, genistein, and daidzein.

Whole seeds may be deep-fried or made into spreads similar in nature to "hummus" (traditionally

made from chickpeas) and falafel (traditionally made from faba beans) and used with salads or breads.

#### **Fermented Foods**

"Tempeh" is a traditional Indonesian food, made by two successive fermentations using soybeans as substrate (see Fermentation: Foods and Nonalcoholic Beverages. Soybean: Soymilk, Tofu, and Okara). A bacterial fermentation during the soaking of cooked de-hulled soybeans is followed by a solid-state fermentation of the bean mass by the mould Rhizopus oligosporus. Tempeh has been successfully made from L. albus, L. angustifolius, and blends of both with soybeans. Indonesian consumers like the taste of lupin-based tempeh but frequently comment that the texture is too firm. The production of second-generation products, such as burgers and patties, from lupin-based tempeh seems to overcome this objection.

"Miso" is a fermented paste made from soybeans, usually mixed with rice (*see* Soybean: Soy-Based Fermented Foods). A traditional process involves preparing a rice "koji" by fermenting cooked rice with a culture of *Aspergillus oryzae* ("tane-koji"), and then adding cooked soybeans and salt to the koji. Lupins have been used experimentally to make miso of comparable quality to soy miso. "Natto," another Japanese fermented food, can also be made from lupin grain.

"Shoyu" is the traditional soy sauce of Japan (see Soybean: Soy-Based Fermented Foods). Some Japanese people now use lupin shoyu as an alternative. Lupins can be used to make sauces similar in flavor and texture to the traditional soy sauces of China, Korea, and Indonesia.

Several thousand tons of Australian sweet lupins have been used commercially in Indonesia for tempeh production, and small commercial batches of lupinbased miso have been sold in Japan.

#### Flour Additive

Lupin kernel flour can be mixed in with wheat or wholemeal flours to make bread more nutritious, by giving a better balance of essential amino acids. Legume grains contain relatively more lysine and less methionine than cereal grains; so adding  $\sim 10\%$  lupin flour to wheat flours will give a product with similar properties to the full-wheat product, but with an improvement in amino acid score from under 40% to over 70% relative to egg albumin. Loaf volume is compromised with increasing lupin content but fortifying with gluten or using harder wheat in the blend can overcome this. In practice, the limit of inclusion is  $\sim 10\%$ . There is an increase in water-holding

capacity. The texture, flavor, and golden color of the lupin-wheat flour is appealing to many consumers. In Australia, some bread manufacturers use lupin-hull flour to provide "bulk" in high fiber breads. Ultra fine kernel flour has an attractive yellow color, good dispersion in aqueous systems, and good emulsifying properties. Albus lupin flour can be added into wheat flour to make the traditional Chilean breads "hallula" and "marraqueta." Up to 10% micronized flour from L. albus cotyledons could be included in breads, biscuits, and cakes, enhancing protein quality, color, and taste. The added lupin flour retards staling, probably by increased water retention and better emulsification properties. Studies have shown a high acceptability of pasta enriched with L. angustifolius kernel flour, and up to 15% substitution of L. albus flour for semolina or durum flours enhanced the protein quality and appearance of spaghetti noodles without affecting sensory qualities. Flour from L. albus has been blended with noodle wheat flour to make unsalted white noodles with an improved protein and dietary fiber contents, and color and texture. Up to 50% lupin flour can be incorporated into a range of cakes and biscuits.

There are several suppliers of lupin flour, and other products in Europe, but there are no available data on sales.

#### **Lupin Kernel Fiber**

The purified cell-wall fraction from lupin kernels is virtually colorless, odorless, and tasteless and can hold up to 8 times its own weight of water. The fiber has cholesterol-lowering properties, and acts as a fecal-bulking agent thus reducing stool transit time and benefiting bowel health. It also enhances satiety, probably due to its high water-holding capacity, and has potential as an antiobesity agent. The soluble fiber fraction can function as a fat replacer. The fiber can be used to supplement breads, pastas, biscuits, mousses, and jellies.

#### **Protein Fractions**

Lupin kernel proteins have some valuable functional properties, combining good emulsifying, foaming, water- and oil-holding properties but with less thermal stability than soy proteins. Nevertheless, lupin-protein concentrates and isolates are used as meat extenders, and refined fractions used to enhance the foaming properties of dairy desserts and other foods.

#### **Vegetable Milk Products**

A lupin-based milk was used in the Chilean program for children's nutrition for several years, and is still used by households in some parts of the country. In making "tofu" (see Soybean: Soymilk, Tofu, and Okara), it is possible to incorporate up to 30% lupin (L. angustifolius) milk with soymilk before the coagulation stage and produce an acceptable product, with the advantage of a lower unit cost of production. Higher levels of incorporation are not possible because the lupin proteins lack the necessary tertiary structures to produce a good curd. Sensory evaluations showed that tofu from a 30/70 lupin/soy blend was equally acceptable as a tofu made from soymilk.

Fermentation of lupin milk produces a yogurt-like product of stable texture and with no beany flavor. It is comparable to the yogurts made from soymilks.

Malted lupins could be used as a health-drink base. The presence of the beneficial isoflavones — genistein and daidzein — in the hypocotyls of developing lupin sprouts is well-reported. Lupin tea, made in the same way as pearl barley tea, has been developed in Japan.

#### **Allergenicity of Lupins**

There are some reports of lupin allergenicity in the medical and scientific literature. Nearly all of the affected subjects had a history of reactions to other protein foods (e.g., peanuts, soybeans, shellfish). The evidence to date suggests that lupin proteins have a lower allergenicity than most other protein foods.

#### **Feed Uses**

#### **Ruminants**

Almost all of the testa can be fermented in the rumen so there is no need to de-hull lupins for ruminant feeds. The whole grain is highly fermentable with a minimal risk of acidosis. It is best to crack the grain open as a minimum action, to increase access for the rumen flora, but any grinding should not be too fine because there can be a loss of material to the hind gut where it is used less efficiently. A significant amount of protein degradation occurs in the rumen, but some protection from this is possible by treating the grain with formaldehyde before feeding. Other methods include flame-roasting the grain, steaming, and rolling the grain into flakes, and coarsely grinding the grain and then extruding the meal (see Extrusion Technologies).

Lupins are a valuable component of diets of feedlot cattle (up to 40% of the diet), and milking cows can readily consume large amounts of (cracked) grain whilst in the milking sheds. Rangeland farmers often supplement their grazing sheep with 50–250 g grain per head per day in dry periods when pastures are low. There is ample evidence for an enhancement of reproductive performance of both

rams and ewes when their diets are supplemented with lupin grain prior to mating. No specific factor has been attributed to this.

Several hundred thousand tons of grain are retained on-farm in Australia each year for grazing supplements for sheep. The greatest consumption of traded lupin grain is by cattle, either in feedlots or under intensive housing when fed formulated diets containing lupin grain.

#### **Nonruminant Animals**

The testa is virtually worthless to all nonruminant species. It is removed whenever economical to do so. Otherwise, it simply supplies some bulk nonfermentable fiber. Processed lupin kernel meal is frequently used in commercial feeds for pigs, poultry, and aquatic species. The actual inclusion levels will depend on price and availability of the meal and alternative ingredients as well as any constraints imposed by any individual ingredient. The meal is low in lysine and methionine relative to most animal needs, but this is not a significant problem. First, this deficiency is reflected in the price of the ingredient; secondly, deficiencies of one ingredient in the formulation will be at least partly offset by other ingredients; and thirdly, crystalline amino acids are readily available to the feeds industry.

Pigs The digestible energy (DE) value of whole lupin grain for pigs is  $\sim 14-15\,\mathrm{MJ\,kg^{-1}}$ ; however there is a lot of hindgut fermentation and the net energy value is  $\sim 10.5\,\mathrm{MJ\,kg^{-1}}$ . Nevertheless, lupins are widely used in the pig industry. Recommended inclusion levels of ASL vary from 100 to 350 g per kg depending on the age and physiological status of the animal. Yellow lupins have a similar energy value and are also widely used. Albus lupins are not used because they contain an inappetence factor for pigs. This reduces feed intake, slows growth, and makes it uneconomic to use such a diet.

The addition of industrial carbohydrase enzymes to lupin-based pig diets can improve their net energy value.

Pigs rank second to cattle in the overall consumption of lupin grain, almost entirely as a component of formulated feeds.

**Poultry** The apparent metabolizable energy (AME) content of both ASL and YL grain is  $\sim$ 8.6 MJ kg<sup>-1</sup>. It is not practical to use more than 100 g per kg feed because of the high water-holding capacity of the undigested carbohydrates, which can cause wet droppings. For layer birds, it is possible to include up to 300 g per kg in the diets, as the wet droppings are less

of a problem. Albus lupins can be readily included in poultry diets. There is some benefit from adding carbohydrase enzymes to poultry diets. The oligosaccharides in lupin meals seem to have a beneficial effect on osmotic stability of the gut enhancing nutrient absorption.

Poultry ranks third as consumer of lupins in formulated feeds.

Finfish and crustaceans Lupins can be used in both high-nutrient density (HND; high protein and oil content) and low-nutrient density (LND; low protein and oil content) diets for finfish. When included in HND diets fed to rainbow trout (Oncorhynchus mykiss), the DE value of lupin kernel meals ranges from  $12.8 \,\mathrm{MJ\,kg^{-1}}$  for L. angustifolius, to  $14.8 \,\mathrm{MJ\,kg^{-1}}$ for L. albus. Protein digestibility of all lupin varieties when fed to fish is high, typically greater than 95%. The low essential amino acid content is less of a problem for finfish fed HND diets than for pigs and poultry because of the high level of protein required in the diets restricting the practical inclusion levels of the meal and also that so much of the dietary protein is metabolized as an energy source. Good acceptance and palatability of HND diets containing L. angustifolius, L. albus, and L. luteus has been reported, with acceptable inclusion levels up to 500 g per kg.

Lupin meals are used commercially in diets for freshwater, estuarine, and marine finfish as well as crustaceans. This is a small but emerging market, as the relative value of the splits seems high for finfish.

See also: Fermentation: Foods and Nonalcoholic Beverages. Grain Crops, Overview. Grain, Morphology of Internal Structure. Grains Other than Cereals, Nonstarch Polysaccharides. Lupin: Breeding; Agronomy. Pulses, Overview. Soybean: Soymilk, Tofu, and Okara.

#### **Further Reading**

Gladstones JS, Atkins C, and Hamblin J (eds.) (1998) Lupin as Crop Plants: Biology, Production and Utilization. Oxon, UK: CAB International. (This book is the definitive work on all aspects of the Genus Lupinus, and was drawn on for this article).

*Nahrung* 45(6) (2002) A special edition covering the processing of lupins and other grain legumes.

Petterson DS (2000) The use of lupins in feeding systems: review. *Asian Australasian Journal of Animal Sciences* 13(6): 861–882.

Van Barneveld RJ (1999) Understanding the nutritional chemistry of lupin (Lupinus sp.) seed to improve livestock production efficiency. *Nutrition Research Reviews* 12: 1–30.

Wink M, Meissner C, and Witte L (1995) Patterns of quinolizidine alkaloids in 56 species of the genus Lupinus. *Phytochemistry* 38(1): 139–153.

#### **Relevant Websites**

http://www.rala.is — This gives a brief background of The International Lupin Association and provides a link to details of their scientific proceedings. http://www.agric.wa.gov.au — This website, managed by the Department of Agriculture, Western Australia gives a scientific bibliography on lupins; covering breeding, agronomy, disease and pest problems, composition and utilization of the grain.

# **Breeding**

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#### Introduction

The lupin is a legume plant; its seeds have been used as food and feed from ancient times. The green plant itself has been used extensively in some countries as forage and as an organic material for soil enrichment or in crop rotation. Despite its qualities and potential, it has not become a major world crop. This article will review the main characteristics of breeding, the nutritive value of its seeds, and the uses and potential for more extensive production.

#### The Plant and Its Origin

Lupin or lupine are trivial names for plants of the genus *Lupinus* belonging to the Fabaceae family, subfamily Papilionoideae. This genus is very diverse and contains several known species. It is an annual plant, usually 0.3-2 m in height, with a highly branched stem, digitated leaves, and a shrubby growth pattern. The flowers form high above the leaves, may be of several different colors (white, yellow, purple, blue), and exude a honey-like aroma. The roots are relatively long, usually nodular, and may extend to a depth of 3 m. The lupin has the ability to fix nitrogen and mobilize phosphorus and other elements by the exudation of citrate to the soil. This is very important for soil enrichment in infertile areas. The pods are normally flat and with a hairy aspect on the outside. They vary in length (4-10 cm) and, depending on the species, have different types of seeds that may vary in

**Table 1** Taxonomic and common names of some commercial *Lupinus* species

Species	Common names
L. albus <sup>a</sup>	White lupin, Egyptian lupin, tremoço, <sup>b</sup> altramuz <sup>c</sup>
L. angustifolius <sup>a</sup> L. luteus <sup>a</sup> L. mutabilis <sup>d</sup>	Blue lupin, narrow-leafed lupin Yellow lupin, tremosilla <sup>c</sup> Tarwi, tauri, tarhui, chocho, <sup>c</sup> Andean lupin

<sup>&</sup>lt;sup>a</sup>Mediterranean origin.

size, shape, or color. However, the endosperm is usually yellowish, and the most common seeds are flattened (*Lupinus albus*) or ovoid (*Lupinus mutabilis*) with diameters in the range 2–15 mm. It is a plant well adapted to poor sandy and acid soils and also to high altitudes, and to temperate climates, found in Europe. The most important species that are produced on a commercial scale are *L. albus*, *L. angustifolius*, *L. luteus*, and *L. mutabilis*.

The lupin has its origin in the Old World in the Mediterranean region, as well as in the New World, in North America, and in the Andean highlands. Some representative examples of the former are L. albus, L. angustifolius, L. luteus, and L. consentinii. "Tarwi" is the indigenous name of L. mutabilis, which is the lupin species found in the Andes (Table 1). However, there are probably more than 200 wild species, most of them in the New World. L. albus was cultivated for human consumption by the ancient Romans, Egyptians, and Greeks; however, due to its bitter taste, the mature seed had to be subjected to prolonged washing before consumption. A similar treatment was also used by the ancient inhabitants of the Andes. The seeds of tarwi were boiled, thoroughly washed in a river for several days, and then suitably cooked for immediate consumption or dried for future use. Presently, several different species of lupins are grown in Europe, some parts of Africa, South America, Australia, and New Zealand, including a number of sweet low-alkaloid varieties which are now commercially available.

#### **General Aspects of Lupin Breeding**

Lupin utilization and breeding programs started simultaneously. This explains why *L. albus*, cultivated in ancient Egypt, as well as *L. mutabilis*, an Andean native, have the same nonshattering characteristics. In contrast, the wild forms of both the species have a distinct shattering characteristic. Despite progress in the area of lupin breeding, records of the world

<sup>&</sup>lt;sup>b</sup>Portuguese name.

<sup>&</sup>lt;sup>c</sup> Spanish names: altramuz may be used also to designate other species. <sup>d</sup> Andean origin: tarwi, tauri, and tarhul are from native languages. Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 3623, Elsevier Ltd.

gene resources of the lupin plant are still insufficient and not easily available to plant breeders. This has been recognized as one of the main problems for worldwide lupin cultivation. Detailed information on the features of a particular lupin accession would help breeders. Their particular objective of coordinating specific features of the plant with regional conditions of soil and climate, in order to improve productivity on a commercial level, would be possible. The genus *Lupinus* is very diverse, with many species and varieties originating from distinct regions of the New and Old World. This diversity also contributes to a considerable increase in the difficulties encountered while planning a plant-breeding program. Despite these problems, significant advances have been achieved in the improvement of plant resistance to diseases, in the establishment of stable low alkaloid lines, and in the increase of productivity of the most-used commercial species of cultivated lupins.

Production of lupin may be directed to completely different objectives. It may be used as green forage, particularly for silage making, or for green manuring. In these cases maximum green-matter yield is strongly desirable. The plant when used as green manure improves the soil conditions since it increases the amount of organic matter and also provides accumulation of nitrogen and phosphorus in poor sandy soils. Bitter varieties may be used when the plant is grown for this purpose. However, for ruminant feed, as forage, sweet varieties are preferred due to the low alkaloid contents. Lupin grains are increasingly used as protein supplements for ruminant diet, and in some countries it also finds applications in human nutrition. Breeding for lupin seed production needs a more rigorous selection in order to obtain a satisfactory harvest index, short maturation periods, and disease resistance. Enhanced pod setting and normal branching are also desirable characteristics, particularly to complement short growing seasons found in Mediterranean climates. The high levels of toxic alkaloids originally found in lupin seeds used to be a negative factor to improve the worldwide production. However, low alkaloid varieties have been established for the most important lupin species. The "sweet" forms are natural or induced mutants, that were generally weaker, so backcrossing with more vigorous bitter forms was required to improve them. Several sweet varieties have been established in Germany, Poland, Russia, Australia, and in the last decades in France and Chile as well. Most of them are sown in the spring and the first winter varieties were only more recently obtained in USA, Chile, and France for the species L. albus. These varieties are not only resistant to temperatures as low as  $-10^{\circ}$ C but also show high yields up to  $7.3 \, \text{t ha}^{-1}$ .

Another significant and more recent advance in lupin breeding was plant selection starting from induced mutations of forms with determined growth or epigonals. This was achieved with different species in Germany, Russia, and Poland. However, these mutants were also too weak and backcrossing with vigorous high yielding forms was necessary. In France and in Chile winter cultivars were obtained that are homogeneous in maturity and easy to thresh, but these varieties maintain their tendency to produce lower yields. This has limited their success when sown in the Spring. An exception is a very early maturing variety created in Denmark, with initial material coming from Belarus. Domestication of narrow-leafed lupin in Australia allowed large-scale seed production for animal feeding as a soybean substitute. In fact, Australia has become one of the biggest producers and exporters of lupin seeds in the world. This work was started by J Gladstones, who was responsible for developing early maturing lupin crop with low alkaloid seeds, but at the same time with nonshattering pods. In his breeding work, in order to distinguish desired plants from naturalized types of L. angustifolius which were previously bitter with blue flowers, he selected white flowered plants as a marker showing the desired attributes. Natural mutants were then selected from field populations and were intercrossed in order to combine all desired characteristics. Also, crosses with wild types selected from Mediterranean regions were carried out. The first successful variety obtained was Uniwhite, released in 1967. Many other new varieties have been developed following additional breeding programs. Domestication of rough-seeded lupins was later achieved with L. consentinii, and more recently breeding programs have been developed to domesticate L. atlanticus and L. pilosus. Some rough-seeded lupin species grow wild on Mediterranean soils. These two species show great potential for cultivation in fine-textured alkaline soils since few cultivated species have this characteristic. Work by Buirchell in Australia following previous selection of mutants with low alkaloid content by Gladstones has been developed aiming at domestication of these species. Introgression of domestication genes from previously domesticated L. consentinii and induction of domestication genes by means of mutation were the strategies used. Some lines of L. atlanticus with domestication characters were selected showing potential for large-scale cultivation.

#### **Resistance to Stress and Diseases**

Parallel to the improvement of the yield, great efforts have been made to increase lupin resistance to

diseases. Fungal and virus diseases are the most common forms of lupin crop contamination such as anthracnose, fusariose, and phomopsis. The first caused by the fungus Colletotrichum gloesporoides poses the main threat for lupin cultivation. The latter causes a disease called lupinosis that affects animals fed with infected plants. Because anthracnose is a seed disease, the symptoms may appear early mainly showing cankers on the stem and the plant may die even before flowering. Significant advances to improve lupin plant resistance to anthracnose have been achieved particularly for L. angustifolius in Australia and L. albus in Chile. Selection and intercrossing strategies have helped in developing lines more resistant to anthracnose and gray leaf spot from collaborative work between Australian and American researchers. Varieties derived from wild Mediterranean lupins proved to have moderate resistance to phomopsis. However, until now selected varieties are not completely resistant to the pathogens.

Necrotic and nonnecrotic strains of mosaic viruses may also be responsible for crop damage, but to a lesser extent than fungal diseases. Pleiochaeta setonsa is the main cause of the disease brown leaf spot, favored by cool temperatures. Because the incidence of this fungus is more accentuated in crops sown in European autumn, winter lupin cultivars tolerant to frost has been increasingly used in Europe. Rust caused by *Uromyces lupinicolus* affects plantation mainly in warm and dry summer promoting considerable defoliation. In this case, the use of fungicides based on triazole compounds appears to be quite effective to face this problem. Phorbia platura is an insect that may have a significant negative impact on lupin crop, particularly the white lupin. The larvae usually promote extensive damage to the roots and hypocotyls, and the systemic use of insecticides is the most effective treatment.

Inadequate climate and soil conditions may cause abiotic stress and produce losses in lupin crop. Frost, inadequate soil pH, and other climate conditions are the main causes. Most of *Lupinus* species are not tolerant to high pH. Above pH 7.5, free lime present in the soil may induce iron chlorosis. However, in low pH, below 4.5, aluminum toxicity may be observed. Some structural characteristics of the plant may help in frost resistance. Large roots, which is greatly dependent on genotype, favor plant survival in intense frost conditions. Vernalization requirements and the plant capacity of hardening leaves are other components of frost resistance.

Germ plasm collections, hybridizations, and mutations have been the main sources of genetic variability applied to lupin breeding up to now. Interspecific crossing has also been a useful tool to improve general

characteristics of lupin varieties. This has been possible between species of the same number of chromosomes, e.g., *L. mutabilis*, *L. elegans*, and *L. polyphyllus*. This can be an interesting approach, for example, in the case of *L. polyphyllus*, which has small seeds and a high proportion of hull, but by crossing with *L. mutabilis*, it can produce bigger seeds with increased protein content and less fiber. The interspecific crossing between species of different number of chromosomes has been actively pursued by researchers in Australia using more modern techniques.

The improvement of the chemical composition of the seed has been of increasing importance in the last decades, together with the concept of protein and oil yield per hectare. Until now, the most favorable composition has been achieved with L. mutabilis, but the best yield of protein and oil per hectare with L. albus. However, within the species, different varieties may show significant differences. In the case of L. albus, which commonly contains  $\sim 35\%$  of protein, it is possible to establish lines with 38-40% of protein. In the case of L. mutabilis, some varieties may have up to 50% protein. More recently, great attention has been directed to other seed components such as carotenoids and omega-3-fatty acids when breeding programs are planned.

Lupin seeds have been increasingly used in feed formulation for fish farming, particularly salmon. Due to overexploitation of marine resources, fish farming seems to be a promising source of protein and oil for human nutrition, and the demand for grains, including lupin, used in the rations will certainly increase tremendously. In human nutrition, lupin seeds are an interesting alternative for diabetic persons due to their carbohydrate composition as well as for those who do not tolerate some other sources of protein of animal or plant origin.

#### **Nutritive Value**

Lupin seeds show similar nutritive attributes to soybeans, particularly with respect to protein and fat content. Some species may have protein contents up to 50 g per 100 g and up to 22 g per 100 g of fat in the seeds. The approximate composition of seeds of some relevant lupin species are presented in Table 2. The protein and fat content may be further increased by de-hulling the seeds.

Although nutritive value may be theoretically improved by breeding, the immense chemical diversity of compounds that are considered make this task extremely complex. However, if a specific target is chosen, improvement may be achieved through breeding programs based on selection of particular species and varieties and intercrossing with others, which could

lead to promising combinations of characters for cultivation. Because different biochemical pathways are involved in the synthesis of particular nutrients in the plant, in some cases the improvement of a particular nutrient may be deleterious to others. For example, breeding strategies to increase the protein content of a seed may lead to a decrease in the oil levels. In practice, breeding efforts to increase nutrient levels of lupin seed did not appear to have produced effective results yet.

#### **Protein**

The lupin seed is one of the richest sources of plant protein. The protein content varies largely between

**Table 2** Approximate composition of seeds of different lupin species<sup>a</sup>

Species	TCW (g) <sup>b,c</sup>	Protein <sup>c</sup>	Oil <sup>c</sup>	Ash	Fiber <sup>d</sup>
L. albus	370	38.2	9.5	3.1	14.0
L. angustifolius	165	28.9	6.6	3.2	17.6
L. consentinii	165	32.2	2.6	3.7	
L. hispanicus	71	48.2	5.8	2.9	19.6
L. luteus	164	49.2	4.8	5.3	17.6
L. mutabilis	211	46.6	15.8	3.6	9.0

<sup>&</sup>lt;sup>a</sup>Results expressed in g per 100 g of dry matter (except TCW).

<sup>b</sup>Thousand corn weight.

species, with L. mutabilis and L. luteus presenting unusually high values (near 50 g per 100 g), surpassing most soybean cultivars, whereas L. albus presents an intermediate protein content and L. angustifolius lower values. Seed protein provides all the essential amino acids, but some of them are not found in sufficient amounts for all sectors of the population. When lupin seed is used for animal feeding, such as ruminants or single-stomach animals, the relatively low level of methionine may be complemented by adequate formulation of the ration or by supplementation with the synthetic amino acid. Table 3 shows the amino acid composition of seeds of some relevant lupin species and the amino acid requirements for infants and adults established by a Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) joint committee (1985). When comparing the lupin amino acid profiles with the requirements for adults, all lupin species show ranges of essential amino acids with adequate amounts and this will be observed also for most pulses. However, if the requirements for infants are used for comparison, it is apparent that lupin protein will show many essential amino acid deficiencies, with tryptophan being the most limiting, having a chemical score of 47-59%, followed by methionine plus cystine. The chemical score is the ratio of the content of an individual essential amino acids in food protein to the content of the same amino acid in a reference pattern expressed as a percentage. It must be taken into account that, if requirements for infants are used for comparison, even beef protein will be deficient in leucine, lysine, methionine + cystine, tryptophan, and valine, and egg protein will be deficient in leucine

Table 3 Amino acid composition of lupin seeds

Amino acid		FAO recommendations <sup>c</sup>				
	L. albus <sup>a</sup>	L. angustifolius <sup>a</sup>	L. luteusa	L. mutabilis <sup>b</sup>	Infants	Adults
Histidine	22	27	29	26	26	16
Isoleucine	44	40	37	57	46	13
Leucine	75	71	79	71	93	19
Lysine	47	46	49	58	66	16
Methionine	7	7	7	9		
Cystine	16	18	24	11		
Methionine + cystine	23	25	31	20	42	17
Phenylalanine	35	37	39	38		
Tyrosine	46	34	27	40		
Phenylalanine + tyrosine	81	71	66	78	72	19
Threonine	36	34	32	38	43	9
Tryptophan	8	8	10	8	17	5
Valine	39	35	32	41	55	13

<sup>&</sup>lt;sup>a</sup> Data from Gross R (1988) Lupins in human nutrition. Proceedings of the 5th International Lupin Conference, pp. 51-63.

<sup>&</sup>lt;sup>c</sup> Data from Trugo LC, Almeida DCF, and Gross R (1988) Oligosaccharide contents in the seeds of cultivated lupins. *Journal of the Science of Food and Agriculture* 45: 21–24.

<sup>&</sup>lt;sup>d</sup> Adapted from Muzquiz M, Burbano C, Bouthelier V, Garcia-Aser C, Rodenas I, and R-Marin A (1982) Estudio de los elementos esenciales de destintas variedades de cinco especies del *Genus Lupinus* cultivadas y espontaneas de la Peninsula Iberica. *Proceedings of the 2nd International Lupin Conference*, pp. 173–181.

<sup>&</sup>lt;sup>b</sup> Data from Gross R, Koch F, Malaga I, Mirinda AF, Schoeneberger H, and Trugo LC (1989) Chemical composition and protein quality of some local Andean food sources. *Food Chemistry* 34: 25–34.

<sup>&</sup>lt;sup>c</sup>Data from FAO/WHO/UNU (1985) Necessidades de Energia y Proteinas. Geneva: World Health Organization.

and lysine. However, if the previous FAO/73 amino acid pattern is used as a reference, methionine + cystine will be the limiting amino acids for lupin (56– 71% chemical score), as in most pulses (soybean 83% chemical score). Consequently, it is strongly recommended that the intended nutritional application of lupin seeds or lupin products be defined to allow a more realistic assessment of the protein quality. From Table 3, it can be seen that the amino acid profile varies considerably with lupin species. Large variations are also observed in biological indicators for protein-quality assessment. Nevertheless, protein utilization, as measured by the net protein utilization index, does not correspond exactly to the amino acid chemical score of different lupin species. For example, L. angustifolius shows a relatively high chemical score (60%, FAO/85 for infants) but presents the lowest net protein utilization index (43%). Despite its amino acid composition, active biological components appear to have a negative influence on the net protein utilization index of L. angustifolius protein in rats. L. albus shows the highest net protein utilization index (70%) followed by L. mutabilis (64%). These figures approach the net protein utilization index of the soybean (69%). Overall, lupin protein should be considered a good plant source of protein for animal and human nutrition. However, the appropriate application and the variation encountered between species must be considered for assessing lupin protein quality.

#### Fat

The fat content of lupin seeds varies considerably between species. In some species, the lipid fraction contributes substantially to the total energy value, whereas in others it is present in relatively small amounts (Table 2). *L. mutabilis* has the highest oil content (up to 22 g per 100 g) and is considered to be a potential material for oil production. *L. albus* has a combination of high protein and an intermediate fat content, which are important attributes for a food,

especially if it is to be used in developing countries where protein and energy are scarce. The fatty acid distribution in the oil fraction is also variable between species. However, low levels of saturated fatty acids are normally found. A very high content of linolenic acid is present, particularly in *L. albus* and *L. luteus*. As in other seeds, high levels of oleic acid are common for all species, particularly *L.albus* and *L. mutabilis* (Table 4). The ratio of polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFAs) in lupin seeds is in the range 1.3 to 2.9:1 which is considerably higher than beef but lower than the soybean (4:0). Higher rates are important, since it has been strongly recommended that a reduction of SFAs in favor of an increase of PUFAs in the diet should be achieved, to assist coronary heart disease prevention. Linoleic acid, which is an essential fatty acid, is found in significant amounts in lupin seed, with L. luteus being the richest source (44%). Linolenic acid, which is the most important source of  $\omega$ -3 fatty acids, is found at very high levels in L. albus and L. luteus, but at relatively low levels in L. angustifolius and L. mutabilis (Table 4). L. mutabilis may be commercially used for oil production, and its low linolenic acid content, although not so nutritionally important as in other sources, may be advantageous to improve its conservation in comparison with other vegetable oils. Considering the fatty acid profile of lupin seeds, it is noted that L. mutabilis and L. albus are more similar to peanut oil, whereas L. angustifolius and L. luteus resemble maize oils, although they differ in flavor.

#### **Carbohydrates**

In contrast with many legumes, lupin seeds are practically devoid of starch, and the major carbohydrates in mature seeds are oligosaccharides and nonstarchy polysaccharides, particularly from the cell-wall structure. The oligosaccharides found in the cotyledons are sucrose and nondigestible galactosides of the raffinose

Table 4 Fatty acid composition of lupin seeds in comparison to soybeans

Legume						Perc	entage	of total	fatty ad	cids <sup>a</sup>				
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	22:1	24:0	SFA <sup>b</sup>	<i>MUFA</i> <sup>b</sup>	PUFA <sup>b</sup>
L. albus	0.2	7.2	0.5	2.1	48.6	19.9	13.0	0.8	4.4	2.2	1.2	16	51	33
L. angustifolius	0.3	11.0	0.2	6.2	33.5	39.4	4.6	0.7	2.6	0.2	0.5	21	34	44
L. luteus	0.3	5.5	0.2	2.8	24.3	43.9	10.6	2.4	7.0	1.1	1.0	19	26	55
L. mutabilis Glycine max (soybean) <sup>c</sup>	0.2 0.2	11.5 10.0	0.5 0.2	9.9 4.0	45.7 25.0	27.3 52.0	2.3 7.4	0.9 0.3	1.2 0.1	tr tr	0.3 tr	24 15	46 25	30 60

<sup>&</sup>lt;sup>a</sup> Fatty acids: 14:0, myristic; 16:0, palmitic; 16:1, palmitoleic; 18:0, stearic; 18:1, oleic; 18:2, linoleic; 18:3, linolenic; 20:0, arachidic; 22:0, behenic; 22:1, erucic; 24:0, lignoceric.

<sup>&</sup>lt;sup>b</sup>SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids.

<sup>&</sup>lt;sup>c</sup> Paul AA and Southgate DAT (1979) McCance and Widdowson's The Composition of Food, 294p. London: Her Majesty's Stationery Office. Other data from Muzquiz M et al. (1982) (see Table 2).

family. The main galactosides present in lupin seeds are raffinose (degree of polymerization (DP) of 3), stachyose (DP of 4), and verbascose (DP of 5) (Table 5). These oligosaccharides are derived from sucrose, with increasing numbers of α-galactosyl units linked to C6 of glucose. They are present in most legumes and are not digested by monogastric animals and hence contribute to the flatulent property of legumes. More recently, nondigestible sugars have been used as agents for fermentation in the intestines, thus helping to maintain and regulate the microbial intestinal flora. In this aspect, lupin may be an important raw material for production of oligosaccharides of the raffinose family to be used as functional food ingredients. The polysaccharides are present as major components of the cell wall surrounding the cytosol. Only a small number (1%) of polysaccharides are water soluble, and, together with oligosaccharides, they are important as a source of energy for seed germination. The amount of polysaccharides in lupin seeds is dependent on the species, with high contents found in L. albus and L. angustifolius and the lowest in *L. mutabilis*, due to its thinner seed hull. Due to the polysaccharide composition, lupins are extraordinarily rich sources of fiber. Crude fiber data are presented in Table 2, but even higher figures will be encountered if dietary fiber is considered. Lupin carbohydrates are important sources of energy only for ruminants, but due to their characteristics they may provide useful material to produce special high-fiber food for humans.

#### **Vitamins and Minerals**

The vitamin pattern of lupin seeds is somewhat similar to other legumes. Lupin seeds are good sources of vitamins of the B group, particularly niacin, with levels above 4 mg per  $100 \, \text{g}$ . The thiamin content is  $\sim 0.5 \, \text{mg}$  per  $100 \, \text{g}$  and that of riboflavin  $\sim 0.4 \, \text{mg}$  per  $100 \, \text{g}$ . Carotenoids and tocopherols are present, with the former being mainly responsible for the color of the oil fraction. The mineral composition is similar to other legumes in relation to the major elements,

except for calcium, which is low in all species. However, some differences are found in respect to trace elements. Manganese is found in lupins in unusually high amounts, especially in *L. albus*. In some varieties of this species, it may reach values of 83.5—143 mg per 100 g, and this must be taken into account when it is used in daily animal or human diet. Nevertheless, common figures are in the range 2.5—38.0 mg per 100 g. Lupin seed is also a good source of iron and zinc, with values in the range 2.5—14.0 and 3.0—18.0 mg per 100 g, respectively.

#### **Bioactive Compounds**

Some components found in natural products may present biological activity but no nutritional properties. In fact, some of them may be undesirable for human and animal nutrition. In lupin, some compounds with these characteristics have been reported. Compounds sometimes called "antinutritional factors" are present in lupin seeds in the same range as in other legume seeds, except for trypsin inhibitor, which is virtually absent in the lupin. Allergenic proteins that may cause allergenic food reactions sometimes observed in children are less intense with lupins than with other legume seeds and cow's milk. However, because peanut allergies are quite common, further studies are needed to check if there is any peanut-lupin crossallergy when lupin is used as an ingredient of mixed flour. Some relevant bioactive compounds found in lupin are the alkaloids, phytates, saponins, tannins, and flavonoids. Those bioactive compounds which were usually called antinutritional factors could be considered as targets for breeding programs. This, in fact, happened with the lupin alkaloids. Intensive breeding work was carried out in different countries based on classical methodologies of plant hybridization and selection and they have succeeded in the development of commercial lupin lines with low levels of alkaloids, the sweet lupins. In relation to other antinutritional factors, the situation is quite different. In fact, a lot of controversy exists at the moment in relation to the properties of these compounds.

**Table 5** Oligosaccharide composition of seeds of different lupin species<sup>a</sup>

Species	Sucrose	Raffinose	Stachyose	Verbascose	Galactosides
L. albus	2.9	1.0	6.6	1.1	8.7
L. angustifolius	3.4	1.5	5.2	2.0	8.7
L. consentinii	2.6	0.9	4.9	0.9	6.7
L. hispanicus	0.7	0.9	6.6	1.8	9.3
L. luteus	1.7	1.2	4.9	4.1	10.2
L. mutabilis	2.4	2.5	8.5	1.1	12.1

<sup>&</sup>lt;sup>a</sup>Results expressed in g per 100 g of dry matter.

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Their effects as protective factors for the plant, combined with some confirmed desirable biological properties that have been recently reported for humans, complicate the decision to decrease or in fact increase them in the seeds through breeding work. Some examples are the antioxidant properties of phytates, tannins, and flavonoids, the antitumoral properties of trypsin inhibitors, phytates and saponins, and the protection against cardiovascular diseases of tannins and flavonoids. Although no specific lupin variety has been developed yet by breeding that shows considerably lower levels of those bioactive factors except in relation to alkaloids, selection and intercrossing may be applied to establish lupin lines containing low levels of antinutritional factors. However, it appears that plant transformation would present more potential to show positive results in a shorter time. Current practices based on the use of Agrobacterium tumefaciens vectors, direct uptake into protoplasts, electroporation, and microprojectiles have potential as techniques to be used to introduce specific DNA in the lupin in order to block the expression of a specific bioactive compound. The effect which this could produce in the performance of the plant in the field is still something to be seen in the future.

#### **Alkaloids**

The major alkaloids present in lupin are from the quinolizidine family, although some gramine alkaloids may also be found in L. luteus. Quinolizidine alkaloids have received much attention, because they have a strong bitter taste and may be toxic in high doses. Lupanine, sparteine, lupinine, and some forms of hydroxylated lupanine are some relevant examples. They have a sedative effect on the central nervous system, with sparteine producing the strongest effect. The total alkaloid content in bitter lupins is usually in the range 1.0-4.5 g per 100 g. Intensive breeding work carried out, mainly by Sengbush in Germany, Gladstones in Australia, and Baer in Chile, has led to the establishment of new lupin varieties practically devoid of alkaloids. Presently, sweet varieties of L. luteus, L. angustifolius, L. albus, L. mutabilis, L. consentinii, and L. atlanticus are defined, of which the first three are commercially productive. However, bitter lupins are still largely used in some regions where the new sweet varieties are not well adapted. In those cases, the preliminary soaking and washing procedure before consumption is essential.

#### **Phytates**

Phytate is sometimes considered an antinutritional factor, because it is implicated with the impaired

absorption of minerals. It is found in lupin seeds roughly in the same concentration range as in other pulses, but it is usually lower than that found in soybeans. The average amount found in lupin seed is  $\sim$ 0.8 g per 100 g. The phytate:zinc molar ratio, which may be an indication of zinc bioavailability, is generally lower than in other legume seeds, making lupin a better dietary source of this mineral. The enzymatic degradation of phytate in the digestive tract or in food products submitted to special processing may lead to the formation of inositol phosphates with different degrees of phosphorylation. Some of these derived components may be detected as phytates by nonspecific methods, although they may not present mineral chelation activity. Consequently, more comprehensive information on inositol phosphate composition of different food items is becoming more important to assess the real role of food phytate in the daily diet. More recently, some attention has been directed to the desirable properties of phytates, particularly due to its antioxidative characteristics, which may be beneficial to counteract free-radical activity. Other biological properties may be derived from the lower inositol phosphates since some of them are involved in cell-signaling mechanisms.

# **Saponins**

Saponins are compounds formed by triterpenoids or steroidal aglycones and a carbohydrate moiety by ester or ether linkages. They are present in different classes of plants, particularly in legumes, roots, and some medicinal herbs. Their presence in food products has been considered to be deleterious if consumed frequently. They are toxic to fish and promote retardation of growth in livestock and laboratory animals. They may also produce erythrocyte lysis *in vitro* and may alter intestinal epithelium functional making the mucosa more permeable. Consequently, their continued use in the diet may jeopardize the process of nutrient absorption. Conversely, it has been claimed that they can also be beneficial since they show the ability to lower plasma cholesterol, they have anticancer activity, and they may act as an inhibitor of viral replication. It is not yet clear, though, whether the net effect in the diet would be negative. Some lupin species such as L. luteus, L. mutabilis, and L. angustifolius may present a saponin content of 57-470 mg per kg, but they are not present in L. albus. However, these figures are still low compared to the content present in soybeans, which present values in the range 2000-5000 mg per kg.

#### **Tannins**

Tannins are complex polyphenolic substances found in plants, particularly pulses, with the property to precipitate proteins in aqueous medium. They interact with one or more protein molecules forming large cross-linked complexes that are insoluble in water. This property makes food tanning undesirable since they will make part of the dietetic protein indigestible. There is a wide variation on the content of tannins in legume seeds with higher values being found in faba bean and in peas. Lupin seeds present relatively low values of tannins in the range 0.2–0.5%. No correlation has been found between the tannin content and the bitter taste of some lupin seeds, and the impact of tannin from lupin seed in the diet has not yet been demonstrated both in humans or animals. However, the level of tannin in L. angustifolius has been considered to be low enough for use in pig diets without any kind of problem.

#### **Flavonoids**

Flavonoids are a class of phenolic compounds widely distributed in plants. Quercetin and rutin are among the most largely found flavonoids in a great variety of fruits and vegetables, including tea, coffee, and other grains. As it has been observed with other biologically active non-nutrient components, flavonoids may promote desirable and nondesirable physiological effects in humans. The property of flavonoids to induce goiter has been suggested by studies using peanuts and millet as foods with millet flavonoids presenting a strong inhibition effect on thyroperoxidase activity. The healthy properties of flavonoids may be derived from their antioxidative characteristics as free-radical neutralizers. However, some more specific functions have been reported, including their effect on cancer prevention, antiinflammatory and antiviral activities, and their positive effect on capillary fragility and vascular protection. Data on the presence of flavonoids in lupin are scarce, and although it is not clear whether they are encountered in significant amounts in lupin seeds, different flavonoids have been reported recently in the plants of L. luteus and L. albus, including those with the aglycones apinegin, genistein, and kaempferol.

#### **Food Uses**

At present time, the main applications of lupins are their utilization as green manure and as animal feed. Due to the ability of lupin plants to fix nitrogen and to make insoluble phosphorus available in the soil, they have been increasingly used in the crop-rotation system as an efficient and less expensive means of fertilization. For animal feeding, the whole lupin plant has been used as pasture or forage, or the dry seeds have been used as a direct soybean substitute, or in feeding formulas for pigs, sheep, chickens, dairy cattle, and other livestock. It has been recommended that not more than 10–15% of bitter lupins should be used in pig rations as pigs seem to be more sensitive to alkaloids than chickens. As mentioned earlier, lupin seeds have long been an available protein source for human nutrition. It was a common procedure to submit the grains to prolonged washing to remove the bitter taste, and this washing process is still employed, particularly in some regions of South America where bitter lupin seeds are mainly used. Although the washing process may increase the total cost of lupin products, the water treatment will in fact wash out not only the bitter alkaloids but also the oligosaccharides responsible for the flatulence of legumes. In addition, the end product will show increased relative amounts of protein and oil, on a dry matter basis, due to differences in component solubilities. It is a common procedure in the Andean regions to use leached and cooked bitter lupin seeds directly for consumption as snacks, in soups, salads, and stews. Alternatively, they may be dried and/or milled for future use in several dishes, including baked products. Lupin flour may be used for cereal protein enrichment since lupins and cereals have complementary amino acid compositions. Bread or other bakery products have been made successfully using lupin flour as an additive in the proportion of 10-20%. Many attempts have been made to use lupin seeds as direct substitutes for soybeans, e.g., in fermented products, soy milk, or soybean protein isolate. However, lupin protein does not have exactly the same functional properties as soybean protein, being less stable to heat and forming a milk-like water suspension. Nevertheless, lupin protein may be added to soy products without major modifications to the end product. It is apparent that investigation is still needed to develop a lupinspecific technology and new genuine lupin products for human nutrition.

#### The Potential of Lupin

The world lupin seed production is at present mainly concentrated in Australia, which has produced 1.38 million ton (Mt) in 1993 and around 2 Mt in the year 2000. This represents 2–4% of the world pulse production. Other lupin producers are the countries of the former USSR, Poland and Germany in Europe, and Chile, Bolivia and Peru in South

America, and South Africa. However, the lupin seed shows a real potential for trade in the grain world market due to its nutritional and agricultural properties. The high alkaloid content that used to be one of the major limitations for a wider application of the lupin seed has been gradually overcome by the introduction of commercial sweet varieties, mainly of L. albus and L. angustifolius. Although a new sweet variety of L. mutabilis is already available, work is still needed to establish a productive line with increased seed size and protein content. Besides the improvement in lupin nutritional qualities, research in specific areas such as plant breeding and biotechnology, crop protection, and food lupin technology is vital for lupin to gain a stronger foothold in the international market, with a substantial increase in production in Europe and South America. To consolidate this status an appropriate international price policy along with a strong marketing strategy is mandatory.

See also: Genome Mapping. Genomics. Lupin: Overview; Agronomy. Pulses, Overview. Taxonomic Classification of Grain Species. Variety Registration and Breeders' Rights.

#### **Further Reading**

- Buirchell BJ (1994) Domestication and potential of the rogh-seeded lupins. In: Neves Martins JM and Beirão da Costa ML (eds.) *Advances in Lupin Research*, pp. 19–24. Lisbon: ISA Press.
- Cowling WA, Buirchel BJ, and Tapia ME (1998) Lupin. Lupinus L. Promoting the conservation and use of underutilized and neglected crops. 23. Institute of Plant Genetics and Crop Plant Genetic Research, Gatersleben/ International Plant Genetic Resources Institute, Rome, Italy.
- FAO/WHO/UNU (1985) Necessidades de Energia y Proteinas. Geneva: World Health Organization.
- Fletwood JG and Hudson BJF (1982) Lupinseed a new source of edible oil. *Journal of Food Technology* 17: 11–17.
- Gladstones JS, Atkins CA, and Hamblin J (eds.) (1998) Lupins as Crop Plants: Biology, Production and Utilization. Wallingford, UK: Oxford University Press.
- Gross R (1982) El Cultivo y la Utilizacion del Tarwi. Estudio FAO, No. 36. Rome: Food and Agriculture Organization.
- Gross R (1988) Lupins in Human Nutrition. *Proceedings* of 5th International Lupin Conference. Poznan, Poland.
- Gross R, Koch F, Malaga I, Miranda AF, Schoeneberger H, and Trugo LC (1989) Chemical composition and protein quality of some local Andean food sources. *Food Chemistry* 34: 25–34.
- Hill GD (1977) The composition and nutritive value of lupin seed. *Nutrition Abstracts and Reviews*, B; *Livestock Feeds and Feeding* 47: 511–529.

- Huyghe C (1997) White lupin. Field Crops Research 53: 147-160.
- Muzquiz M, Burbano C, Bouthelier V, Garcia-Aser C, Rodenas I, and R-Marin A (1982) Estudio de los elementos esencials de destintas variedades de cinco especies del Genus Lupinus cultivadas y espontaneas de la Peninsula Iberica. *Proceedings of the 2nd International Lupin Conference*, pp. 173–181.
- Nelson P (1994) The development of the lupin industry in Western Australia and its role in sustainable farming systems. In: Neves Martins JM and Beirão da Costa ML (eds.) *Advances in Lupin Research*, pp. 565–576. Lisbon: ISA Press.
- Paul AA and Southgate DAT (1979) McCance and Widowson's The Composition of Food, 294p. London: Her Majesty's Stationery Office.
- Trugo LC, Almeida DCF, and Gross R (1988) Oligosaccharide contents in the seeds of cultivated lupins. *Journal of the Science of Food and Agriculture* 45: 21–24.
- von Baer E (1994) Lupin development policy in South America. In: Neves Martins JM and Beirão da Costa ML (eds.) *Advances in Lupin Research*, pp. 577–580. Lisbon: ISA Press.
- von Baer E (2002) Requirement of Lupin Protein as Salmon Feed. *Proceedings of the 10th International Lupin Conference*. Laugarvatn, Iceland.

#### **Relevant Websites**

http://www.publish.sciro.au. http://www.grdc.com.au

# **Agronomy**

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#### Introduction

This article surveys the agronomy of the three most commonly grown lupin species — narrow-leafed lupin (NLL, *Lupinus angustifolius* L., also known as the Australian sweet lupin, or blue lupin); white lupin (WL, *Lupinus albus* L.); and yellow lupin (YL, *Lupinus luteus* L.). It describes briefly the Australian and European farming systems in which they are grown, why each species is grown where it is, and the principles underlying the management of lupin crops. Current agronomic recommendations for growing lupin are also described.

# Types of Farming Systems in Which Lupin is Grown

About 85% of the world's lupin is produced in Australia, and  $\sim 80\%$  of this is produced in the grain belt of Western Australia, where NLL is grown in rotation with wheat and barley. Throughout the 1990s, canola was also increasingly used in rotations. Lupin is grown mostly on deep, coarse-textured (<10% clay), mildly acid soils which, in their natural state, are very infertile. NLL is an ideal grain legume under these circumstances because it is deep-rooted, quite acid-tolerant (as is its associated Bradyrhizobium), and modern cultivars yield well. It is preferred on these soils to field pea, the other major grain legume grown in Western Australia, because, despite producing less valuable grain, its strong erect stems and robust stubble mean that it can be harvested readily with machinery used for cereals, and it protects the fragile soil from erosion during long, dry, windy summers.

Lupin does not yield as well as wheat in this system, and farmers do not generally make as much money per hectare from lupin as from wheat. However, lupin contributes indirectly to the productivity of other farm enterprises. Important contributions include providing fixed atmospheric N2 for subsequent crops, breaking disease cycles in other crops, and providing high-quality supplementary feed for sheep over summer. In the early days of the Western Australian lupin industry, being able to use highly effective selective grass herbicides was an advantage, resulting in much fewer grass weeds in a crop following lupin than following wheat. Western Australia's worst agricultural weed, annual ryegrass (Lolium rigidum Gaud.), has since developed widespread resistance to many of these herbicides, and NLL's poorer competitiveness with ryegrass, compared to wheat, is a disadvantage to some farmers. Much of this also applies to other areas of southern Australia, where substantial areas of NLL are grown. However, NLL is not as prominent as in Western Australia, largely due to soil differences.

Outside Australia, the most commonly grown species is WL. The most sophisticated industry based on this species is in Europe, largely in France, where it is grown in rotation with cereals and oilseeds on neutral to mildly acid soils. WL is preferred to other lupin species because, if it is sown early enough in autumn, the vegetative crop can survive the winter in much of the UK, France, and even in parts of Denmark. NLL and YL do not tolerate freezing and in many areas of Europe can only be grown when planted in spring.

There has recently been renewed interest in lupin in Europe with the release of more agronomically suitable varieties and increased emphasis on protein crops in general. However, European farmers face much the same constraints as their Australian counterparts. In a survey of farmers' attitudes to grain legumes (of which lupin is one) in six European countries, rotation and soil improvement was given as the major reason for growing them, and poor profitability in relation to other crops, particularly cereals, as the main reason for not growing them.

The species *L. mutabilis* Sweet., known locally as "tarwi" or "chocho," has been grown and consumed by the inhabitants of the Andean regions of Peru and Ecuador since pre-Columbian times. Attempts to develop this species for commercial crop production outside the Andes have had limited success, and commercial lupin production in Chile is based on WL and NLL.

# **Growing a Lupin Crop**

#### Which Species?

The species generating the greatest income will generally be chosen. There is little evidence that rotational benefits differ between lupin species, so this will be determined by grain yield, grain price, and the cost of production.

Grain yield is a consequence of adaptation. John Gladstones identified NLL as having wider physical adaptation than other lupin species in Western Australia in the 1950s, and the considerable effort that has gone into its improvement has only reinforced its advantages over WL and YL. There are instances, though, where other lupin species might be grown in Australia. WL is more competitive with NLL on fertile loamy soils than on sandplain. YL tolerates soil aluminium (Al, the major constraint to plant growth on strongly acid soils), waterlogging, and the diseases brown leaf spot (caused by *Pleio*chaeta setosa [Kirchn.] Hughes) and Eradu patch (caused by an as yet unnamed species of *Rhizoctonia*) better than NLL or WL. In central and eastern Europe considerable effort has gone into improving YL which is adapted to the sandiest and most acid soils, so it has predominated until recently. WL tolerates soil freezing better than YL or NLL, so it can survive the northern European winter. This allows it to be sown in autumn, which results in higher yields than spring sowing. NLL has the advantage of greater anthracnose (Colletotrichum gloeosporiodes [Penz.] Penz. & Sacc.) tolerance.

Although WL is a minor component of traditional cuisine in some Mediterranean countries, and some components of lupin fiber and protein are being developed as additives for the food industry, lupin grain is mainly used to feed livestock, so the price it

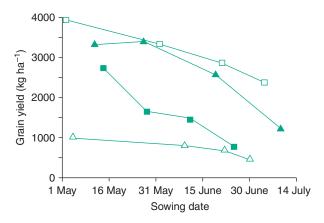
commands should reflect its composition. YL contains more protein than WL, which in turn contains more protein than NLL. YL also contains more S-amino acids, typically deficient in legume storage proteins, than WL or NLL. Consequently YL attracts a higher grain price than WL or NLL, but in Western Australia this is barely sufficient to induce farmers to grow it in preference to NLL, given the lower yield potential of current cultivars. WL has higher oil in its grain, and therefore has greater metabolizable energy than YL or NLL.

Lupin species, and cultivars within a species, may differ in their cost of production. Differences in disease tolerance may mean that one species may not need fungicides necessary for other species. Differences in aphid attractiveness are important in Western Australia: the only agronomically adapted YL cultivar is extremely sensitive to aphid feeding damage, making necessary at least one, if not two, more insecticide sprays than NLL would need.

#### **Sowing Time**

Sowing at the correct time is crucial to growing a successful crop, but there are different constraints on sowing time in different parts of the world. In the Mediterranean environments of southern Australia, matching the crop's life cycle to the rainfall pattern is the most important consideration. In early autumn, the soil is typically too dry to support plant growth until the season "breaks" with the onset of winter rains. A cool, wet winter follows; in spring, temperature rises rapidly and rain ceases. To maximize the time available for crop growth, lupin should be sown to germinate on the earliest winter rains. Delaying sowing will mean that the reproductive growth occurs under hotter, drier conditions. This reduces lateral branch production and hence pod numbers, curtails seed filling, and reduces yield. In much of Western Australia's agricultural areas, delaying sowing at the beginning of winter results in a linear yield decline, with a slope as high as  $40 \text{ kg ha}^{-1} \text{ day}^{-1}$  (Figure 1). The yield penalty for delayed sowing is usually, though not always, greater in high yield potential situations.

Early sowing has been a central dogma of lupin growing in Western Australia, and sowing the crop into dry soil, anticipating the break of season, is widely practiced. However, there are good reasons for not sowing too early. In eastern Australia, and the south of Western Australia, sowing before the end of April encourages excessive lateral branch growth at the expense of pod set, especially in older cultivars. In drier environments, the first winter rains are sometimes followed by a long period



**Figure 1** Grain yield response of NLL cultivar Belara to sowing date at four locations in Western Australia: closed squares – Esperance 1998, closed triangles Wongan Hills 1998; open squares – Mingenew 2000; open triangles – Merredin 2000.

without further rain, severely stressing the young crop. In extreme cases the crop may die, but more often the potential to form lateral branches is restricted and the crop is shortened, which makes complete yield recovery while harvesting difficult. The probability of this happening is low if there is already water in the soil from summer rain. An increasingly important reason for not sowing too early is the developing resistance of important weeds to selective herbicides. As the effectiveness of selective herbicides wanes, alternative methods of weed control must be sought. One is to wait after the season breaks until weeds germinate, and then kill them with a nonselective herbicide, or cultivation, before sowing the crop.

In northern Europe, sowing time is chosen so that the crop either survives, or avoids, the harsh winter. When WL plants are old enough, their roots are sufficiently lignified to survive soil temperatures as low as  $-3.5^{\circ}$ C, which would kill younger plants. As the plants age further, and stem elongation begins, the stems become frost sensitive and can be killed by aerial freezing. WL sown too early in autumn also tends to produce too many lateral branches at the expense of pod set, and to grow too tall so that the crop lodges. Physiological models of lupin development have been used to define sowing windows for different locations in the UK, where the sowing window extends from mid-August to early September in Scotland; and from early September to early October in south-west England.

Many of the YL and NLL cultivars grown in Europe have a vernalization requirement for flowering and must be sown early enough in spring to satisfy it. In Germany and Poland, they must be sown before mid-April to ensure that they flower at the correct time and are ready to harvest in August. If sown later, they will be taller and more prone to

lodging, have fewer pods and lower yields, and exhibit delayed ripening. This will interfere with sowing winter wheat, which usually follows lupin, in September.

#### **Optimum Plant Densities**

Plant density has a profound effect on the growth and structure of lupin crops. The same general principles apply to each lupin species and in each growing environment, but the target densities may be quite different.

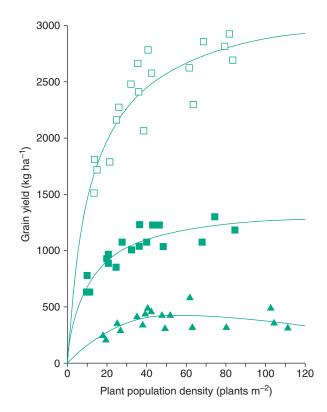
Dense lupin crops achieve canopy closure faster than thin ones, and therefore intercept more radiation earlier, grow faster, and compete with weeds better. Increasing plant density suppresses lateral branch growth and pod set on low-order branches so the number of pods per plant is reduced, but not usually enough to reduce grain yield per unit area. The relationship between grain yield and plant density is usually asymptotic, often well described by a hyperbola. Grain yield sometimes reaches a maximum and declines at higher densities, though.

In Western Australia, NLL grain yield will sometimes respond to increasing density above 80 plants m<sup>-2</sup>, and sometimes the response is saturated at less than 40 plants m<sup>-2</sup> (Figure 2). Generally, yield will respond to higher densities when yield potential is high, but there are exceptions.

Modern NLL cultivars are more responsive to plant density than older ones, and Western Australian farmers plant denser lupin crops now than in the past. A target of 50 plants  $\rm m^{-2}$  is common. In Germany, sowing 80-100 seeds  $\rm m^{-2}$  is recommended for YL and NLL, although restricted branching NLL should be sown at 120-140 seeds  $\rm m^{-2}$ . Spring sown WL should be sown at 60-70 seeds  $\rm m^{-2}$ . In the UK, 20 plants  $\rm m^{-2}$  is optimum for autumn-sown WL, but sowing 40 seeds  $\rm m^{-2}$  is recommended because  $\sim 50\%$  of plants are lost over the winter season.

# **Row Spacing and Seed Depth**

Lupin is generally sown in rows with the same machinery used for cereals and oilseeds. In Australia, these rows were traditionally 18 cm apart, but in the past decade many lupins (and cereals) have been sown in wider rows, most commonly 22–25 cm. This trend has been driven by changing herbicide practices in wheat cropping, but lupin can benefit from rows perhaps as far apart as 50 cm. Growing lupin in wide rows means more stubble from the previous crop can be retained, which reduces the spread of the *Pleiochaeta* disease organism. Lupin in wide rows also grows taller due to increased intrarow competition between individual plants, improving



**Figure 2** Grain yield response of NLL cultivar Danja to plant population density at three locations in Western Australia: open squares — Beverley 1987; closed triangles — Cadoux 1987; closed squares — Hyden 1988. (Reproduced with permission from French RJ, Smart WL, and McCarthy K (1994) Optimum plant population densities for lupin (*Lupinus angustifolius* L.) in the Western Australian wheat belt. *Australian Journal of Experimental Agriculture* 34: 491—497.)

harvestability. Some farmers are also beginning to experiment with spraying nonselective herbicide between wide rows to combat herbicide resistance problems. Crop plants are protected by hoods around the nozzles. Finally, some agronomists claim that lupin grown in wide rows exhibits a more efficient pattern of water use than when grown in narrow rows, but this has yet to be firmly established. On the other hand, growing lupin in wide rows could encourage more aphid landings, and hence virus spread, unless sufficient stubble is retained to cover bare ground between the rows, and weeds growing between the rows experience less competition from the crop in wide than in narrow rows. No interaction between response to plant density and row spacing has been observed in either NLL or YL in Western Australia.

Lupin cannot be sown as deep as many other largeseeded legumes. The ideal sowing depth for NLL and WL in Western Australia is 5 cm. They were once commonly sown 2–3 cm deep, but this is now discouraged as it encourages *Pleiochaeta* root rot. YL should be sown no deeper than 3–5 cm in Western

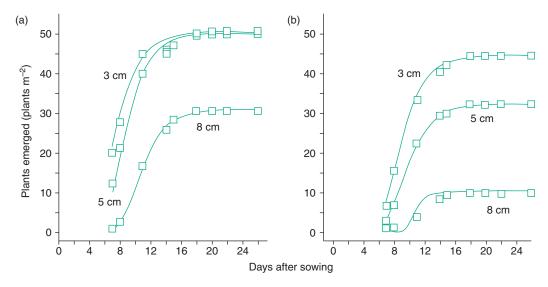


Figure 3 Effect of sowing at 3, 5, or 8 cm depth on emergence of lupin at Merredin, Western Australia in 1998: (a) NLL cultivar Merrit and (b) YL cultivar Wodjil.

Australia (Figure 3). In Europe, it is recommended that lupin is sown 2-5 cm deep.

#### **Crop Nutrition**

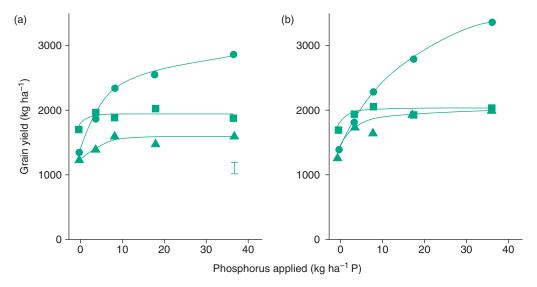
Lupin evolved on very infertile soils and is more efficient at acquiring nutrients than many crops. There is no need to use N fertilizer if the crop is well nodulated. Lupin forms root nodule symbioses with Bradyrhizobium species, which do not nodulate other temperate grain legumes. Inoculation of the seed with the appropriate bacterial culture is therefore necessary if lupin is being sown on land without a lupin history. It is rarely necessary to inoculate seed of subsequent lupin crops in Western Australia, even if the previous crop was 5 or more years ago, since lupin Bradyrhizobium is very robust on neutral to acid soils. In Germany, inoculation is only recommended for NLL if lupin has not been grown in the preceding 8 years. Seed should be inoculated within a few days of sowing, as the bacteria do not survive well on stored seed, and fungicide seed dressings are usually toxic to the inoculum. There is rarely any advantage in using starter doses of N.

**Phosphorus** (P) The main fertilizer requirement for lupin in Australia is phosphorus. NLL is more responsive to phosphorus than either YL or WL (**Figure 4**), which have evolved mechanisms that allow them to solubilize forms of soil phosphorus unavailable to most plants. In these species, phosphorus application is unnecessary unless soil phosphorus levels are very low. In NLL, yield responses have been observed to as much as 40 kg ha<sup>-1</sup> P on some soils, but farmers in

Western Australia typically apply 5-15 kg ha<sup>-1</sup> P. On the more fertile soils where WL is grown in Europe, it is often most profitable not to apply phosphorus to lupin, and replace the phosphorus it removes elsewhere in the rotation (see **Table 1** for the amount of plant nutrients removed in lupin grain).

The way phosphorus is applied can have a large impact on its effectiveness. Australian farmers have traditionally either broadcast phosphorus fertilizer on the soil surface, or drilled it at seed depth with their crops. Many soils quickly immobilize applied phosphorus into insoluble forms and little applied phosphorus reaches the subsoil. It is therefore unavailable to the crop late in the season when the soil surface dries out. This has little effect on cereals, which take up most of their phosphorus requirement early in the season, but NLL maintains a high requirement for phosphorus following flowering. It was discovered in the late 1980s that placing fertilizer up to 10 cm below the seed (called "banding") increased the effectiveness of the fertilizer on high-fixing soils (Figure 4). Banding fertilizer away from the seed is also important when planting lupin in wide rows to avoid toxic osmotic effects of the greater concentration of fertilizer in each drill run.

Potassium (K) and sulfur (S) Lupin grain contains considerable amounts of K (Table 1) but it is usually most profitable to apply it to more responsive crops in the rotation in both Europe and Australia. Increasing amounts of K fertilizer are being used in Australia on cereals and canola but in lupin there have been few economic K responses observed in trials, even where wheat responds strongly. Similarly, lupin appears



**Figure 4** Response of lupin to applied phosphorus on a high phosphorus fixing soil at Mt. Barker, Western Australia. Circles — NLL cultivar Merrit; squares — YL cultivar Teo; triangles — WL cultivar Kiev Mutant. In panel (a) phosphorus was drilled with the seed, in panel (b) it was drilled 8 cm below the seed. (Reproduced with permission from Bolland MDA Sweetingham MW, and Jarvis RJ (2000) Effect of applied phosphorus on the growth of *Lupinus luteus*, *L. angustifolius* and *L. albus* in acidic soils in the south-west of Western Australia. *Australian Journal of Experimental Agriculture* 40: 79—92).

**Table 1** Average amount of plant nutrients removed by 1 ton of lupin grain

Nutrient	Narrow-leafed lupin	White Iupin	Yellow Iupin	
Nitrogen (kg)	60	67	70	
Potassium (kg)	8.1	9.8	9.7	
Phosphorus (kg)	3	3.6	5.1	
Sulfur (kg)	2.3	2.4	4.6	
Calcium (kg)	2.2	2	1.5	
Magnesium (kg)	1.6	1.4	2.1	
Zinc (g)	35	30	56	
Manganese (g)	17	835	17	
Copper (g)	5	5	9	

Data from Petterson DS (1998) Composition and food uses. In: Gladstones JS, Atkins CA, Hamblin J (eds.) *Lupins as Crop Plants. Biology, Production and Utilization*. Wallingford, UK: CAB International; and unpublished data of Sipsas S, Department of Agriculture, Western Australia.

unresponsive to applied S on many soils where wheat is responsive.

Trace elements Manganese (Mn) is the trace element most commonly deficient in lupin. This is principally a problem of NLL, causing "split seed," in which the crop can remain green at maturity and the seed does not develop properly. Both WL and YL are more efficient at acquiring Mn than NLL. In fact, WL grain can have high enough Mn levels to cause nutritional problems when fed to animals. In NLL, Mn deficiency can be treated by applying a foliar application of 4 kg ha<sup>-1</sup> MnSO<sub>4</sub> following flowering. In parts of Western Australia where the

disorder occurs frequently, this is routine. Lupin growing on alkaline soils can experience Fe deficiency, but it is rarely grown on such soils.

#### **Weed Management**

Lupin is not as competitive against weeds as cereals, and the availability of effective herbicides played a crucial role in establishing the lupin—wheat rotation in Western Australia. Without herbicides early sowing would be impossible since crop establishment would have to wait until germinated weeds could be killed by cultivation.

Any weeds growing prior to sowing the crop are controlled with a nonselective herbicide or by cultivation. Most lupin crops in Australia have simazine, or a combination of simazine and atrazine, applied prior to or immediately after sowing. The safe rate depends on soil type and weather conditions. NLL is more tolerant than YL or WL, and modern cultivars are more tolerant than old ones, having been selected against a background of herbicide use. There are also a number of herbicides that can be applied postemergent to lupin crops. Table 2 gives a list of herbicides used for weed control in lupins in Australia and Europe.

The heavy reliance on herbicides in the Australian lupin-based farming system has encouraged the development of increasingly severe herbicide resistance in some of its most troublesome weeds, most notably annual ryegrass and wild radish (*Raphanus raphanistrum* L.). Considerable effort is being devoted to developing new weed management strategies to

Table 2 Herbicides used for weed control in lupin

Herbicide	Places of use
Preemergent herbicides	
Atrazine	Australia
Glyphosate	Australia, Europe
Paraquat/diquat	Australia
Pendimethalin	Australia, Europe
Simazine	Australia, Europe
Terbuthylazine/terbutryn	Europe
Tri-allate	Australia
Trifluralin	Australia, Europe
Postemergent herbicides	
Diflufenican	Australia
Metosulam	Australia
Picolinafen	Australia
Pyridate	Europe
Simazine	Australia
Various aryloxyphenoxypropionates	Australia, Europe
Various cyclohexanediones	Australia, Europe

Registration details should be checked with the relevant pesticide registration authority. Information on weeds controlled, safe rates and application methods is found on herbicide labels.

complement the existing herbicides, and these should become more prominent over the next decade. Examples include towing a chaff cart behind the harvester to collect weed seed rather than returning it to the ground, "crop topping" where a low rate of nonselective herbicide (usually paraquat) is sprayed onto the maturing crop to disrupt weed seed development, and spraying nonselective herbicide between crop rows using specially shielded nozzles to prevent the herbicide contacting the crop.

#### **Diseases**

Brown spot Brown spot, caused by Pleiochaeta setosa, was until recently the most damaging lupin disease in Australia, a status it now shares with anthracnose (see below). It is also significant in Europe and other parts of the world. It commonly causes leaf lesions that lead to defoliation and, if severe enough, crop death. The organism can also cause a root rot. The disease can be seed borne, but the main source of infection is spores in the soil remaining from previous infected crops. Seed treatment with dicarboximide fungicides (iprodione or procymidone) provides good protection for up to 6 weeks after sowing, and retention of stubble from a preceding cereal crop prevents spores on the soil surface from being splashed onto lower leaves by rain drops, which is the main way infection spreads. Spore populations in the soil decline over time if lupins are not grown, so the recent trend in Western Australia for longer periods between lupin crops on the same land has contributed to fewer problems with brown spot.

The root rot mode of *Pleiochaeta* is managed by sowing deep enough so that developing roots do not come into contact with the concentrated band of spores at the soil surface (hypocotyl tissue is not affected), and by not mixing spores through the top 5 cm of the soil by cultivation. YL is more tolerant of *Pleiochaeta* than NLL or WL.

Leaf rust Leaf rust (*Uromyces lupinicolus* Bub.) does not affect lupin in Australia, but is currently the most common lupin disease in the UK, and occurs elsewhere in Europe, North Africa, and South America. It usually occurs on plants approaching maturity, but is most damaging if infection begins before flowering. A preflowering application of tebuconazole or cyproconazole is recommended in the UK to protect WL against early infection, and a further application may be necessary at the end of flowering. NLL, WL, and YL are all susceptible.

Anthracnose Anthracnose, caused by *Colletotrichum gloeosporiodes*, is the world's most important lupin disease. It is serious in Europe, South America, and, since 1996, Western Australia. It has yet to spread in eastern Australia. Infection is primarily seed borne, but infected plants rapidly produce secondary inoculum, which can be spread through a crop by wind and rain splash.

Its effects are minimized by using disease-free seed but, since in the right conditions damaging outbreaks can arise from less than 1 plant in 10 000, seed should also be treated with thiram or carbendazim. Various foliar fungicides can also be effective against anthracnose, but the economics of these are dubious. In Western Australia, control of naturalized populations of *Lupinus cosentinii*, which can act as another source of infection, is an important component of anthracnose management.

Lupin species differ considerably in their susceptibility to anthracnose. NLL has much greater tolerance than either YL or WL, and this explains why the area of NLL has recently grown at the expense of YL in central and eastern Europe. Within NLL, there is considerable variation in tolerance between cultivars, and in Western Australia this is an important consideration in choosing a cultivar in areas likely to experience an anthracnose outbreak.

Virus diseases The two most important virus diseases of lupin are bean yellow mosaic virus (BYMV) and cucumber mosaic virus (CMV). Within a crop, these viruses are spread from plant to plant by aphids, but the initial source of infection can vary. In YL and WL, BYMV is seed borne, as is CMV in NLL and YL. Plants arising from infected seeds are the

Table 3 Invertebrate pests of lupin

Common name	Scientific name	Type of damage	Places of occurrence
Seedling pests			
Cutworm	Agrotis spp.		Australia
Brown pasture looper	Ciampa aritaria		Australia
Wireworm	Agriotes spp.		Europe
Red-legged earthmite	Halotydeus destructor	Feeding on emerged seedlings	Australia
Lucerne flea	Sminthurus viridus		Australia
Slugs	Deroceras reticulatum		Europe
	Arion hortensis		Europe
Bean seedling maggot or bean root maggot	Delia platura	Feeding on root and hypocotyl of emerging seedlings	Australia and Europe
Vegetative and reproductive	pests		
Green peach aphid Blue green aphid Cowpea aphid Lupin aphid	Myzus persicae Acyrthosiphon kondoi Aphis craccivora Macrosiphum albifrons	Feeding on growing point and flowers	Australia Australia Australia Europe
Thrips	Frankliniella occidentalis Thrips angusticeps	Feeding on flower buds and leaves	Australia Europe
Native budworm	Helicoverpa punctigera syn. Heliothis punctigera	Feeding on pods and seeds	Australia
Mirid bugs	Lygus spp.	Feeding on young pods	Europe

initial source of infection: aphids pick up the virus when feeding on them, and carry it to healthy plants. BYMV is not seed borne in NLL, but infected pasture legumes such as red clover and subterranean clover act as sources of the virus, again spread by aphids. CMV does not affect WL.

Management of these diseases involves the reduction of virus source and its spread. Where the virus is seed borne, virus-free seed should be used. Management that encourages early canopy closure, such as early sowing, high sowing rates, and narrow row spacing, is beneficial because they shade out weak virus-infected plants and discourage aphid landing. This reduces the chance of the infection spreading. Cultivar choice is also important since there is variation in both YL and NLL in how readily CMV is transmitted to the seed in infected plants. Spraying aphids to prevent virus spread is rarely economical.

#### **Invertebrate Pests**

Lupin crops are subject to damage from a number of invertebrate pests, the most important of which are listed in Table 3. They can all be controlled by chemical pesticides, although this is not always worthwhile economically. There are some differences in susceptibility between species: YL is more susceptible to redlegged earthmite than NLL, which is more susceptible than WL; and YL and WL are both more susceptible to native budworm than NLL is. There are also differences in susceptibility to some pests between cultivars within a species. Some Australian NLL cultivars are more susceptible to aphid damage than

others, and the YL cultivar Wodjil is also extremely sensitive to aphids. Correct agronomy can help reduce damage from some pests: planting lupin after pasture increases the chance of damage from red-legged earthmite and lucerne flea, which multiply on pasture plants. Deep-sowing predisposes lupin to damage from bean root maggot, and taking measures to ensure rapid canopy closure reduces aphid damage.

#### Harvesting

Lupin is harvested with the same machinery (combine harvesters) as cereals. In Australia, this is usually done when the seed is at 12% moisture or drier, but in Europe crop maturity can be slow and sometimes lupin is harvested with moisture as high as 30%. Obviously this grain needs drying before storage. Lupin is sometimes desiccated with diquat in Europe to hasten maturity, but care must be taken that seed growth is sufficiently complete, or the grain may begin to rot in the pod. Desiccation with glyphosate has led to loss of seed viability in Australia.

Harvesting losses are usually greater in lupin than in cereals. Lupin pods are very brittle when dry, and pod-bearing lateral branches must often be cut a long way below the pods. The shaking induced by the cutter bar is an important source of pod loss. Such losses are smaller in restricted-branching cultivars, because they have shorter branches, and can also be minimized by fitting harvesters with double-density knife guards. Another source of loss is the large bulk of awkwardly shaped material that will overflow from the front if not taken into the harvester quickly enough. A number

Lupin grain is fragile compared to cereals, and must be treated gently, especially if it is to be used for seed. The drum or rotor speed should be as low as possible and the concave closed only enough to ensure pods are threshed. Harvesting in the early morning or at night reduces the amount of seed damage. It also reduces shattering losses as pods and stems are tougher in the more humid atmosphere. If it is to be kept for seed, grain should be handled as little as possible after harvest, to prevent further mechanical damage. In Australia, though, any green material from wild radish should be removed promptly. Green pods of this weed release toxic isothiocyanates that can dramatically reduce germination percentage.

See also: Lupin: Breeding. Pulses, Overview.

# **Further Reading**

Articles on lupin agronomy are frequently published in Australian Journal of Agricultural Research and Australian Journal of Experimental Agriculture.

Much information is also contained in the proceedings of the International Lupin Conferences, held biennially, and published by the International Lupin Association. Recent conferences have been held in Iceland (2002), Germany (1999), and California (1996).

- Bolland MDA, Sweetingham MW, and Jarvis RJ (2000) Effect of applied phosphorus on the growth of *Lupinus luteus*, *L. angustifolius* and *L. albus* in acidic soils in the south-west of Western Australia. *Australian Journal of Experimental Agriculture* 40: 79–92.
- Dracup MN and Kirby EJM (1996) *Lupin Development Guide*. Perth, Western Australia: University of Western Australia Press.
- French RJ, Smart WL, and McCarthy K (1994) Optimum plant population densities for lupin (*Lupinus angustifolius* L.) in the Western Australian wheat belt. *Australian Journal of Experimental Agriculture* 34: 491–497.
- Howieson JG, Fillery IRP, Legocki AB, et al. (1998)
  Nodulation, nitrogen fixation and nitrogen balance.
  In: Gladstones JS, Atkins CA, and Hamblin J (eds.)
  Lupins as Crop Plants: Biology, Production and
  Utilization, pp. 149–180. Wallingford: CAB
  International.

- Nelson P and Delane RJ (1991) Producing Lupins in Western Australia. Bulletin 4179. Perth, Western Australia: Department of Agriculture.
- Perry MW, Dracup M, Nelson P, Jarvis R, Rowland I, and French RJ (1998) Agronomy and farming systems. In: Gladstones JS, Atkins CA, and Hamblin J (eds.) *Lupins as Crop Plants: Biology, Production and Utilization*, pp. 291–338. Wallingford: CAB International.
- Petterson DS (1998) Composition and Food Uses. In: Gladstones JS, Atkins CA, and Hamblin J (eds.) *Lupins as Crop Plants. Biology, Production and Utilization*, pp. 353–384. Wallingford: CAB International.
- Riethmuller G and Blanchard E (1995) Lupin harvesting modifications *Farmnote* 19/95. Perth, Western Australia: Department of Agriculture (available at http://www.agric.wa.gov.au).
- Siddons PA, Jones RJA, Hollis JM, et al. (1994) The use of a land suitability model to predict where autumn-sown, determinate genotypes of the white lupin (*Lupinus albus*) might be grown in England and Wales. *Journal of Agricultural Science*, Cambridge 123: 199–205.
- Snowball K and Robson AD (1986) Symptoms of nutrient deficiencies: lupins. Soil Science and Plant Nutrition. Perth, Western Australia: University of Western Australia.
- Sweetingham MW, Jones RAC, and Brown AGP (1998)
  Diseases and pests. In: Gladstones JS, Atkins CA, and Hamblin J (eds.) *Lupins as Crop Plants: Biology, Production and Utilization*, pp. 263–289. Wallingford: CAB International.

#### **Relevant Websites**

http://www.general.uwa.edu.au — The Centre for Legumes in Mediterranean Agriculture (CLIMA, University of Western Australia) is a collaborative center bringing together expertise from the WA Department of Agriculture, CSIRO, the University of Western Australia, and Murdoch University. It describes lupin research of a more strategic nature than that on the Department of Agriculture website, as well as research on a broad range of crop and pasture legumes.

http://www.agric.wa.gov.au — WA Department of Agriculture, this website contains much practical information on growing lupin (mainly NLL) as well as summaries of a great deal of applied agronomic research.

http://www.grainlegumes.com — European Association for Grain Legume Research, this site gives general background information on the production and utilization of grain legumes, including lupin, and an archive to Grain Legumes, the Association magazine.

# M

# **MAIZE**

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# **Genetics**

**E A Lee**, University of Guelph, Guelph, ON, Canada © 2004, Elsevier Ltd. All Rights Reserved.

Maize (Zea mays L. ssp. mays), in addition to being an economically important crop plant, is a model plant for studying genetics. The intent of this brief article is to acquaint the reader with some of the unique contributions that maize has made to the science of genetics. Information regarding mode of propagation, controlled pollinations, heterosis, quantitative genetics, and breeding are covered in Maize: Breeding. Several excellent comprehensive reviews of the general subject are available. A recent book - "Mutants of Maize" – gives an extensive pictorial overview of numerous maize mutants, while another - "Maize Genetics and Breeding in the 20th Century" – gives an overview of the more prominent maize geneticists of the twentieth century and their contributions to maize genetics.

#### **Origin of Maize**

Maize, a diploid plant with 10 chromosomes (x = 10, 2n = 20), is a member of the grass (Poaceae) family, which includes crops such as rice (*Orzya sativa*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), sugar cane (*Saccharum* ssp.), pearl millet

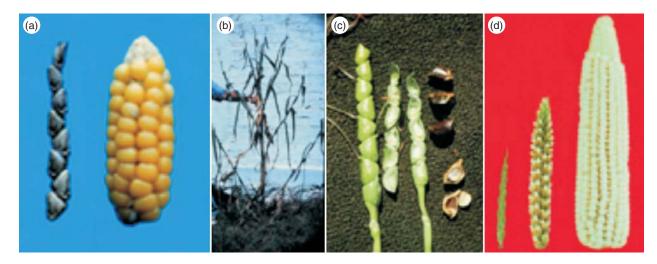
(Pennisetum glaucum), and sorghum (Sorghum bicolor). The maize genome appears to be an ancient segmental allotetraploid (i.e., hybridization of two species whose genomes are partially alike). Many genes in maize exist as two unlinked copies (i.e., homologs), each of which occur on duplicated chromosomal segments (i.e., linkage blocks). Over time some of these genes have developed slightly different expression patterns (i.e., paralogs), such as the anthocyanin genes - colored 1 (r1) and colored plant 1 (b1), or colored aleurone1 (c1) and purple plant1 (pl1). For some of these genes, one copy (i.e., pseudogene) may have become nonfunctional due to mutations. While other homologs have not diverged from one another leading to redundancy, such as the orange pericarp1 (orp1) and orange pericarp2 (orp2) genes. Comparing divergence times for 14 pairs of homologs enabled the maize genome to be subdivided into two subgenomes. Molecular marker studies of the two apparent subgenomes of maize suggest that one of the subgenomes is more closely related to sorghum than it is to the other maize subgenome.

Two contrasting theories regarding the origin of maize existed for many years, the "teosinte hypothesis" put forth by George Beadle and the "tripartite hypothesis" championed by Paul Mangelsdorf. Today, the most widely accepted theory is that maize originated from an annual teosinte (Z. mays ssp. parviglumis),  $\sim 7000-12\,000$  years ago (teosinte hypothesis). Teosinte parviglumis (x=10, 2n=20) is a wild grass native to Mexico and Guatemala. Maize  $\times$  T. parviglumis hybrids are completely

fertile, vigorous plants. At the chromosome, gene structure, and nucleotide sequence level, maize and T. parviglumis exhibit as many differences as would be observed between any two maize varieties. While 7000-12 000 years is considered an extremely short period of time in terms of evolution, dramatic morphologically changes in plant architecture and kernel structure occurred between maize and teosinte in that time (Figure 1). Genetic evidence suggests, however, that as few as five mutations may be responsible for these dramatic morphologically differences. Two genes have been identified that change maize morphology to teosinte morphology. The teosinte glume architecture1 (tga1) locus controls differences associated with kernel structure and teosinte branched1 (tb1) controls differences in plant

architecture (Figure 2a). Both *tga1* and *tb1* are believed to be genes that were important in the domestication of maize from teosinte.

Molecular mapping of the various grass genomes has led to two interesting observations. Gene content of the various grass family members does not vary greatly, even though variation in DNA content is considerable. Most of the differences in genome size are due to variation in the amount of noncoding DNA (i.e., DNA other than genes). Noncoding DNA consists of highly repetitive DNA sequences, made up of fragments retrotransposon sequences. The other observation is that gene order is semiconserved across the grass species. This phenomenon is generally referred to as collinearity or synteny. Chromosomal rearrangements have occurred that break up the



**Figure 1** (a) Teosinte ear on the left and "reconstructed" primitive maize ear on the right. George Beadle created the latter by crossing teosinte with Argentine popcorn and then selecting the smallest segregants. This maize ear resembles the earliest archaeological corn recovered from the Tehuacán valley in Mexico (photo by John Doebley). (b) Mature teosinte plant showing lateral side branches (photo by Hugh Iltis). (c) Immature ears of *Zea diploperennis* whole and sectioned with a few mature fruitcases one of which is cracked open to expose the grain (photo by Hugh Iltis). (d) Teosinte ear (*Zea mays* ssp. *mexicana*) on the left, maize ear on the right, and ear of their F<sub>1</sub> hybrid in the center (photo by John Doebley).

Figure 2 Examples of the range of mutations that have been identified and studied in maize. (a) teosinte branched1 (tb1) mutation indicated by the arrow adjacent to normal maize plants. (b) An ear segregating for anthocyanless 1 (a1) and shrunken 2 (sh2). a1 and sh2 are tightly linked (0.2 cM) genes, with a1 affecting aleurone color and sh2 affecting starch accumulation in the endosperm. (c) brown mid-rib3 (bm3) mutant leaf on the left and a normal leaf on the right. (d) lethal leaf spot1 (lls1) mutant leaf on the left and a normal leaf on the right. (e) An ear segregating for opaque2 (o2) and normal kernels. o2 kernels indicated by the arrow are lighter colored with a chalky endosperm. (f) dwarf1 (d1) plant on the leaf and a normal maize plant on the right. (g) Example of a feminized tassel with silk tissues (stigmatic tissues) developing from the male flower. This is due to a mutation in the tassel seed1 (ts1) gene. (h) Culm of a plant with functional purple plant1 (pl1) and booster1 (b1) alleles permitting anthocyanin accumulation in the sheath and leaf tissues. (i) salmon1 (sm1) silks. Nonmutant silks are green. (j) Leaves of a knotted1 (Kn1) plant showing extra cell divisions at the vascular bundles. (k) Ears representing four different p1 alleles, from left to right p1-rr(functional in both pericarp and cob tissues), p1-wr(functional only in cob tissues, non-functional in pericarps), p1-rw (functional only in pericarps, non-functional in cob tissues) and p1-ww(nonfunctional in both cob and pericarp tissues). The first letter in the allele designation refers to functionality in pericarps and the second letter refers to functionality in cob tissues. (I) An ear segregating for the red aleurone 1 (pr1) gene. pr1 kernels are red in appearance while nonmutant kernels are bluish-black. (m) An ear segregating for collapsed pericarp2 (cp2) and nonmutant kernels. cp2 kernels, indicated by an arrow, are extremely thin poorly developed kernels. (n) collapsed pericarp2 (cp2) seedling on the left and normal seedling on the right. (o) yellow endosperm1 (y1) mutant ear in the lower left corner and a functional Y1 ear in the upper right corner. (All photos are courtesy of Maize DB.)

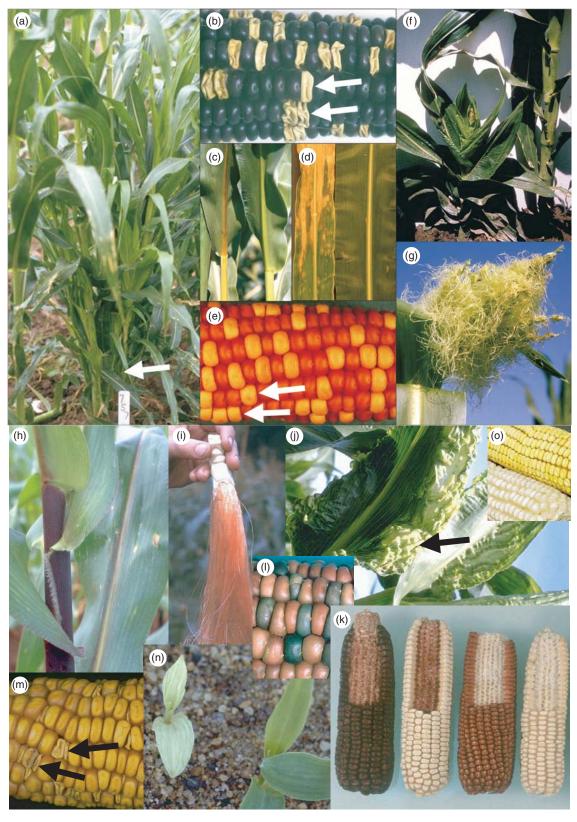
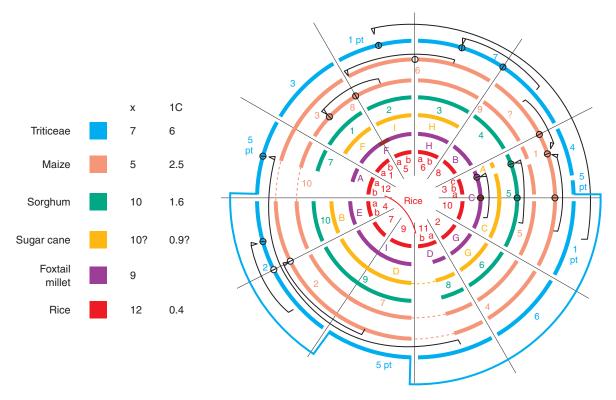


Figure 2

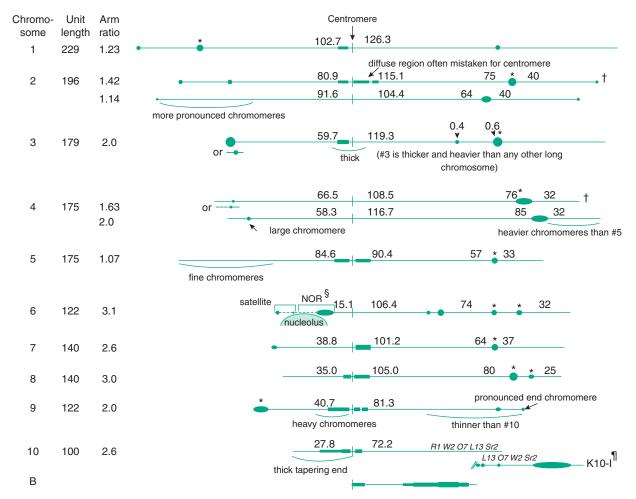


**Figure 3** Alignment of the genomes of six major grass crop species with 19 rice linkage segments, whose order reflects the circularized ancestral grass genome. The thin dashed lines correspond to the duplicated segments. Inversions of sets of sequences within a linkage segment (such as the inversion of segments 3a and 3b in maize chromosome 5) are not shown. Linkage segments forming parts (pt) of Triticeae chromosome 5 are shown as a series of segments connected by colored lines. The alignment is based on the genetic map of the D genome of wheat. The red line indicates the duplicated segments shown as blocks 11b and 12b. Chromosomes formed by the insertion of one segment into another are shown by black lines with arrows indicating the direction and point of insertion. The points of chromosome breakage involved with insertion events are indicated by black bisected circles. The "haploid" chromosome number of each species is shown in the column marked "x." The haploid DNA content of each species, shown in the column of 1C values, is per  $10^9$  bases. (Reproduced with permission from Moore G, Devos KM, Wang Z, and Gale MD (1995) Grasses, line up and form a circle. *Current Biology* 5: 737–739, 169–174. © Elsevier.)

collinearity between grass species. These rearrangements tend to involve translocations, inversions, duplications, or deletions. The syntenic relationships between the maize, rice, and wheat genomes are represented in Figure 3. For example, chromosome 9 of rice contains genes that are contained on chromosomes 7 and 2 of maize, those genes are also found on chromosome 5 of wheat (Figure 3). Generally, collinearity between the grass genomes exists on a macroscale. When collinearity is examined on a microscale, there can be quite a few exceptions to the expected gene order. Synteny is a powerful tool that permits maize geneticists to utilize genetic resources from other grass species. For example, the genome of rice has been recently sequenced. Because of the syntenic relationship that exists between the maize and rice genomes, maize geneticists can potentially identify the rice ortholog for any maize gene and then use the rice ortholog's DNA sequence to isolate the maize gene.

## **Cytogenetics**

Historically, one of the major contributions of maize genetics was to the field of cytogenetics. Maize cytogeneticists were the first to demonstrate that chromosomes are individually recognizable by their lengths, arm ratios, and other physical features, physical exchange of chromosome segments accompanies genetic recombination, and that telomeres are necessary for maintaining fidelity during chromosome duplication and division. Barbara McClintock was the first geneticist to characterize the ten maize chromosomes. Working with mitotic chromosomes, she used initially overall chromosome length and the position of the centromeres relative to each chromosome arm (i.e., arm ratio) to describe each chromosome. Information regarding the presence of chromosome "knobs" (i.e., dark staining heterochromatic regions), nucleolus organizer region (NOR), and chromomeres were later added to McClintock's original description of the chromosomes (Figure 4). The



<sup>\*</sup> Knobs found in more than 50% of races

**Figure 4** Cytological map of the ten maize chromosomes drawn to scale with major distinguishable features such as arm ratios, chromosome knobs, NOR, and chromomeres. (Reproduced with permission from Neuffer MG, Coe EH, and Wessler SR (1997) *Mutants of Maize*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.)

maize chromosomes are numbered in order of size, chromosome 1 is the longest and chromosome 10 is the shortest. The NOR region is located at the end of the short arm of chromosome 6.

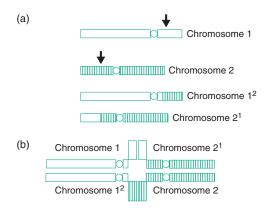
There are two types of reciprocal translocations that have been developed in maize that have proven to be extremely useful, A—A translocations and B—A translocations. These translocations enabled geneticists to assign molecular marker based linkage groups to chromosomes and orient them, as well as assigning recessive and dominant mutations to chromosome arms. Reciprocal translocations involve exchange of chromosome segments between nonhomologous chromosomes. To create reciprocal translocations requires (1) chromosome breaks to occur in two different chromosomes, followed by (2) rejoining of

the fragments to produce two novel chromosomes (Figure 5). The first reciprocal translocations were spontaneous, however most of the reciprocal translocations in maize were generated using ionizing radiation. A-A translocations involve interchanges between the 10 maize chromosomes (i.e., A chromosomes). B-A translocations involve interchanges between one of the 10 maize chromosomes and a supernumerary chromosome called the B chromosome. The B chromosome is relatively small ( $\sim$ 60% the size of chromosome 10) and is comprised almost entirely of heterochromatic DNA. There are several interesting features that are unique to the maize B chromosome. It divides normally during cell division (mitosis) and during the development of the megaspore (female gamete). However, its behavior during

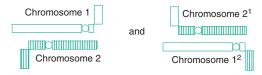
More common form of chromosomes 2 and 4

<sup>§</sup> Secondary constriction + NOR heterochromatin

<sup>¶</sup> Only in certain stocks (i.e., "abnormal 10"); inverted gene order is shown for the region that is in common with normal Smallest dots represent prominent chromomeres



#### Products of alternate disjunction



#### Products of adjacent disjunction

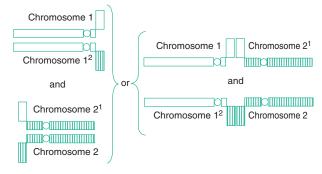


Figure 5 Reciprocal A-A translocations are reciprocal exchanges between two nonhomologous chromosomes (1 and 2). (a) Chromosome breaks occur in the two chromosomes (denoted by the arrows) followed by rejoining of the two fragments to generate two novel chromosomes (12 and 21). Chromosome 12 contains the intact long arm of chromosome 1, along with the centromere of chromosome 1 and a portion of the short arm of chromosome 1, to which is attached the chromosome fragment of chromosome 2. Likewise, chromosome 2<sup>1</sup> contains the intact long arm of chromosome 2, along with the centromere of chromosome 2 and a portion of the short arm of chromosome 2, to which is attached the chromosome fragment of chromosome 1. (b) In meiosis reciprocal A-A translocation heterozygotes (i.e., one copy each of chromosomes 1, 2, 1<sup>2</sup>, and 2<sup>1</sup>) pair in a Chi arrangement. Only when alternate disjunction occurs are the resulting gametes viable. Adjacent disjunction results in chromosome duplications and deficiencies.

development of the microspore (male gamete) and subsequent fertilization are what distinguish the maize B chromosome from other B chromosomes. (1) Nonconcordance of the two sperm nuclei arising from the second mitotic-like division of microsporogenesis occurs. This phenomenon, also called nondisjunction, is the result of one of the sperm nuclei receiving two copies of the B chromosome while the other sperm nuclei is lacking the B chromosome.

(2) The megaspore is preferentially fertilized by the sperm nuclei containing the B chromosomes. These two phenomenon result in an accumulation of B chromosomes in each generation. It is a unique, yet unknown, feature of the B chromosome's centromere that is responsible for nondisjunction and it is this feature that makes the B–A translocations particularly useful for mapping recessive mutations (Figures 6 and 7).

# Transposable Elements

Probably the largest contribution that maize has made to the field of genetics was the discovery and genetic characterization of "jumping genes," for which Barbara McClintock was awarded the Nobel Prize in Medicine in 1983. Transposable elements (TEs) (i.e., "jumping genes") are pieces of DNA that can move around the genome. Transposable elements are the single largest component of most eukaryotic genomes, accounting for 50-80% of the DNA sequence in some grass family genomes. However, only a small fraction represent sequences that are still active. The TE that McClintock identified and studied was a site that was vulnerable to chromosome breakage, hence she named it Dissociation (Ds). Ds, however, could break chromosomes and transpose only when a second factor, called Activator (Ac), was present. Ac/Ds represent one TE family in maize, Ac is the autonomous element (i.e., capable of moving by itself) and Ds is the nonautonomous element (i.e., not capable of moving by itself). Part of Ac's DNA sequence contains a gene that encodes an enzyme required for transposition, transposase. The DNA sequence of Ds elements is identical to Ac, except that the transposase gene is partially or completely deleted (Figure 8).

Transposable elements display some Mendelian properties. They are associated with chromosomes and they can be transmitted from parent to offspring. Phenotypically, their only effect is through their affect on the expression of other genes. Many TEs preferentially insert into genes when they move. The insertion of a TE generally disrupts the gene's normal pattern of expression, for example, altering tissue specificity or knocking out expression altogether. Transposable elements also display some very "non-Mendelian" characteristics. Their genome position is not constant, rather they are capable of moving (i.e., transposing) around the genome. They cause the appearance of variable expression of genes when they "hop-out" of a gene and restore functionality during development. This is normally observed as sectors of expression in cell lineages, either streaks or spots are the common types of sectors observed. The larger the

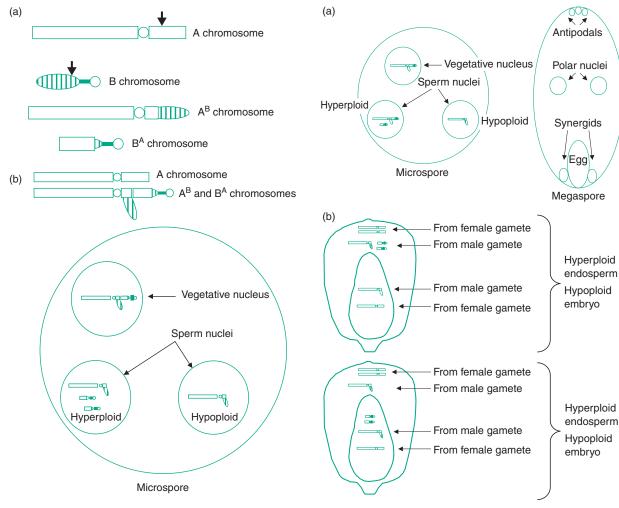


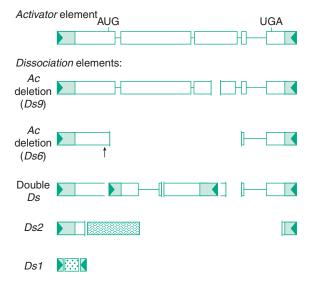
Figure 6 B-A translocations involve interchanges between one of the ten maize chromosomes (A chromosomes) and the supernumerary chromosome called the B chromosome. (a) Chromosome breaks occur in the two chromosomes (denoted by the arrows) followed by rejoining of the two fragments to generate two novel chromosomes (AB and BA). Chromosome AB contains the intact long arm, the centromere, and a portion of the short arm of the A chromosome, to which is attached the chromosome fragment of the B chromosome. Likewise, chromosome BA contains the centromere of the B chromosome and a portion of the arm of the B chromosome, to which is attached the fragment of the A chromosome. (b) Chromosome pairing in a B-A translocation heterozygote and the consequences of non-disjunction during the second mitotic like division of microsporogenesis. During microsporogenesis, 50% of the viable pollen grains (i.e., microspores) contain the non-translocation A chromosome, but the other 50% of the microspores non-disjunction occurs during the second mitotic like division. After meiosis the microspore undergoes two mitotic like divisions. The first division gives rise to the vegetative and generative nuclei. The vegetative nucleus governs pollen tube development, but the generative nucleus undergoes a second division to give rise to the two sperm nuclei. It is during this second division that the B chromosome centromeres fail to disjoin following duplication. This results in one sperm nucleus lacking the portion of the A chromosome translocated to the B centromere (i.e., BA), a condition referred to as hypoploidy, while the second sperm nucleus has two copies of BA chromosome, a condition referred to as hyperploidy.

**Figure 7** Fertilization of a normal megasporocytes with a microsporocyte containing a B—A translocation. (a) One of the sperm nuclei fuses with the egg to form the embryo (2n), while the other sperm nuclei fuse with the two polar nuclei to give rise to the endosperm (3n). (b) If the hypoploid sperm nucleus fuses with the egg, any recessive mutations in the female that are present in that A chromosome segment translocated to the B centromere will be visible. If the recessive mutation affects kernels characteristics such as aleurone color or starch formation, then those phenotypes will be visible when the hypoploid sperm nuclei fuse with the two polar nuclei to for the endosperm.

sector, the earlier in development (i.e., establishment of the cell lineage) the TE excised from the gene and restored function (Figure 9).

#### **Mutants of Maize**

One of the features that made maize an attractive plant with which to study genetics was the wealth of mutations that visibly alter some aspect of the plant and the ease with which controlled crosses between two genotypes can be made (*see* Maize: Breeding). There have been four types of mutants in maize that have been



**Figure 8** *Ac/Ds* family of transposable elements. *Ac* elements are 4565 bp in length, consisting of 10 bp terminal inverted repeats (TIRs) at the ends (green arrowheads), exons (green boxes), introns (connecting lines), nontranscribed regions (green boxes), and one open reading frame (AUG start codon through UGA stop codon) that encodes the transposase. The *Ds* elements are structurally diverse and consist of internal deletions of the *Ac* element. *Ds1* and *Ds2* also contain unique DNA sequences (wavy and dotted boxes). (Reproduced from Neuffer MG, Coe EH, and Wessler SR (1997) *Mutants of Maize*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.)

extensively studied: those that affect kernel properties, those that affect pigmentation, those that affect plant form, and those that affect fertilization. Several of these mutants have become economically important, such as the genes used in "sweet corn."

#### **Mutants Affecting Kernel Properties**

There are two groups of mutants that affect the kernel, mutations affecting carbohydrate, oil, or protein composition and content (Table 1) and mutations that affect kernel development (Table 2). While studying kernel mutations, it is important to remember that the kernel is actually a mixture of maternal tissues (e.g., pericarp) and zygotic tissues (e.g., embryo, aleurone, and endosperm), and that the embryo is diploid (2n) while the aleurone and endosperm are triploid (3n). The triploid nature of the aleurone and endosperm result from two identical maternal gametes (i.e., polar nuclei, the result of megasporogenesis) fusing with one paternal gamete (i.e., sperm nuclei, the result of microsporogenesis) (Figure 7).

The typical maize kernel is composed of 70-75% starch, 8-10% protein, 4-5% oil, 1-3% sugar, and 1-4% ash. Most of the starch is associated with the endosperm (<90%), while the embryo contains high levels of protein ( $\sim$ 26%), oil ( $\sim$ 83%), sugar ( $\sim$ 70%),

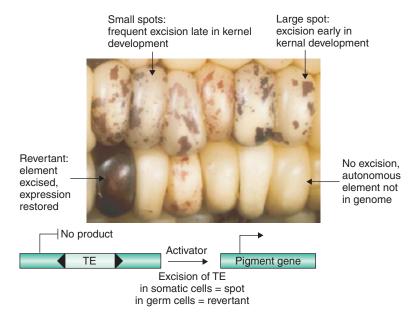


Figure 9 Phenotypes of kernels from a genotype with an *Ac* element in the genome and a *Ds* element inserted in an anthocyanin gene. The kernels represent several different times of *Ds* element excision during development, from extremely early events resulting in completely colored kernels, to mid-development resulting in large colored spots, to late in development resulting in small colored spots. Completely colored kernels are termed revertants, meaning that the TE excised from the germline. Completely colorless kernels indicate that the autonomous element, *Ac*, is not present in the germline, but the *Ds* element is still interrupting the anthocyanin gene in the germline. Spotted or sectored kernels represent somatic excision events and indicate that the *Ac* element is still present in the germline and that the *Ds* element is still interrupting the anthocyanin gene in the germline. (Reproduced with permission from Freschotte C, Jiang N, and Wessler SR (2002) Plant transposable elements: where genetics meets genomics. *Nature Reviews Genetics* 3: 329–341. © 2002 Macmillan Magazines Ltd.)

Table 1 Mutations affecting carbohydrate or protein composition of the kernel. Mutation abbreviations are in parentheses

Mutation	Gene product	Kernel phenotypic description	Economic use
Carbohydrate composition n	nutations		
amylose extender1 (ae1)	Starch branching enzyme II	Glassy appearance, high amylose content	Highly linear starch used for food, films, fibers, and other industrial purposes
brittle endosperm1 (bt1)	Amyloplast adenylate translocator	Collapsed, often translucent and brittle	Used in sweet corn
brittle endosperm2 (bt2)	ADP glucose pyrophosphorylase	Collapsed, often translucent and brittle	Used in sweet corn
dull1 (du1)	Starch synthase	Glassy appearance	
shrunken1 (sh1)	Sucrose synthase	Inflated, transparent kernels collapse on drying	
shrunken2 (sh2)	ADP glucose pyrophosphorylase	Inflated, transparent, sweet kernels collapse on drying	Used in "super-sweet" sweet corn
sugary1 (su1) sugary2 (su2)	Isoamylase Starch branching enzyme	Wrinkled and translucent Glassy, translucent, sometimes wrinkled	Used in sweet corn
sugary enhancer1 (se1)	Unknown	Observed only in <i>su1</i> lines light yellow, slow drying	Used with <i>su1</i> sweet corn to boost sugar content 50–100%
waxy1 (wx1)	NDP-glucose-starch glucosyltransferase	Opaque endosperm, high in amylopectin	Highly branched starch, used for food gels, adhesives, and other industrial purposes
Protein composition mutation	ns		The second secon
floury1 (fl1)	Unknown	Soft, opaque endosperm	
floury2 (fl2)	Alpha zein	Soft, opaque endosperm, reduced protein levels	
floury3 (fl3)	Uknown	Soft, opaque endosperm, reduced production of the prolamine fraction, and enhanced lysine content not used commercially due to poor germination	
opaque endosperm1 (o1)	Unknown	Soft, opaque endosperm	
opaque endosperm2 (o2)	Unknown regulatory protein of zein proteins and pyruvate orthophosphate dikinase1 (pdk1)	Soft, opaque endosperm, higher in lysine and tryptophan	
opaque endosperm2 (o2)	Unknown	Soft, opaque endosperm, higher in lysine	
gamma zein modifier1 (gzr1)	Unknown	Modifies the hardness of o2 endosperm	
mucronate1 (mc1)	Unknown	Dominant, opaque endosperm, higher in lysine	
proline responding1 (pro1)	Unknown	Crumpled, soft, opaque endosperm, proline auxotroph	

Table 2 Mutations affecting kernel development

Mutation	Gene product	Phenotypic description
defective kernel1 (dek1)	Calpain superfamily member	Germless kernel that can not be embryo rescued because only the root primordia are present, the shoot primordia are absent. Floury, white endosperm with missing aleurone layer
dek2 to dek3	Not yet identified	Similar to dek1 above
cp2, cp3	Not yet identified	Collasped pericarp
emp1, emp2	Not yet identified	Empty pericarp
ren1, ren2, ren3	Not yet identified	Reduced endosperm
miniature seed1 (mn1)	Cell wall invertase	Small ( $\sim$ 1/5 the size of a normal kernel), fully viable kernel
mn2-cp1	Not yet identified	Small kernel, loose pericarp, defective appearing, but fully viable kernel

and ash ( $\sim$ 80%). Mutations that affect kernel composition either can alter total content or composition of the protein, starch, or oil fraction. Starch is a homopolymer of glucose molecules linked together in either  $\alpha$ -1,4 or  $\alpha$ -1,6 linkages. There are two types of starch molecules.

- 1. Amylose is a linear molecule composed of  $\alpha$ -1,4 glucose linkages. For about every 200 glucose molecules, there will be an  $\alpha$ -1,6 glucose linkage that gives the starch molecule a small degree of branching. The amylose molecules are highly variable in length, ranging from 100 to 1000 glucose molecules.
- 2. Amylopectin molecules are larger, consisting of up to 200 000 glucose molecules and have a higher degree of branching with  $\sim 4-5\%$  of the glucose molecules in  $\alpha$ -1,6 glucose linkages.

Amylose makes up 25-30% of the starch, while amylopectin comprises 70-75% of the starch in a typical maize kernel. There are two mutations that shift this ratio rather dramatically, amylose extender1 (ae1) results in high amylose content while waxy1 (wx1) results in high amylopectin content. Both the wx1 and ae1 mutations are used in specialty maize to produce commercial specialty starches for food and nonfood industrial uses. In addition to these mutations, there are numerous mutations that interfere with starch formation, resulting in "sweet" rather than "starchy" kernels (Table 1). Of these mutations, three are used commercially in "sweet corns" to confer sweetness: *shrunken2* (*sh2*) (Figure 2b), *sugary1* (*su1*), and sugary enhancer1 (se1). The typical fatty acid profile of a maize kernel is 50% linoleic acid, 1% linolenic acid, 40% oleic acid, 12% palmitic acid, and 2% stearic acid. Most of the genetic research on maize kernel oil has focused on total content rather than composition, the inheritance of which is highly quantitative, however, one single gene mutant effecting oil composition has been identified. Linoleic acid content is controlled by a single recessive mutation, linoleic acid1 (ln1). Maize kernel proteins are very high in sulfur-bearing amino acids (i.e., methionine and cystine), but are deficient in the essential amino acids, lysine, and tryptophan. The endosperm contains the gluten proteins, glutelin, and zein. Changes in protein content of a kernel generally involve changes in the gluten proteins, primarily the zein component. Zeins, devoid of lysine and tryptophan, are the proteins that hold the starch granules in a matrix. Increased zein levels are generally associated with an increase in endosperm hardness. There are two families of mutations that affect the zein genes in maize, floury and opaque (Figure 2e) mutants. These kernel mutants have extremely soft endosperm and are either higher in lysine and tryptophan or have an altered protein profile (Table 1). The *opaque2* (*op2*) mutation has been used to develop high lysine specialty maize, referred to as quality protein maize (QPM).

It has been estimated that more than 350 genes affect kernel development. Mutations in these genes generally result in poorly filled, collapsed, crumpled, germless, small, or loose-pericarped appearing kernels, hence the name defective kernel (dek) mutants. Some of these mutations are completely lethal (i.e., defective kernels will not germinate), others such as collapsed pericarp2 (cp2) are seedling lethals (i.e., defective kernels germinate, but the seedling dies) (Figures 2m and 2n), while other mutations are not lethal (i.e., defective kernels germinate and the resulting plant is fully fertile). Lethal mutations can be further divided into those that can be rescued by excising the developing embryo and culturing it on either basal or enriched tissue culture medium. The rescued embryos produce seedlings that are viable long enough to observe altered seedling phenotypes. The lethal mutations generally represent developmental failures, which are specific to a particular stage of embryo development. For example, dek1 embryos when rescued will develop into seedlings that are missing the shoots. In other words, the *dek1* mutation interferes with shoot primordia formation, but not root primordia formation in the embryo.

# Mutants Affecting Pigmentation (Kernel and Plant)

Phenolic-based compounds and carotenoid compounds comprise two of the major classes of pigments in maize. The phenolic-based compounds arise from the phenyl-propanoid pathway through either the flavonoid pathway or the lignin-biosynthesis pathway. The carotenoid compounds are derived from fatty acids. Carotenoid mutations are visible either through their effect on the accumulation of carotenoids in the kernel (i.e., endosperm color or lack of dormancy of the embryo) or through their effect on chlorophyll stability (i.e., albino, pale green, or delayed greening of seedlings). Some of the mutations affect both endosperm and seedling phenotypes, while others are specific. Endosperm color can be altered from yellow to white by the mutation yellow endosperm1 (y1), that results in a lack of  $\beta$ -carotene (provitamin A) (Figure 20). This mutation is used commercially to produce white endosperm maize, and subsequently, white starch. In addition to v1, there are over 20 genes that alter carotenoid content of the kernel. Some of these genes impart a lack of embryo dormancy (i.e., vivipary), causing mature embryos to germinate on the ear.

The lignin pathway has four mutants associated with it that either reduce the total lignin content of the plant or alter the lignin composition. Lignin is a polymer composed of three different monolignols, p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. These monolignols polymerize to form lignin, which is part of the cell wall. Phenotypically, mutations in the lignin-pathway genes result in a browning of the midvein (i.e., midrib) of the maize leaf, hence the name brown mid-rib (bm1) mutants (Figure 2c). Economically, these mutants are of interest as a means of increasing the digestibility of the stover that is fed to ruminant livestock. The *bm1* mutant, which encodes the enzyme cinnamyl alcohol dehydrogenase, reduces lignin content of the stover at maturity by  $\sim 86\%$ . However, bm1 does not result in increased stover digestibility. The mutant brown midrib2 (bm2) alters lignin composition, greatly reducing the guaiacyl (i.e., polymerized coniferyl monolignol) content, but increasing the levels of the syringyl monolignols. Mutations in brown mid-rib3 (bm3), which encodes caffeate O-methyltransferase, result in increased stover digestibility when fed to livestock. The fourth mutant, brown mid-rib4 (bm4) has not been extensively characterized. The bm3 mutation is being used commercially in maize hybrids developed specifically for the North American silage market.

Flavonoid mutants were probably the earliest and the most extensively studied set of mutants in maize. The flavonoid pathway in maize produces at least four types of flavonoid compounds: flavones, flavonols, anthocyanins, and phlobaphenes. Only the latter two are visible compounds. Anthocyanins are blue/black pigments and phlobaphenes are reddishbrown pigments. The flavonoid pathway branches off the phenyl-propanoid pathway at the 9-carbon compound, 4-coumaroyl CoA. Chalcone synthase, regarded as the first committed step in the flavonoid pathway, is responsible for the synthesis of chalcone

from three molecules of malonyl-CoA and the 9-carbon phenyl-propanoid pathway product. Maize has two genes that encode chalcone synthase, colorless2 (c2) and white pollen1 (whp1). c2 and whp1 are examples of homologs that have evolved slightly different expression patterns. Only *c*2 is expressed in the aleurone, while both whp1 and c2 are expressed in cob, pericarp, and other plant parts. The effect of the whp1 mutation on pollen color is only observed when *c*2 is nonfunctional. Mutations in both flavonoid-pathway structural genes, such as c2, and regulatory genes have been identified. In addition to c2 and whp1, mutations in the structural genes a1 (Figure 2b), a2, bz1, bz2, pr1 (Figure 21), sm1 (Figure 2i), and sm2 have been identified (Table 3). These mutations have been ordered in the context of the flavonoid pathway, in some instances appearing to be involved in multiple branches of the flavonoid pathway. The regulatory genes governing the flavonoid pathway are composed of primarily myb-like (c1, pl1, p1, and p2) and myclike (r1, b1, and in1) oncogene transcription factors (Table 4), and unlike the structural genes, appear to be relatively pathway-branch specific. For example, r1/b1 and c1/pl1 are only involved in activating the anthocyanin branch of the pathway. But activation of the pathway requires both the presence of a functional r1 along with a functional c1 for aleurone pigmentation, and b1 with pl1 (Figure 2h) for plant pigmentation. p1 is responsible for activation of the phlobaphene pathway, with various alleles of p1 imparting to tissues specific expression of the phlobaphene pathway (Figure 2k).

## **Mutants Affecting Plant Form**

There is an impressive array of mutations that affect plant form. For example, the *dwarf* (*d*) mutations affect plant stature (Figure 2f), the *lesion mimics* (*les*) mutations mimic leaf diseases (Figure 2d), the

Table 3 Mutations affecting the flavonoid pathway structural genes

Mutation	Gene product	Phenotypic description
anthocyaninless1 (a1)	NADPH dihydroflavonol reductase	Colorless aleurone, when all other anthocyanin factors are present, brown pericarp and cob tissues with p1 alleles that function in pericarp and cob tissues
anthocyaninless2 (a2)	Leucoanthocyanidin dioxygenase	Colorless aleurone, when all other anthocyanin factors are present
bronze1 (bz1)	Flavonol 3-O-glucosyltransferase	Bronze/brown aleurone tissues, when all other anthocyanin factors are present
bronze2 (bz2)	Glutathione S-transferase	Bronze/brown aleurone tissues, when all other anthocyanin factors are present
colorless2 (c2)	Chalcone synthase	colorless aleurone, when all other anthocyanin factors are present
red aleurone (pr1)	Flavonoid 3'-Hydroxylase	Red rather than blue pigmented aleurone tissue
salmon silk1 (sm1) salmon silk2 (sm2) white pollen1 (whp1)	4,6-Dehydratase Rhamnosyl transferase Chalcone synthase	Salmon colored silks, with $p1$ alleles that are functional in silk tissues Salmon colored silks, with $p1$ alleles that are functional in silk tissues White pollen, with recessive $c2$

Table 4 Mutations affecting the flavonoid pathway regulatory genes

Mutation	Gene product	Phenotypic description
colorless1 (c1)	myb-like oncogene	Anthocyanin accumulation in the aleurone tissue
purple plant1 (pl1)	myb-like oncogene	Anthocyanin accumulation in vegetative plant parts
pericarp color1 (p1)	myb-like oncogene	Phlobaphene accumulation in pericarp and cob tissues, and flavone accumulation in silk tissues
colored plant1 (b1)	myc-like oncogene	Anthocyanin accumulation in vegetative plant parts
colored1 (r1)	myc-like oncogene	Anthocyanin accumulation in the aleurone tissue, anthers leaf tips, and brace roots
intensifer1 (in1)	myc-like oncogene	Intensifies the accumulation of anthocyanins in the aleurone tissue
leaf color1 (lc1)	myc-like oncogene	Anthocyanin accumulation in nodes, auricles, leaf blades, and coleoptiles
scutellar node color1 (sn1)	myc-like oncogene	Anthocyanin accumulation in pericarps, nodes, leaf blades, silk tissues, and coleoptiles

knotted (kn) mutations affect organ/tissue identity (Figure 2j), and the tassel seed (ts) mutations result in feminization of the tassel (Figure 2g). These types of mutations have been extensively used in the field of developmental genetics to understand the biology of plant development. The dwarf mutations in maize: anther ear1 (an1), dwarf1 (d1), dwarf2 (d2), dwarf3 (d3), dwarf5 (d5), and dwarf8 (D8) have similar distinctive "cabbage-like" phenotypes (Figure 2f). All but D8 are recessive and respond to exogenous applications of the plant hormone gibberillic acid (GA). The recessive dwarf mutants result from mutations in specific steps in the GA biosynthesis pathway, while the dominant dwarf mutant, D8, is the result of a mutation in a GA receptor.

#### **Mutants Affecting Fertilization**

The final classes of maize mutations that will be discussed in this article are those that affect fertilization, either through gamete preference or through control of fertility. Several factors influence how readily gametes can successfully fuse to form a zygote. Both megaspores (i.e., female gametes) and microspores (i.e., male gametes) are not very tolerant of chromosomal deletions. However, there are some viable deletions, but they are only transmitted through the megaspore and generally at a lower frequency. Deletions arise through several means, including chromosomal breakage (e.g., ionizing radiation, dicentric bridges), crossing over in inversion heterozygotes, chromosomal nondisjunction, and adjacent segregation in translocation heterozygotes. In addition to deletions, there are genes that influence gamete preference. The gametophyic factor (ga) class of mutations affects the functioning of individual pollen grains. For example, Ga1 pollen grains compete equally with ga1 pollen grains when the female is homozygous recessive ga1. (Remember that pollen grains are haploid.) However, when the female contains at least one Ga1 allele, > 90% of the fertilizations will involve Ga1 pollen grains. The ga1 pollen grains are mostly incompatible when the female possess a Ga1 allele and in some genetic backgrounds ga1 pollen grains are completely incompatible.

Recessive mutations have been identified that affect both male and female flower fertility (e.g., dsy1, dsy2, pam1, pam2, as). Each of these mutations involves defects in various stages of meiosis. Another class of mutations results in male flower sterility (i.e., failure to develop functional pollen grains), but do not affect female flower fertility. This group is collectively referred to as male sterile mutations. Only some of these phenotypes are the result of mutations to a gene that is nuclear encoded. Male sterility in maize can be due solely to mutations in nuclear encoded genes, or it can result from a rearrangement in the mitochondrial genome in a genetic background that is not able to compensate for it with the appropriate nuclear encoded genes. This second type of male sterility is referred to as cytoplasmic male sterility (CMS).

The cytoplasmic genomes, mitochondrial, and plastid, are maternally inherited. That is they are not transmitted through the pollen. Nuclear encoded genes can compensate for the defect caused by the rearrangement in the mitochondrial genome of CMS cytoplasms. These genes, termed restorer factors (rf), are dominant and restore male fertility in CMS cytoplasm genotypes. In normal (N) cytoplasm genotypes, rf genes are not required for male fertility. There are several CMS cytoplasms, however the one that was used quite extensively in commercial North American hybrid seed production fields until the early 1970s was CMS-T. To produce hybrid seed, inbred line parents were either deemed female or male. The male parents carry the dominant Rf gene and a normal cytoplasm (N). The female parents need to have two different genotypes. One genotype needs to possess normal cytoplasm (N) and the recessive rf gene and

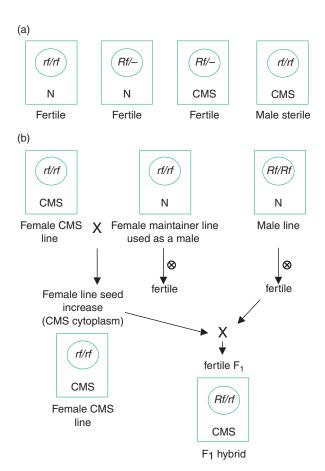


Figure 10 Cytoplasmic male sterility (CMS) and restorer gene (rf) system. (a) Four possible combinations of cytoplasms (N normal vs. CMS - male sterile) and dominant, nuclear encoded restorer genes (rf/rf - not capable restoring male fertility vs. Rf/ - capable of restoring male fertility). (b) System for using CMS to produce hybrid seed. There are two versions of the female inbred. the CMS containing line and the maintainer line. These lines are genetically identical except for the mitochondrial genomes. The maintainer line contains a normal (N) cytoplasm, while the CMS line contains the male sterile cytoplasm. The maintainer line is male fertile, while the CMS is male sterile. The maintainer line is used as a male parent in crosses with the CMS line to produce more CMS seed. The CMS line is used as the female in hybrid seed production fields and the male inbred line is a unrelated inbred line with a different genetic constitution. The male contains a normal cytoplasm and is homozygous for the dominant Rf allele. The resulting F<sub>1</sub> hybrid will contain the CMS cytoplasm, but will be heterozygous for the Rf gene and thus will be male fertile. Note: ⊗ is genetic's shorthand for self-pollination.

the other genotype possesses the CMS cytoplasm and the recessive *rf* gene. The first genotype is male fertile and is used to maintain both of the female inbred genotypes, while the second genotype is male sterile and is used as the female parent in hybrid seed production fields (**Figure 10**). Unfortunately, the mitochondrial genome rearrangement that led to male sterility rendered those genotypes susceptible

to the fungal leaf disease — northern corn leaf blight (NCLB) (Exserobilum turcicum, previously called Helmithosporium turcicum). A NCLB epidemic in the US in the early 1970s devastated the maize crop. Use of CMS to produce hybrid seed quickly fell out of favor, and is no longer widely used.

Nuclear encoded male sterile genes (*ms*) are quite numerous, with over 45 unique mutants that confer the *ms* phenotype characterized. Most of the *ms* mutants are recessive, however two dominant *Ms* mutants (e.g., *Ms1*, *Ms2*) have been isolated. The *ms* genes appear to affect all aspects of gamete development from breakdown of early meiotic events (e.g., *ms8*, *ms9*), after meiotic prophase (e.g., *ms17*, *ms23*), during vacuolation of the microspore (e.g., *ms17*, *ms23*), during microspore mitosis (e.g., *ms14*), or after microspore mitosis (e.g., *ms5*, *ms11*). Nuclear encoded *ms* genes have not been used in hybrid seed production, due to the difficulty in generating large quantities of homozygous recessive *ms* female seed.

#### **Maize Genetics Resources**

The Maize Genetics Cooperation was formally organized in 1932 by the maize geneticists attending the sixth International Genetics Congress. They agreed to establish a cooperative enterprise to further the advance of maize genetics, specifically by collecting and disseminating unpublished data and information and maintaining and distributing of genetic stocks (e.g., mutants, translocations, etc.). Information was exchanged through the medium of an informal Maize Genetics Cooperation – News Letter (MNL). The newsletter is still compiled and published 71 years later. There are several excellent publicly available resources for maize genetics. For seed of maize mutants and lines containing chromosomal rearrangements, the Maize Genetics Cooperation – Stock Centre, located in Champaign-Urbana, Illinois, USA maintains a collection of over 80 000 pedigreed samples. It is the main repository for maize mutants utilized in research by scientists worldwide. The Stock Centre is located at the University of Illinois and is part of the National Plant Germplasm System and is supported by the US Department of Agriculture, Agricultural Research Service (USDA/ARS). MaizeDB (http://www.agron.missouri.edu/index.html) is a web-based database containing historical and up-tothe-minute information about the maize genome. The database is administered at the University of Missouri-Columbia and is funded through support from the USDA-ARS, National Science Foundation (NSF), and the University of Missouri.

See also: Cereals: Overview; Evolution of Species. Genetically Modified Grains and the Consumer. Maize: Breeding.

## **Further Reading**

Carlson WR (1988) The cytogenetics of corn. In: Sprague GF and Dudley JW (eds.) Corn and Corn Improvement, pp. 259–343. Madison, WI: ASA-CSSA-SSA Publishers.

Coe EH, Neuffer MG, and Hoisington DA (1988) The genetics of corn. In: Sprague GF and Dudley JW (eds.) Corn and Corn Improvement, pp. 81–258. Madison, WI: ASA–CSSA–SSA Publishers.

Freschotte C, Jiang N, and Wessler SR (2002) Plant transposable elements: where genetics meets genomics. *Nature Reviews Genetics* 3: 329–341.

Galinat WC (1988) The origin of corn. In: Sprague GF and Dudley JW (eds.) *Corn and Corn Improvement*, pp. 1–31. Madison, WI: ASA–CSSA–SSA Publishers.

Moore G, Devos KM, Wang Z, and Gale MD (1995) Grasses, line up and form a circle. *Current Biology* 5: 737–739 169–174.

Neuffer MG, Coe EH, and Wessler SR (1997) *Mutants of Maize*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Peterson PA and Bianchi A (1999) Maize Genetics and Breeding in the 20th Century. Singapore: World Scientific.

Walbot V and Messing J (1988) Molecular genetics of corn. In: Sprague GF and Dudley JW (eds.) Corn and Corn Improvement, pp. 389–429. Madison, WI: ASA—CSSA—SSA Publishers.

White S and Doebley J (1998) Of genes and genomes and the origin of maize. *Trends in Genetics* 14: 327–332.

# **Relevant Websites**

http://www.agron.missouri.edu – Maize Genetics Cooperation – News Letter (MNL).

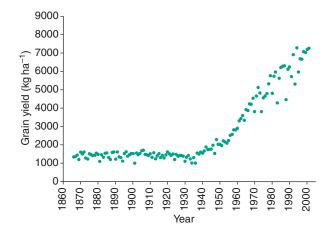
http://w3.ag.uiuc.edu — Maize Genetics Cooperation — Stock Centre, Champaign-Urbana, Illinois, USA.

# **Breeding**

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Prior to the introduction of maize hybrids in the early 1930s (US) and 1940s (Canada), maize grain yields were static. Since then average on-farm maize grain yields have risen steadily from prehybrid level of  $\sim 1000 \text{ kg ha}^{-1}$  to the present level of  $\sim 7000 \text{ kg ha}^{-1}$  (Figure 1).



**Figure 1** Maize grain yields in US from 1866 to 2001. (Source: USDA, National Agricultural Statistics Service.)

The intent of this brief article is to familiarize the reader with some of the unique aspects of maize breeding, and how these approaches have contributed to the steady increase in North American maize grain yields during the hybrid era. Although the perspective of this chapter will be that of a northern temperate zone, North American breeding program, the underlying concepts are applicable wherever maize is bred.

There are several types of maize grown commercially, each with very different end uses: "number 2 yellow maize," for animal feed and some industrial uses such as ethanol, corn oil, citric acid, and high fructose corn syrup production, makes up the largest proportion of maize acreage; "sweet corn" for the fresh market, canning and frozen food industries; "popcorn" and "white and yellow food-grade maize" for the snack food industry and general human consumption; "silage maize" and "high oil maize" for animal feed; "waxy maize" for animal feed and industrial specialty starches; and "high amylose maize" for industrial specialty starches. Breeding methods for "number 2 yellow maize" will be discussed in this article; however, methodologies that are applied to specialty types of maize are essentially the same. Whenever possible we have included references to more comprehensive treatments of the topic. Several excellent reviews of the general subject are available, and a recent book ("Specialty Corns") covers breeding for specific types of maize.

There are two general approaches to maize breeding: pedigree breeding and population improvement. Population improvement has been practiced for centuries by both indigenous peoples and farmers, and, in a more sophisticated form, continues to be used in modern maize breeding; pedigree breeding is a more recent approach that appeared when the potential of heterosis (hybrid vigor) was realized.

## **Mode of Propagation**

Maize is a monoecious plant with separate male (tassel) and female (ear) flowers on the same plant. The tassel produces ~25 million pollen grains while the ear produces ~1000 silks (stigmatic and stylar tissue), each leading to an ovule and a potential seed. Separation of the male and female flowers greatly facilitates controlled crossing. The tassel emerges from the uppermost leaf whorl and primary ear shoots emerge from the leaf sheaths. Once visible the ear shoots are covered, prior to the emergence of the silks, with a glycine bag called a shoot bag. The shoot bag prevents pollen from landing on the silks and unwanted fertilization from occurring. When the tassel is mature, anthers are extruded from the spikelets, followed by dehiscence of the anthers to release the pollen grains. Pollen shed usually lasts for 5-8 days, depending upon the size of the tassel and weather conditions. Peak shedding time is generally mid-day, but is dictated by temperature and humidity. Pollen grains remain viable for no more than 24 h, even under "ideal" conditions. For controlled pollinations, a tassel that is shedding pollen is covered with a brown paper bag called a tassel bag, the day before the pollination is made. The bag is folded securely around the base of the tassel and stapled. The only viable pollen in the bag will be from the tassel. Also the day before the pollination is made, the silks are cut back to the tip of the ear shoot. The next day the tassel bag is removed with the newly released pollen grains in it. The shoot bag is briefly removed from the female plant and the pollen in the bag is sprinkled onto the regrown silks. The shoot bag is replaced, the tassel bag is placed over the shoot bag with the edges wrapped around the stalk and stapled together. Notes regarding the pollination date and the male genotype are written on the tassel bag. Kiesselbach gives an excellent detailed description of the structure and reproduction of a maize plant.

#### Adaptation

Maize genotypes tend to be adapted to relatively narrow maturity zones compared, for example, to other cereal crops. Ideally, material adapted to temperate zones should flower late enough to produce maximal leaf area to intercept the incident solar radiation, yet early enough that the grain reaches physiological maturity before the first killing frost. Several systems have been developed to aid breeders and producers to place genotypes into the correct adaptation zones. In N. America, the Minnesota relative maturity (RM) system, growing degree days (GDDs), and Ontario corn heat units (OCHUs) are commonly used, while

Table 1 Maize relative maturity rating systems: RM, GDDs, OCHUs, and FAO

Minnesota relative maturity (days)	US growing degree days (GDDs)	Ontario corn heat units (OCHUs)	FAO (units)
	(GDD3)	(001103)	
70	1650	2100	100
75	1750	2300	
80	1850	2500	200
85	1950	2600	
90	2050	2700	300
95	2150	2800	
100	2250	2900	400
105	2350	3200	
110	2450	3400	500
115	2550	3500	
120	2650	3700	600
125	2750	3900	
130	2850	4100	700
135	2950	4300	
140	3050	4500	800

Adapted from Troyer AF (1999) Background of US hybrid corn. *Corn Science* 39: 601–626.

in Europe the Food and Agriculture Organization developed the FAO system (Table 1). Both flowering date and rate of moisture loss from the grain influence maturity; in practice, breeders monitor grain moisture at harvest to assess maturity. Selection for hybrids that can take full advantage of the available growing season in the various maturity zones has resulted in very specific adaptation for a given hybrid.

#### Germplasm

The genetic base of North American hybrid maize industry represents only a small portion of the entire Zea mays gene pool. There are 250-300 races of maize, of which only one, the Corn Belt Dent, is the predominant source of commercial germplasm. Of the hundreds of open-pollinated varieties of Corn Belt Dent that were grown up to the 1940s, only half a dozen or so can be considered as significant contributors to current inbred lines. But the overwhelming majority of the inbred lines trace their pedigree back to only two open-pollinated varieties, Reid Yellow Dent and Lancaster Surecrop. All North American hybrids have at least one parent from Lancaster or Reid. The great majority of hybrids are, in fact, crosses of a Reid line derivative with a Lancaster line derivative. Virtually all commercial North American hybrids involve six inbred lines or their close relatives: Lancaster-type inbreds C103, Mo17, and Oh43 and Reid-type lines B37, B73, and A632. Further, these lines are used worldwide in maize breeding programs, either directly as a parent or parents of a commercial hybrid or as a component in an inbred

development program. Concern about the narrow genetic base underlying the hybrid corn industry has led to programs designed to diversify breeding germplasm. The program on germplasm enhancement of maize (GEM) (additional information on GEM can be found at <a href="https://www.public.iastate.edu/~usda-gem">www.public.iastate.edu/~usda-gem</a>) represents one of these efforts.

# Pedigree Breeding: The Inbred-Hybrid Concept

Hybrid maize traces its roots back to experiments on heterosis and inbreeding conducted by G. H. Shull at Cold Spring Harbor Laboratories in New York and E. M. East at Connecticut State College. They observed that when maize plants were self-pollinated (i.e., inbred) in successive generations, their vigor and grain yield rapidly deteriorated. However, when two inbred lines from unrelated populations were crossed, both vigor and grain yield of the F<sub>1</sub> hybrid often exceeded that observed for the original source populations. It was these observations, made around 1908– 09, and methodology outlined by Shull that gave rise to the modern hybrid maize industry. Today most of the maize acreage grown in North America, Europe, and South America is planted to hybrid maize, with an increasing percentage of the acreage in Asia and Africa moving from open-pollinated populations, improved synthetics, and variety crosses to hybrids. There are several types of hybrids (Figure 2). Single-cross hybrids occupy the largest percentage of the acreage in North America. However, double-cross hybrids were grown more widely in the early years of hybrid maize because of seed production problems with the early inbred lines.

This section outlines the primary breeding methodology used to produce inbred line parents for hybrids. In a typical commercial maize breeding company,

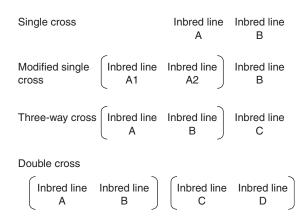
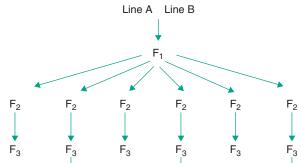


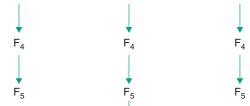
Figure 2 Types of hybrids grown commercially in North America.

 $\sim$ 90% of the breeding effort is focused on developing inbred lines and testing them in hybrid combinations using pedigree breeding. Pedigree breeding (Figure 3) starts with a breeding cross (line A  $\times$  line B), that cross (F<sub>1</sub>) is self-pollinated to produce F<sub>2</sub> seed, and individual F<sub>2</sub> plants are selected and self-pollinated to establish individual lines.

Inbreeding via self-pollination continues until the lines are nearly fully homozygous (i.e.,  $F_5$  (~94%) homozygous) or  $F_6$  ( $\sim$ 97% homozygous)). During each generation of inbreeding, visual selection in the breeding nursery is practiced to remove lines that are not favorable. Because the aim of an inbred development program is to create inbred lines that when crossed to unrelated inbred lines will result in superior hybrids, testing of the lines during development must involve crossing them to elite unrelated inbred line testers. In maize, early generation testing is conducted at either F<sub>3</sub> or F<sub>4</sub> to eliminate poor performing genotypes. Early in the hybrid era, breeders observed that grain yields tended to be greater when lines from unrelated genetic backgrounds were crossed, rather than lines from similar genetic backgrounds. This led to the use of heterotic patterns. Several heterotic patterns have arisen during the hybrid era. In northern Europe, the heterotic pattern is typically "flint" crossed to



Cross each line onto two different inbred tester lines. Grow test-crosses out in replicated multilocation yield trials. Select superior  $F_3$  lines based on their performance in the testcrosses.



Cross each line onto two different inbred tester lines. Grow testcrosses out in replicated multilocation yield trials. Select superior  $F_6$  lines based on their performance in the testcrosses.



**Figure 3** Simplified pedigree breeding scheme, involving a two parent breeding cross.

"dent," with "flint" referring to maize from the Northern Flint race and dent referring to maize from the Corn Belt Dent race. While in North America and southern Europe, the heterotic patterns generally fall within the "dent" category. Patterns such as Iowa Stiff Stalk Synthetic (BSSS), Lancaster Surecrop, and Iodent all trace back to either early synthetic breeding populations or open-pollinated populations. Most commercial companies will have multiple heterotic patterns in which they are actively developing inbred lines, and within a heterotic pattern, there are multiple inbred line families that have been established. For example, within the BSSS heterotic pattern there are at least three inbred line families, B14, B37, and B73, and within the Lancaster heterotic pattern there are at least three inbred line families, C103, Mo17, and Oh43. The hybrid maize industry worldwide is dominated by North American germplasm, but there are specific areas where other backgrounds are grown, e.g., the "orange flints" in Argentina and the "white maize" of southern Africa.

In the early 1980s, commercial maize breeding companies were surveyed regarding their breeding approaches for inbred line development. The information presented below is a synopsis of Bauman's survey. While it is slightly dated, most of the approaches have not changed. The philosophy of the commercial maize breeding industry is twofold: (1) genetic recombination presents opportunities for creating superior genotypes, and (2) evaluation of large numbers of breeding crosses and large numbers of individuals from each breeding cross increases the odds of creating and identifying those superior genotypes. This philosophy results in most maize breeders preferring to avoid breeding strategies that involve backcrossing and instead using strategies that involve creation of new linkage groups, through multiple crosses, and examining large numbers of progenies from each cross. The types of breeding crosses used are narrowbased populations (about six elite parents), elite inbred populations (elite inbred crossed to about six other sources so that 50% of the population is composed of alleles from the elite inbred), double crosses (four inbred parents with equal contribution of alleles), single crosses (two unrelated inbred parents), related line crosses (two related inbred parents), one backcross (two inbred parents, with the F<sub>1</sub> crossed with one of the parents = BC1), and two backcrosses (two inbred parents, with BC1 crossed a second time to the same inbred parent = BC2). Starting with one of these sources, the breeder begins by self-pollinating several plants ( $S_0$  (=  $F_1$ ) plants) (**Table 2**).

Once these S<sub>0</sub> plants are self-pollinated, the seed is called S<sub>1</sub> seed, or seed from one generation of selfpollination. Typically 500 S<sub>1</sub> plants will be grown and selected plants will be self-pollinated to establish S<sub>2</sub> families. Considerable visual selection occurs at this stage, with about 180 S<sub>1</sub> plants being selected for further inbreeding. S2 families are then grown and maintained in what is referred to as ear-to-row, meaning that the S<sub>2</sub> seed from one ear is grown in a single row (20–30 plants) in the breeding nursery. Again considerable visual selection occurs both within an S<sub>2</sub> family and between S<sub>2</sub> families, with about 80 S<sub>2</sub> families being self-pollinated and the "best" ear from each family being selected to advance family to the S<sub>3</sub> generation. As mentioned previously, the aim of an inbred line development program is to produce an inbred line that will result in a high-yielding hybrid. Unfortunately, inbred line grain yield is not indicative of hybrid yield. Therefore, selection for grain yield during inbred line development must be done by crossing the families onto inbred lines from unrelated heterotic groups (i.e., testcrosses). Selection for grain yield in testcrosses can successfully begin in an early generation. Some breeding programs begin this process with S<sub>2</sub> families; however, most programs wait until the  $S_3$  or  $S_4$  generation to begin testcross evaluation. Early testing during inbred development typically involves: (1) using two testers, usually elite inbred lines although occasionally single-cross hybrids are employed, (2) testing at a limited number of locations ( $\sim$ 4), usually for only 1 year and with no more than three (and often fewer) replications per location, and (3) current commercial hybrids that are similar in maturity are typically included in the trials as "checks." Only families that in testcross combinations produce hybrids with performance equivalent or superior to the "checks" will be advanced to the next generation. Superior families are selected and the inbreeding and visual selection process continues until the  $S_5$  or  $S_6$  generation, where again the families are evaluated in testcross combinations. Testing at this stage may involve more testers and environments (year and location combinations). Once an inbred line is developed by this process, it will then enter a hybrid development program where it is crossed to numerous

**Table 2** Relationship between generation designations  $F_n$ and  $S_n$  and expected average level of homozygosity from selffertilization, for all loci at which the F<sub>1</sub> (S<sub>0</sub>) was heterozygous

	Generation	Expected homozygosity		
F <sub>1</sub>	S <sub>0</sub>	0		
F <sub>1</sub> F <sub>2</sub> F <sub>3</sub> F <sub>4</sub> F <sub>5</sub>	$S_0$ $S_1$	50		
$F_3$		75		
$F_4$	$oldsymbol{S_2} oldsymbol{S_3}$	87.5		
F <sub>5</sub>	$S_4$	93.8		
$F_6$	$S_5$	96.9		

inbred lines and evaluated extensively in an attempt to identify commercial caliber hybrids for sale to farmers.

# **Population Improvement**

Although most of the inbred parents of commercial hybrids have been bred via pedigree selection, some have been selected from populations improved through cyclic recurrent selection. Indeed, some of the families extensively used in pedigree selection originated from inbred lines developed from populations improved through cyclic recurrent selection. Notable examples are the B14, B37, and B73 families, all of which originated from various cycles of recurrent selection in the population BSSS. Cyclic recurrent selection is a procedure whereby several desirable individuals are selected from a genetically diverse population and these individuals, or their progeny, are intermated to generate an improved population (Figure 4).

The procedure is repeated for as many generations as the breeder deems necessary, each generation representing a cycle of selection. The average performance of the population for the traits(s) of selection is expected to improve with each successive cycle of selection as favorable genes for the selected trait(s) accumulate, or, conversely, the frequency of less desirable genes is deceased (Figure 5). Recurrent selection can be effective for many cycles; for example, BSSS was formed in the 1930s and progress remains satisfactory after 15 cycles of recurrent selection.

In breeding programs where hybrids are the product of interest, selections made as parents of the next cycle

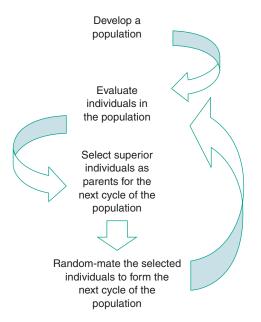
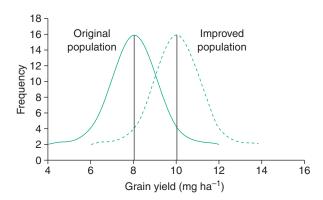


Figure 4 Cyclic steps involved in recurrent selection.

can be used as well to breed new inbred lines. However, in developing countries where hybrids may not yet be available, the improved population itself may be the product used by farmers. International organizations such as International Maize and Wheat Improvement Center (CIMMYT) and International Institute of Tropical Agriculture (IITA) have been very successful in this endeavor, although they now are also emphasizing the development of hybrids based on inbred lines derived from improved populations.

Cyclic recurrent selection is utilized to improve traits that are controlled by genes at several loci, i.e., quantitative traits. The rate of improvement and duration of effective selection will depend on such factors as initial genetic diversity for the traits, the number of individuals selected as parents in each cycle and their genetic diversity, and heritability, i.e., the proportion of variability due to genetic factors, of the selected traits. Most recurrent selection programs will result in progress for the selected traits, but the desired level of performance can be achieved more rapidly if the initial germ plasm already has segregants with a relatively high level of performance for these traits. For this reason, synthetics formed from elite inbreds often are used to initiate a recurrent selection program. For example, BSSS, the highly productive synthetic from Iowa State University, was formed by intercrossing 16 elite inbred lines with good stalk quality.

Several cyclic recurrent selection procedures have been used to improve population performance (Table 3). For a detailed description of recurrent selection procedures, including expected genetic gain per cycle of selection for the various procedures, the reader is referred to Hallauer and Miranda, 1988. Examples of two recurrent selection procedures, S<sub>2</sub> progeny and half-sib tester, are presented in Table 4. In general, all recurrent selection procedures have given progress for



**Figure 5** Idealized objective of recurrent selection: improvement of the mean performance of the population while maintaining genetic variation within the population.

the selected trait(s). Several studies have compared some of the procedures, but the results to date are not definitive because of such factors as different germ plasms and their levels of genetic diversity among studies, different procedures being compared in different studies, switches in selection procedures over time, varying number of cycles of selection among the studies, different selection intensities, etc. For example, mass selection might be effective in the early cycles of selection in a population with extensive genetic variability, whereas in later cycles, when genetic variability is reduced, selection methods requiring progeny testing might give greater progress. Selfed progeny selection is expected to be effective because selfing results in deleterious recessive genes being revealed as homozygotes instead of being masked from selection by a dominant gene in the heterozygous state. In contrast, half-sib tester recurrent selection can result in the tester genes masking deleterious genes in the population; however, the tester, especially an elite inbred tester, helps to ensure that lines derived from the population will combine well with elite inbreds to produce a superior hybrid. Visual selection among  $S_1$  lines in the breeding nursery, which is common to both selfed progeny and half-sib tester methods, helps to reduce the frequency of deleterious recessive genes. Also, testcrossing of the selected lines in the inbred development phase of selfed progeny testing helps to enforce the desired combining ability in that procedure.

Some breeding systems for population improvement integrate two or more cyclic recurrent selection

Table 3 Some methods of cyclic recurrent selection for improvement of maize populations

Mass. It is the oldest method of recurrent selection. Individual, open-pollinated plants are selected from populations grown in isolation. Bulked seed from the selected plants are planted in the next cycle. An often-used variation is to impose a grid on the isolation block and then select the best plant within each component of the grid. Grid selection reduces the effect of variable growing conditions in the isolation plot. Mass selection requires one season per cycle and is most effective in populations with high genetic variability for the trait(s) of interest and especially with highly heritable traits.

Ear-to-row. Seeds from selected individuals in a population are planted on an ear-to-row basis, i.e, each row is from seed of one selected plant. The plot is grown in isolation and the plants are open pollinated. The best individuals in the best rows are selected for ear-to-row planting in the next cycle. A common variation is modified ear-to-row selection, whereby the individual ear-to-row plots are detasseled before pollen is shed and male (pollinator) rows from bulked seed of all the entries are planted at regular intervals throughout the isolation block. Undesirable plants in the male rows can be rogued before pollination. In addition, the entries are tested in one or more replicated trials to identify the best performing entries. From the isolation block, the best individuals in the entries selected from the replicated trial data are chosen for the next cycle of modified ear-to-row selection. Each cycle requires one season.

Half-sib family. Individual ear-to-row families are selected based on data from replicated trials at two or more locations. Residual seed of the selected entries is then used for systematic intercrossing of the selections. Thus, unlike mass or ear-to-row recurrent selection, the pollen sources also are only from the selected entries. Individual plants are selected within each family in the intercrossing block for entries in the next cycle. Each cycle requires two seasons, i.e., one year if both summer (for testing) and winter (for intercrossing) facilities are available.

Selfed progeny. Selected plants (S<sub>0</sub>) within a population are self-pollinated to produce S<sub>1</sub> lines. The S<sub>1</sub> lines are tested in replicated trials at two or more locations, and residual S<sub>1</sub> seed of the selected entries is planted for intercrossing to produce the next cycle of the population. Three seasons are required per cycle, or two years if a winter nursery is used. An alternative procedure is to plant the S<sub>1</sub> lines in the breeding nursery and perhaps in a pest nursery as well. Visual selection is practiced among the S<sub>1</sub> lines, and selected plants within the selected lines are selfed to produce S<sub>2</sub> seed. S<sub>2</sub> lines are then evaluated in replicated trials. This procedure increases the efficiency of costly replicated trials by eliminating visually undesirable families beforehand. Furthermore, if a winter nursery is used, a second generation of intercrossing of the selected entries can be done, thus enhancing genetic recombination, and therefore genetic variance, before initiating the next cycle of selection. Six seasons are required per cycle, or three years with a winter nursery. Table 4 presents a season-by-season description of S<sub>2</sub> selfed progeny recurrent selection.

Half-sib tester. The procedure is similar to selfed progeny recurrent selection except that the S<sub>1</sub> (or S<sub>2</sub>) lines are topcrossed to a common tester, e.g., a population, a hybrid, or, most frequently, to an elite inbred line. The topcrosses are then evaluated in replicated trials and residual S<sub>1</sub> (or S<sub>2</sub>) seed of the selected entries used to plant the intercrossing block. Compared to the selfed progeny procedure, an extra season is required in order to make topcrosses. Thus, four seasons are required if S<sub>1</sub> lines are topcrossed, and seven seasons if S<sub>2</sub> lines are used. However, if a winter nursery is used, a cycle still can be completed in 2 years with S<sub>1</sub> lines and in 3 years with S<sub>2</sub> lines. Table 4 presents a season-by-season description of half-sib tester recurrent selection using S<sub>2</sub> lines and an inbred tester.

Full-sib. Selected plants in the population are crossed in pairs to produce seed of full-sib progenies. The full-sib progenies are evaluated in replicated trials and the selected full-sib progenies are intercrossed to form a new cycle. Each cycle requires three seasons or 2 years if a winter nursery is used.

Reciprocal recurrent. This procedure is designed to improve the cross-performances of two complementary populations. It is essentially the same as half-sib tester, but the tester for each population is the other population or an inbred line from that population. A full-sib version uses plant-to-plant crosses between the two populations.

Table 4 A season-by-season description of S2 selfed progeny (S) recurrent selection and half-sib tester (HS) recurrent selection with an elite inbred tester when a winter breeding nursery is available and the breeding program is located in a temperate zone

Winter 1. For both S and HS: self-pollinate 400-600 selected plants from a population of 1000 or more plants to produce S<sub>1</sub> seed. Select again at harvest.

Summer 1. For both S and HS: grow out S<sub>1</sub> progeny rows in breeding and pest (if needed) nurseries. Select among S<sub>1</sub> progeny rows and self-pollinate selected S<sub>1</sub> plants within the selected rows to produce S<sub>2</sub> seed. Select again at harvest to give 100 or more S<sub>2</sub> entries for subsequent evaluation.

Winter 2. No activity in S. For HS: cross the selected S2 lines to an elite inbred tester.

Summer 2. (1) For S, evaluate the 100 (or more) S2 lines in replicated trials at two or more locations. Select 20 or more S2 lines as parents for the next cycle. The HS program is the same except that testcrosses of the 100S2 lines are evaluated. (2) Continue selfing and selection among and within the 20 (or more) S2 lines to produce S3 seed.

Winter 3. For both S and HS, use residual seed of S2 lines selected from evaluation trials for first intercrossing.

Summer 3. (1) For both S and HS, make a second intercrossing using bulked seed of each line from first intercrossing. This will complete cycle. The next cycle of selection will be initiated in the next winter nursery (as per Winter 1). (2) Keeping only the S<sub>3</sub>s, of the best of the 20 (or more) selected lines from the evaluation trials, continue selfing and selecting to produce S4 seed. Also testcross the S<sub>3</sub> lines to elite inbred testers. Subsequent testcross evaluation trials will determine which lines will continue to be selfed for potential use as an inbred parent in a commercial hybrid.

methods in order to maintain or even enhance genetic variability while shaping this diversity into populations with agronomic/breeding potential. For example, the hierarchical, open-ended, population enrichment (HOPE) system employs three levels of performance to include a highly diverse population at the low-level in which mass selection is used, an intermediate level using selfed progeny recurrent selection, and two elite level populations using fullsib-reciprocal recurrent selection. Introductions are added on a continuing basis and selected entries can be moved upward in the hierarchy. Inbred lines are developed from the elite level populations.

# **Incorporating GMO Events into Inbred Lines**

Since the 1990s, commercial maize hybrids containing transgenic events have been widely grown in North America. These genetically modified organisms (GMOs) involve expression of Bt proteins against European corn borer (Ostrinia nubilalis), or resistance to the herbicides – Liberty<sup>TM</sup> and Round-Up<sup>TM</sup>. For all of these events, only one parent of a single-cross hybrid needs to contain the event, because the events on the market to date are all psuedodominant, i.e., the event has been added to the genome of the GMO parent; therefore, the gene does not have a counterpart on the homologous chromosome segment from the non-GMO parent. To transfer the GMO event from one inbred background to another inbred background, rapid backcrossing is employed. In rapid backcrossing, the GMO parent (donor parent) is crossed to the inbred line of interest (recurrent parent), and then the  $F_1$  is crossed to the recurrent parent (BC1). At this point, the BC1 generation, only 50% of the individuals will contain the GMO event and the average genetic constitution of a BC1 individual is 75% of the recurrent parent's genotype. However, within the BC1 population there are individuals that contain less than 75% of the recurrent parent's genotype and there are individuals that contain considerably more that 75% of the recurrent parent's genotype. Individuals containing the GMO event are identified using an antibody-based assay such as ELISA or spraying with the herbicide. Molecular markers are used to identify the individuals that contain as much of the recurrent parent's genotype as possible. Those individuals that contain the GMO event and a high proportion of the recurrent parent's genome will be backcrossed again to the recurrent parent (BC2) and those BC2 individuals that contain the GMO event again will be identified as well as those individuals that contain >95% of the recurrent parent's genotype. Using this approach and off-season nurseries, breeders can rapidly introduce transgenic events into new inbred lines.

# **Production of Commercial Hybrid Maize Seed**

Hybrid maize seed planted by farmers is produced and sold by commercial seed companies. The production of hybrid seed is an intricate process, because seed quality, seed purity, and cost of production are all critically important factors. Wych provides a detailed description of this process. Most hybrid seed production now is of single crosses (two inbred parents) or modified single crosses (three inbred parents, two of which are closely related), but three-way crosses (three inbred parents) or double crosses (four inbred parents) have been popular in the past (Figure 2). The minimum isolation distance that a seed production field must be from other maize fields is 200 m to ensure purity of the

seed. The typical pattern of planting that is used in a seed production field is 4:1, i.e., 4 rows of the female line and 1 row of the male line. This pattern is repeated systematically throughout the field so that a female row is no more than two rows away from a male row.

Plant densities in seed production fields range from 54 000 to 64 000 plants/ha, which is a lower plant density than that normally found in commercial production (~70 000 plants/ha) in North America. The lower plant density is used to achieve maximum yield of saleable kernels. Pollen control in seed production fields is achieved by removing the tassels (detasseling) from the female rows prior to anthesis. Detasseling can be done either by hand or mechanically using cutter bars or pullers. An option to detasseling is to use the cytoplasmic male sterile system with restorer genes. However, this practice fell out of favor in the early 1970s when susceptibility to southern corn leaf blight (Bipolaris maydis) was associated with the use of the Texas male sterile cytoplasm (cms-t). Soon after pollination, typically the male parent rows are removed to reduce competition with the female row and the risk of seed contamination at harvest. The female rows will be harvested at  $\sim 30-38\%$  grain moisture, i.e., just before they reach physiological maturity. Timely harvest is essential to maintain yield and seed quality. Freezing damage to the kernels and damage due to insects and diseases can reduce the germination percentage of the seed, while delays in harvest will increase the risk of dropped ears. Harvesting is done by machine using mechanical ear pickers. The ears are sorted to remove diseased or undesirable ears and are then dried in low-temperature dryers at temperatures ranging from 35°C to 40°C until the kernels have reached a moisture content of 12-13%. The ears are then shelled, the seed is cleaned to remove foreign material and broken kernels, and the seed is then sized. Sizing involves sorting the seed into lots of uniformly sized kernels. The sized seed is treated with a fungicide or a combination fungicide/insecticide and bagged. Hybrid maize seed is bagged in units either weighing 50 lbs or containing 80 000 kernels.

#### Summary

The maize breeding methods and philosophy described in this article have been successfully practiced for over 90 years with very little modification to the basic principles: (1) creation of new linkage blocks through recombination; (2) simultaneous evaluation of large numbers of breeding crosses and families within breeding crosses; and (3) progeny testing in testcross combinations using replicated trials in multiple locations. Innovations to maize breeding during those 90 years have involved incorporating new

technologies such as mechanical harvesters and planters, computers, electronic data capture devices, and databases. All of these technologies have aided the maize breeder to create and identify superior genotypes more efficiently and thus continue to contribute to the steady grain yield increases in N. America during the hybrid era (Figure 1). Most likely any future technologies, such as genomics, will continue to aid maize breeders to meet this objective, rather than replace the methods and philosophies that have proven to be so successful.

See also: Maize: Genetics.

## **Further Reading**

Brown WL and Goodman MM (1977) Races of corn. In: Sprague GF (ed.) Corn and Corn Improvement, pp. 49-88. Madison, WI: American Society of Agronomy.

Hallauer AR (1987) Maize. In: Fehr WR (ed.) Principles of Cultivar Development, vol. 2, Crop Species, pp. 249–294. New York: MacMillan.

Hallauer AR (2001) Specialty Corns, 2nd edn. Boca Raton, FL: CRC Press.

Hallauer AR, Miranda JB, Fo (1988) Quantitative Genetics in Maize Breeding, 2nd edn. Ames, IA: Iowa State University Press.

Hallauer AR, Russell WA, and Lamkey KR (1988) Corn breeding. In: Sprague GF and Dudley JW (eds.) Corn and Corn Improvement, pp. 463-564. Madison, WI: ASA-CSSA-SSA Publishers.

International Institute of Tropical Agriculture, www. iita.org, IITA c/o Lambourn (UK) Limited, Carolyn House, 26 Dingwall Road, Croydon CR9 3EE, UK.

International Maize and Wheat Improvement Center, www.cimmyt.org, CIMMYT, Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico.

Kiesselbach TA (1949) The Structure and Reproduction of Corn. Lincoln, NB: University of Nebraska Press.

Troyer AF (2001) Temperate corn: background, behavior, and breeding. In: Hallauer AR (ed.) Specialty Corns, pp. 393-466. Boca Raton: CRC Press.

Walden DB (1978) Maize Breeding and Genetics. New York: Wiley.

Wych RD (1988) Production of hybrid seed corn. In: Sprague GF and Dudley JW (eds.) Corn and Corn Improvement, pp. 565–607. Madison, WI: ASA-CSSA-SSA Publishers.

#### **Relevant Websites**

http://www.iita.org. http://www.cimmyt.org.

# Quality Protein Maize

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# Introduction

Maize is an important source of protein. Globally, it contributes  $\sim$ 42 million tons (Mt) of protein a year –  $\sim$ 15% of the world annual production of food-crop protein. Therefore, the need to improve maize protein in quality and quantity has been recognized for a long time. Maize protein is mainly stored in both the endosperm and the germ – the two principal parts of maize kernels. But there are large differences in the characteristics of protein present in the two parts. Generally, the germ contains 35% of high-quality protein, whereas the endosperm has only 9% of poor-quality protein. Normally, in maize, the endosperm accounts for 80–85% of the total dry weight of kernels, while the germ constitutes the remaining 15–20%. Hence,  $\sim$ 80% of the total protein content in kernels may be contributed by the endosperm. The relative amounts of protein are also dependent on the type, texture, size of kernels, genotypes, and the environments in which the maize is grown.

According to the solubility in different solvents, maize proteins in kernels are divided into several fractions. The prolamins, referred to as the zeins, are the major protein fraction. They are soluble in alcohol, comprising 52% of kernel nitrogen. The glutelins, which account for 25% of kernel nitrogen, are soluble in dilute alkaline solutions, while the water-soluble albumins comprise 7% of kernel nitrogen. The globulins are soluble in salt solutions and consist of 5% kernel nitrogen. Maize proteins have a low nutritional value because they are deficient in essential amino acids such as lysine and tryptophan. An attempt to improve protein quantity was initiated at the University of Illinois in 1898 with a long term selection. After the 1970s selection, the protein contents in Illinois high-protein (HP) strains reached 26.6%. In contrast, the average contents for proteins in normal maize ranged between 9% and 15%. Unfortunately, the protein yield in Illinois HP strains was not improved in proportion, and the increase in endosperm protein was associated with an increase in the low-quality zeins. Currently, major efforts in most breeding programs are focused on improving the quality of proteins, rather than on developing high-protein maize. This is so especially in developing countries where people depend heavily on maize as a food, and adequate supplies of protein supplements for feed are not produced.

## **Historical Background**

In the 1950s, Mertz's group at Purdue University began research on improving the nutritional value of maize proteins. First, they screened a number of maize germplasm from US corn belt, and Central and South America for low levels of zeins, since they are almost devoid of the two essential amino acids, lysine and tryptophan. None of the varieties screened exhibited a high lysine content. Comparison of Illinois HP (18%) and low protein (LP, 4%) strains at Purdue University by Nelson showed that lysine levels were lower in vitreous high-protein endosperms than in floury, low-protein endosperms. He suggested that "flouriness" might be associated with a high lysine content. He analyzed four floury mutants of endosperm, floury-1, floury-2, opaque-1, and opaque-2. Ultimately, it was found that the opaque-2 endosperm not only contained a high level of lysine, but also a much higher level of tryptophan. After this discovery, similar mutants with high lysine were found in other important cereal crops, such as barley and

This remarkable discovery led to an improvement in protein quality using the opaque-2 mutant in maize breeding programs. In the late 1960s, many countries, such as USA, Brazil, China, South Africa, and even the International Maize and Wheat Improvement Center (CIMMYT) initiated breeding programs for developing high-lysine-content hybrids or varieties. The opaque-2 gene (o2) was widely transferred into different inbred lines, and varieties or populations through simple backcrosses. Some high-lysine-content hybrids with o2 were released in USA, China, and South Africa, while some were also developed in CIMMYT. However, some practical problems and limitations of o2 maize became quite obvious when they were gradually applied. A primary failure of o2 maize was the decrease in grain yield. In general, the grain yield of o2 maize hybrids was only 85-92% of the normal hybrids' yield. There was a decrease in kernel weight and density of o2 maize. Also, the kernels of o2 maize were prone to damage by insects and ear and kernel rots were common both in the field and in storage. Its kernels maintained a high moisture level during the development period and tended to dry slower than the normal kernels during the maturity period. In addition, its kernel, which appeared chalky and nontransparent, was not accepted by markets. All these limitations can be attributed to a soft endosperm, which was a characteristic of the standard o2 maize. Therefore, application of the standard o2

maize declined in the 1970s. However, o2 maize is still in use in a few of the adapted areas world over, where the weather is dry and cool.

In order to overcome the defects of o2 maize, many scientists in different countries explored various methods. The major efforts were focused on hardening endosperm textures in o2 maize. Several approaches were suggested, such as recurrent selection and double mutants, using modifier genes. Practically, the third approach may show a great potential. Actually, wide differences in endosperm textures in o2 genotypes were observed when the o2 gene was backcrossed into a series of maize inbred lines. With regard to endosperm structure, some are completely soft, whereas others are partially hard, which is caused by modified genes for o2 locus. Scientists in CIMMYT have developed tropical and subtropical hard-endosperm germplasm combining o2 gene with modified genes during extensive and sustained breeding efforts. They called their hard-endosperm o2 germplasm as quality protein maize (QPM) so that it is distinguished from soft-endosperm o2, which is called as standard o2. The kernels of QPM are almost similar to those of normal maize, but they are homozygous for the o2 allele. Yield improvement, observed in some QPM populations released by CIMMYT, is related to hard or semihard endosperm. The moisture contents and dry-down time of these QPM populations resemble those in normal maize. Ear rot in OPM is also substantially reduced. Recently, germplasm has been used as an essential resource for developing QPM hybrids.

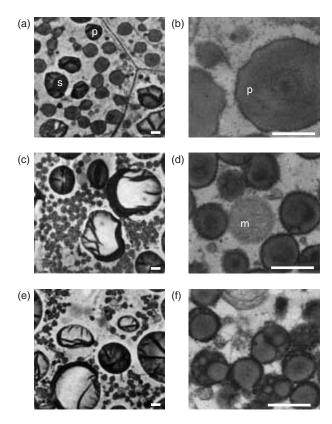
#### **Genetic and Biochemical Basis**

The inheritance of o2 gene follows a typical Mendelian pattern, located on chromosome 7 in the maize genome. It is a recessive mutant and its expression requires the homozygous genotype, i.e., o2/o2. The genetic segregation for the phenotype can be observed on a single F2 ear with a ratio of 3 (normal) to 1 (opaque). The o2 gene was initially cloned by Schmist et al. and later by Motto et al. in the 1980s. More recently, its molecular mechanism has been described. The o2, as a dominant gene, encodes a DNA-binding protein belonging to the basic leucine zipper class, which is involved in the transcription of zein proteins. This protein can bind the 5' flanking sequences of the genes encoding the 22 kDa α-zeins and serve as a transcriptional activator during the transcription. When O2 mutates into o2, the transcription of  $\alpha$ -zeins is significantly reduced, resulting in a remarkable alteration in the amino acid content. But amino acids are not completely eliminated in o2/o2 mutants, since o2 is only one of the transcription factors involved in the expression of  $\alpha$ -zein genes. The increase in the lysine and tryptophan contents results from the decreasing quantities of zein protein.

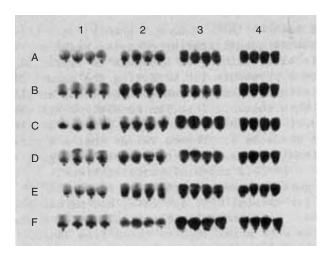
Traditionally, maize zein proteins were classified by molecular weights. A new classifying method is proposed based on their primary genes and amino acid sequences when many genes of storage proteins were found. According to this method, they are divided into four groups,  $\alpha$ -zein (22 and 19 kDa),  $\beta$ -zein (14 kDa),  $\gamma$ -zein (27 and 16 kDa), and  $\delta$ -zein (10 kDa). The α-zein protein group is the largest among them, consisting of  $\sim 80\%$  of the total proteins. The maize zeins are synthesized by membrane-bound polyribosomes, and then are moved to the lumen of the endospermic reticulum, where they are assembled into insoluble protein bodies. There are obvious differences in the composition and size of protein bodies between the aleurone and endosperm of kernels. The small protein bodies containing  $\beta$ - and  $\gamma$ -zeins exist in the subaleurone, whereas the large protein bodies are found in endosperms, containing  $\alpha$ -,  $\beta$ -, and  $\gamma$ -zeins. The quality of proteins in the o2/o2 genotype is improved with the decrease of  $\alpha$ -zeins, which contain no lysine. For the same reason, the sizes of protein bodies in the endosperm of o2/o2 are significantly smaller than those in normal endosperms (Figures 1c and 1d). As a result, the endosperms of kernels turn into being nontransparent and soft.

Modifying genes played an important role in converting soft endosperms of standard o2 maize into hard endosperms of QPM. These may be defined as a series of genes, which, on their own, could not have any effect when o2 gene dose did not exist, but they modify kernel phenotypes of the o2/o2 when it is homologous. Generally, inheritance of modifying genes expresses the quantitative nature of this effect. In a segregation generation, there is a normal distribution for endosperm texture, varying from soft to hard. Some researches indicate that the additive effects of modifying genes seem to be more important in controlling kernel vitreosity for o2 maize in this genetic system. Others suggest that there may be significant effects on kernel vitreosity for modifying genes. Extensive studies at CIMMYT demonstrate that there are a number of o2 modifying genes and that several different types of genetic variations are likely to exist in maize germplasm.

In QPM, the biochemical effects of modifying genes tend to increase zein proteins, but high-quality proteins could maintain nominal changes. There are relatively minor differences between o2 and QPM protein in staining density and morphology (Figures 1d and 1f). The protein bodies in QPM have multiple, nonconcentric areas and a large number of dark-stain regions, which are rich in  $\gamma$ -zeins. In addition, the



**Figure 1** Transmission electron micrographs of normal (a, b), o2 (c, d), and QPM maize (e, f) in subaleurone tissue. m, mitochondrion; p, protein body; s, starch grain. (Reproduced with permission from Paulis JW, Bietz JA, Felker PC, and Nelsen TC (1992) Evaluating quality protein maize genotypes by reversed-phase high-performance liquid chromatography. In: Mertz ET (ed.) *Quality Protein Maize*, 135p. St. Paul, MN: American Association of Cereal Chemists.)



**Figure 2** Kernel phenotypes of QPM and o2 lines and their reciprocal crosses. Columns 1-4 represent parent 1 (P1), parent 2 (P2), P1 × P2, and P2 × P1, respectively. (Reproduced with permission from Larkins BA and Lopes MA (1992) A genetic, biochemical, and ultrastructural analysis of modified opaque-2 maize. In: Mertz ET (ed.) *Quality Protein Maize*, 182 p. St. Paul, MN: American Association of Cereal Chemists.)

studies by Larkins's group on immunocytochemistry reveal that the most significant change in QPM endosperm is the increased synthesis of  $\gamma$ -zeins. This increase could result in a higher concentration of protein in endosperm cells, so that there is less airspace in the endosperm, resulting in a hard and translucent phenotype.

Since the endosperm comprises triploid genotypes, dosage effects of modifying genes are observed through analysis of F1 hybrids from reciprocal crosses between modified and unmodified o2 parents. As can be seen in Figure 2, the parent lines and their reciprocal crosses perform a phenotypic gradient toward the types of increasing vitreousness. The xenia effects of modifying genes are also observed in the hardness of endosperm.

#### **Nutritional and Process Values**

Repeated experiments on infants, children, adults, and various animals have demonstrated that the nutritional value of o2 maize is superior to that of normal maize. Recently, increasing evidence has indicated that the nutritional values are still maintained in QPM when the limitations of o2 are obviously improved upon. As can be seen in Table 1, the volumetric weight and density in QPM are similar to those in normal grains. Protein, lysine, and tryptophan contents are significantly higher for QPM than normal maize, whereas QPM and o2 maize have the same contents. On an average, QPM has ~40% more lysine and tryptophan than normal maize.

Based on the evaluation of digestibility and nitrogen balance for infant feed, nitrogen-retained intakes from

**Table 1** Comparisons of nutrition among QPM, normal, and o2 maize

Item	QPM	Normal	Standard o2
Test weight (kg hl <sup>-1</sup> )	0.79	0.75	0.71
Density (g cm <sup>-3</sup> )	1.31	1.25	1.18
Moisture (%)	11.1	12.7	9.4
Content protein (N×6.25) (%)	9.8	9.1	8.9
Energy (kcal g <sup>-1</sup> )	3.99	3.99	4.08
Arginine	0.66	0.51	0.59
Histidine	0.37	0.28	0.31
Isoleucine	0.32	0.34	0.30
Leucine	0.91	1.14	0.80
Lysine	0.40	0.31	0.40
Methionine	0.17	0.17	0.14
Phenylalanine	0.41	0.47	0.39
Threonine	0.36	0.35	0.32
Tryptophan	0.074	0.054	0.065
Valine	0.52	0.46	0.48

Reproduced with permission from Knabe DA, Sullivan JS, and Burgoon KG (1992) QPM as a swine feed. In: Mertz ET (ed.) *Quality Protein Maize*, 228 p. St. Paul, MN: American Association of Cereal Chemists.

QPM and normal maize in children are 32% and 22%, respectively, indicating a higher efficacy of nitrogen utilization for infant food with QPM. In the animal growth trials, pigs fed the QPM diets consumed 1.97 kg of feed daily and gained 0.75 kg per day, whereas pigs fed normal maize diets, which contain the same level of soybean meal supplementation, consumed 1.86 kg of feed and gained 0.63 kg daily. Other experiments indicated that milk productions of dairy cattle increased when QPM was used as silage.

The wet-milling and dry-milling properties of QPM were also evaluated in order to enhance its utilization in industry. Maize protein is the major by-product of the wet-milling industry, which is used entirely in animal feed. Lysine contents of maize protein for QPM reached 3.06 g per 100 g protein, which is significantly higher than that for normal maize (2.43 g per 100 g protein). Maize protein with a high lysine content can be beneficial in diet formulation for animals since it is an appropriate substitute for the expensive protein supplements. In wet-milling process, starch in QPM endosperm may be easily released from protein matrix since it produces a lower-bound yield, namely, 1.1 g per 100 g protein. On the other hand, there is no significant difference between QPM and normal maize in the physico-chemical properties of starch and in the characteristics of maize oil for wet-milling process. In the dry-milling experiments, the total yield of grits and other products from QPM, such as low-fat meal and low-fat flour, are comparable to those from normal maize. But dry milling of QPM may provide products with improved nutritional value.

#### **Genetic Improvement**

The purpose of genetic improvement of QPM is to develop hybrids or cultivars combining o2 gene with the modifying genes, which has not only a higher lysine content but also semihard or hard endosperm. In order to reach this goal, it is very important for breeders to have adaptable germplasm containing modifying genes. The kernel modifications controlled by modifying genes can be divided into two patterns, regular and irregular. In the regular pattern, the hard fraction of endosperm increases progressively from the crown towards the base of the kernels. But in the irregular pattern, the translucent fractions may be distributed among the whole kernel as a band, scattered, resembling a bridge, or vitreous base. Regular modification is more important for genetic improvement of QPM because it is more stable.

Practically, recurrent selection is the most useful breeding method for the development of QPM germplasm. The first stage for QPM improvement is to screen the modifying genes from different resources and develop QPM donor stocks. Extensive studies at CIMMYT indicate that several o2 populations may have a higher frequency of modifying genes, especially in flint varieties from Caribbean Sea and South America. In general, there are two principal ways to develop QPM donor stocks. Intrapopulation selection is used when resources express a considerably higher frequency of modified o2 ears. The other way is to combine partially modified o2 families from different genetic backgrounds into one donor stock. The QPM donor stocks can be used as the basic populations for recurrent selection. A full-sib selection for several cycles is often employed to accumulate modifying genes. First, full-sib families are evaluated for kernel phenotypes among different testing sites. Then, top 10-20 family lines with more vitreous endosperm are chosen and are recombined into the next cycle. Another more efficient but quite complicated approach is backcross-cum-recurrent selection program, since QPM is involved in two genetic systems, the o2 gene and modifying genes. The procedure includes backcrossing o2 gene into modified family lines and accumulating modifying genes within modified family lines. In addition, other traits are also selected in this program. A series of improved QPM populations have been released by CIMMYT.

Recently, the development of QPM hybrid has been given more attention all over the world because hybrids exhibit more advantages than improved populations, such as higher yield performance and more uniform and stable traits. The breeding procedure for QPM hybrids includes three basic steps. The development of elite QPM inbred lines is the vital step. They could be bred with QPM germplasm as in conventional breeding. Then, the combining abilities of QPM inbred lines are measured and superior combinations are selected for adapted planting areas. Finally, QPM hybrids are released into commercial production.

During developing QPM inbred lines, analysis for protein quality is essential because vitreousness in endosperm may have a negative relationship with protein quality. In order to maintain high lysine contents, endosperm analysis is preferred since the modification in OPM only involves changes in endosperm.

#### **Future Prospects**

More recently, molecular marker technique has provided a powerful tool to facilitate breeding practices. In QPM breeding, sequence tagged site (STS) markers for o2 gene could be designed on the basis of its sequence. With this molecular marker, o2/o2 genotypes could be precisely selected at any stage even though hard endosperm QPM is very similar to normal endosperm. Another application for molecular marker technique in QPM breeding is to map modifying genes. Molecular markers will allow the screening of whole maize genome to identify the modifying gene loci. These molecular markers linked with modifying genes could be employed to conduct marker-assisted selection (MAS) for modifying genes. In addition, with the development of functional genome, further investigation will reveal modifying gene function and improve the understanding of the biosynthesis of  $\gamma$ -zeins in QPM endosperm. These processes could offer the potential to improve QPM by genetic engineering.

There is increasing interest in QPM developments in the developing world, where QPM has performed well. The development of QPM hybrids has especially enhanced heterosis utilization, which could raise QPM yield and improve its tolerances to stress significantly. There is no doubt that QPM will make great progress in the near future.

See also: Animal Feed. Cereals: Overview; Protein Chemistry. Genetically Modified Grains and the Consumer. Grain, Morphology of Internal Structure. Maize: Genetics; Breeding; Dry Milling; Wet Milling; Foods from Maize. Nutrition: Beriberi, A Deficiency Related to Grains. Starch: Synthesis.

# **Further Reading**

Boyer CD and Hannah LC (2001) Kernel mutant of corn. In: Hallauer AR (ed.) *Specialty Corns*, pp. 1–32. Boca Raton, FL: CRC Press.

Knabe DA, Sullivan JS, and Burgoon KG (1992) QPM as a swine feed. In: Mertz ET (ed.) Quality Protein Maize, 228p. St. Paul, MN: American Association of Cereal Chemists.

Larkins BA and Lopes MA (1992) A genetic, biochemical, and ultrastructural analysis of modified opaque-2 maize. In: Mertz ET (ed.) Quality Protein Maize, 182p. St. Paul, MN: American Association of Cereal Chemists.

Mertz ET (1992) Quality Protein Maize. St. Paul, MN: American Association of Cereal Chemists.

Neuffer MG, Coe EH, and Wessler SR (1997) *Mutant of Maize*. New York: Cold Spring Harbor Laboratory Press.

Paulis JW, Bietz JA, Felker PC, and Nelsen TC (1992) Evaluating quality protein maize genotypes by reversed-phase high-performance liquid chromatography. In: Mertz ET (ed.) *Quality Protein Maize*, 135p. St. Paul, MN: American Association of Cereal Chemists.

Vasal SK (2001) High quality protein corn. In: Hallauer AR (ed.) *Specialty Corns*, pp. 85–125. Boca Raton, FL: CRC press.

#### **Relevant Websites**

http://www.maizegdb.org. http://www.cimmyt.org.

# **Dry Milling**

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#### Introduction

Dry milling is often used to describe three different processes when talking of maize. The first process, stone grinding, is the oldest and is also known as whole kernel dry milling or full fat dry milling. Whole kernel dry milling does not seek to fractionate the maize kernel but to grind it into uniform size particles, usually flour or meal. The term "stone ground" refers to the use of two stone grind plates between which the maize is ground into a flour or meal. Other devices for particle size reduction can be used to produce this meal or flour, including hammer mills, pin mills, and disk mills. The defining characteristics of this type of dry milling is that nothing is separated in the process and the resultant product is used for human nutrition. The products are usually referred to as "full fat" flour or "full fat" meal because the germ has been left unseparated from the other components. Full fat products have a fuller flavor but a much shorter shelf life than degermed products due to the potential for rancidity of the oil.

The second process refers to the production of ethanol from maize. A hammer mill is used to grind maize to the desired particle size prior to jet cooking, liquefaction, saccharification, and fermentation. Recently, this process is being referred to by the name "Dry Grind Ethanol" in order to prevent confusion.

The third process referred to as maize dry milling is the process we are considering in this article. More accurately referred to as degerminated dry milling, this process attempts to remove the germ and coarse fiber (or pericarp) from the collection of grits, meal, and flour that is left. Degermination improves the shelf life of the endosperm products by removing the bulk of the oil in the maize kernel ( $\sim$ 75%). For information

on the structure and composition of maize kernels, see Maize: Wet Milling.

#### **Process Overview**

Maize, which has been mechanically cleaned to remove broken pieces of maize, weed seed, other grains, and any other adulterant, is tempered. Numerous tempering methods exist, but a single stage tempering would be to first spray 6-8% water on the kernels as they are dropped into a low rpm horizontal screw conveyor or similar type of mixing device. Residence time in this screw conveyor would be 2–10 min. The surface wet maize is then dropped into the top of a tall, small-diameter mass flow bin, which feeds the degerminator (Figure 1) for a total tempering time of 15–40 min.

The degerminator is fed at a constant rate and results in one or two streams containing a mixture of detached germ, pericarp pieces, and endosperm pieces of varying sizes. In degerminators with two streams, the objective is to get the germ and pericarp to go in one stream with small endosperm pieces ("thru stock") and to get large endosperm pieces ("tail stock") to go in the other.

Exploiting the density, size, and aerodynamic differences that exist in the different particles, it is possible to make a separation between pericarp pieces, germ, and endosperm and to separate out different size fractions of endosperm. To accomplish these tasks, a variety of equipment can be utilized including most of the equipments described in Wheat: Dry Milling. Because of the differences in objectives, maize dry millers generally do not find it cost-effective to use equipments other than sieves, aspirators, roller mills, and gravity tables. Sieves, aspirators, and roller mills are almost essential components of a maize dry mill but good germ separation can be achieved without using gravity tables as shown in Figure 1. The figure shows the process flow for a simple, lowcost dry mill using a Beall-type degerminator. The initial and maintenance cost of gravity tables make recovery of the germ using a roller mill and sieve attractive. However, worldwide there are many maize dry mills still using gravity tables to separate out germ.

In Figure 1, the tempered maize is split into two fractions by the degerminator. The first fraction (40-80%) is the "thrus" referring to germ, endosperm, and pericarp fiber that passes through the

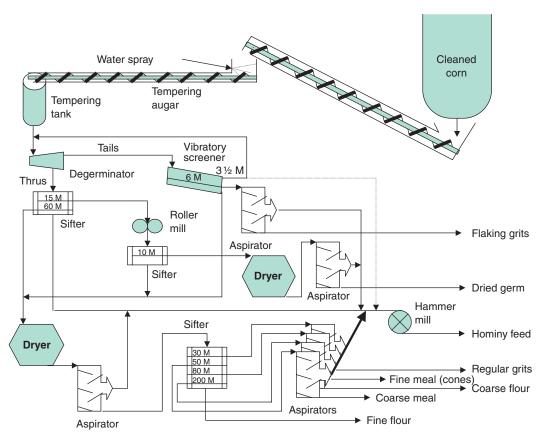


Figure 1 Process flow diagram for simple maize dry mill.

screen on the degerminator. The second fraction, the tails, is usually smaller (20-60%) and is a function of maize quality and genetics. The tails, being predominately flaking grits, some recycle and flour, can be screened with a vibratory screener using a 5.6 mm screen as a scalper to recover oversized grits with attached germ and unbroken kernels. This scalped material can be recycled or disposed into the hominy feed fraction. The vibratory screener also has a 3.55 mm screen for recovery of flaking grits (-5.6 mm + 3.55 mm; this designation means)"through a 5.6 mm screen and on a 3.55 mm screen). The material which goes through the 3.55 mm screen is mixed with the  $-1.25 \text{ mm} + 250 \mu \text{m}$  fraction sieved from the thrus. The flaking grits are then aspirated, with the aspirate going to the hominy feed fraction. A stationary sieve (as shown in Figure 2) can be used in place of the vibratory screener with comparable results. The stationary sieve can often be built locally for lower cost and operates with no energy input and minimal maintenance.

Screening devices come in many configurations and sizes but employ the same basic principle for achieving separation, size difference. Particles smaller than the screen opening can fall through, while those larger than the screen opening remain on the screen. The probability that any piece of maize material will pass through the screen is not just a function of screen opening size but is also affected by the length of exposure the piece of maize has to the screen opening. Three major factors affect the length of exposure: (1) the depth of the material on the screen, (2) the distance

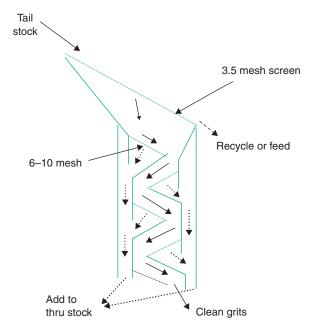


Figure 2 Static sifter.

the particles must transverse (basically the length of the screen), and (3) the amount of angle at which the screen is operated. Most screens used in corn dry milling are metal wire screens with the size openings given in millimeters or micrometers.

Most commercial screens enhance the separation process by using some type of mechanical action. There are a variety of reciprocating actions, that are used to help the material to be removed, work its way to the screen surface. The action also serves to increase the exposure of the maize material to the screen openings, since the particles are agitated or turned to expose different orientations to the screen openings. This increases the likelihood of the particle falling through the screen.

Although it is often said that aspiration will separate light or less dense material, material with a density or weight greater than the maize fraction may actually be removed using aspiration because the aerodynamic characteristics of a particle depends upon shape, texture, and weight or density and not just density or weight alone. There are two primary forces which act upon a particle in an air stream. The downward gravitational force experienced by the particle is due to its total weight (total mass multiplied by the gravitational constant) and not due to its density. The force which counteracts the gravitational forces is the upward drag force. It is made up of two components: form drag and frictional drag. The amount of frictional drag is dependent upon surface texture and total exposed surface area. Form drag, which is dependent upon the projected area of a particle and upon its shape characteristics, dictates the amount of separation that will occur in an aspirator.

The rate at which a particle will move up or down in an air stream depends upon the difference between the drag force and the gravitational force. If the two forces are equal, the particle will remain stationary. If the drag force is greater than the gravitational force, the particle will be carried upwards by the air stream.

Aspirators are designed to force an air stream across the path of flowing mill products, usually at a 90° angle to the flow. The less aerodynamic fiber particles are the first to be removed by aspiration. The small, soft, floury endosperm pieces are usually the next particle group to be removed. Larger/heavier particles can also be removed, if enough aeration is used in the system for them to reach incipit fluidization. Multiple pass aspirators, where the flowing particle stream is exposed sequentially to multiple air streams, are generally preferable to a single air stream aspirator.

The thrus fraction from the degerminator is sieved into three fractions, a +1.25 mm, -1.25 mm + 250  $\mu$ m, and -250  $\mu$ m. The +1.25 mm fraction contains large grits and grits with attached germ or

pericarp and germ. This fraction is passed through a roll stand with a gap setting which breaks the large (-3.55 mm + 2 mm) grits and grits with attached germ or pericarp. The germs, containing ample water, do not break but rather are squeezed or compressed into flat flakes. The germ can now be sieved out of the endosperm material, dried, and aspirated to recover any pericarp and small endosperm material. The aspirate is added to the hominy feed. The germ can be pressed on-sight, recovering ~14-21 kg oil per MT or delivered to solvent extraction facilities where approximate recovery of oil can vary from 21 to 23.6 kg oil per MT.

The bulk of the thru material  $(-1.25 \text{ mm} + 250 \mu\text{m})$ is dried, aspirated, sieved into various fractions, and reaspirated to achieve a variety of low fat endosperm products. If necessary in order to achieve mill balance, larger grits and flaking grits can be reduced in size by roller milling to achieve a more marketable granulation. The  $-250 \, \mu m$  fraction is known as degerminator fines or degerminator flour and is usually added to the hominy feed due to its high fat content.

A roller mill consists of a pair of parallel cylindrical rolls made out of hardened steel, which rotate opposite to each other, rotating so as to pull material into the nip between the two rolls. The rolls generally range in size from 10 to 40 cm (diameter) and up to 1.3 m in length. The roll faces can be corrugated with a variety of cuts or can be smooth face; however, smooth face rolls are generally not used in maize milling. The gap setting between the two rolls is adjustable and the rolls are operated with a slight rpm differential. The larger the particle size being roller milled, generally the fewer the corrugations per cm on the roll face. As the particle size decreases, the corrugations per cm increase.

## **Tempering**

Tempering maize is much different than tempering wheat and other cereal grains milled into flour. The purpose of tempering wheat is to induce a moisture gradient in the kernels that causes the endosperm to stress crack and the bran layer to release from the endosperm. Wheat is often tempered overnight to allow the added moisture to distribute uniformly throughout the kernel and then 10-30 min before processing the wheat, it is tempered an additional 4-6% moisture to enhance germ and bran layer recovery. The moisture added in the overnight temper is beneficial to keep the flour particles from becoming too dry due to pneumatic handling and the heat from the break and sizing rolls. The stress cracking lowers the energy required to produce flour and helps insure that it will be easier to separate from the bran.

In maize dry milling, tempering is done to create differential swelling resulting from the germ and pericarp of the maize absorbing moisture and swelling faster than the endosperm. This swelling loosens the connecting tissue between the pericarp and the aluerone layer of the endosperm and between the germ and the endosperm. In tempering maize, the objective is to not increase the moisture content of the endosperm. Increasing the moisture of the endosperm risks the chance of creating stress cracks, which will lower the yield of large grit material, and means that the moisture absorbed by the endosperm must be removed by drying, thereby requiring more capital and energy. In scientific literature, maize tempering is usually shown as a two- or three-stage process. Industrial practice in the USA for the last 40+ years has been to use a singlestage temper (6-8%, wb; 10-40 min). For very dry maize (<10% moisture, wb) single stage tempering is still used. It is preferable to buy maize at 12–4.5% moisture content than to use such low moisture maize.

Steam or hot water can be used to increase the rate of absorption and decrease temper times, especially in cold weather climates. Steam alone can be difficult to control without overheating the kernel surface and gelatinizing starch. A combination of steam and hot water is recommended in such cases.

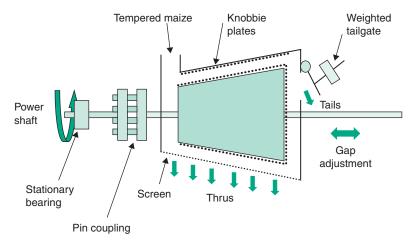
# Types of Degerminators

There are many "brands" of degerminators used around the world with the majority of them being emulations or modifications of a few basic designs. With some exceptions, the patents on most commercially used degerminators have expired and reverse engineering has become an accepted practice in the industry. For example, the patent for the Beall degerminator was granted in 1901 and it is not uncommon to see similar degerminators for sale by competing major manufactures. Parts are sometimes interchangeable although they may not be of the same quality. This reverse engineering keeps prices for degerminators competitive and makes it difficult for new degerminators with slightly improved performance to enter the market. The various degerminators in use can be classified into six categories.

#### Kernel to Kernel Shear (Beall Type)

The Beall degerminator is one of the oldest maize degerminators but is still recognized as the best when the objective is to produce low-fat flaking grits (-5.6 mm + 3.55 mm). The design for the Beall has been widely copied and is one of the most used degerminators worldwide (Figure 3).

The basic Beall design is a truncated cone covered with hemispherical nodules, known as "pearling



**Figure 3** Diagram of a Beall-type degerminator.

knobs" or "knobbies," of  $\sim$ 2.5 cm in diameter, inside an outer truncated cone, half of which is screen surface, and the other half knobby surface. The inner cone is driven counter clockwise and can be moved in and out, relative to the outer cone as a means of controlling gap spacing between the two cones. Tempered maize is fed into the degerminator at the top of the small end of the cone (Figure 3). Spiral ridges on the small one-third of the cone (not shown) moves the kernels away from the opening and helps fill the cavity between the inner cone and the screens. Discharge from the degerminator occurs when the kernel breaks into pieces. The smaller pieces work their way to and then go through the screen, while the large flaking grits and unbroken kernels are shoved out the tailgate. The thrus consist of whole and broken germ pieces, pericarp and smaller endosperm pieces. A 6.4 mm round hole screen is common but different size screens can be used depending upon the size of the maize kernels and the separations desired.

At a steady state, the degerminator is half to twothirds full and the knobbies on the surface of the rotating cone create shearing action between adjacent layers of kernels. It is this kernel-to-kernel shear which breaks open the kernel and releases the germ. Because the knobbies are rounded, there is no significant direct impact forces exerted on the kernels by the degerminator. Once the kernel is broken, the germ and pericarp are released due to continued shearing action. Eventually the broken particles reach the screen surface where all but the largest grits can easily be discharged. A high percentage of the thru material passes through the first two-thirds of the screen surface. If the tempering time was too short or too much water was added, the pericarp can become gummy and clog up the screen. Very quickly the whole degerminator will plug and the drive belts will slip or the direct drive shear pins will break. The degerminator must then be disassembled and cleaned. The last one-third of the screen surface and the tail area (between the largediameter end plate of rotor and tail plate surface) is a polishing area where the large grits are abrading each other, removing loose, soft endosperm material, attached germ and pericarp, and breaking down weak grits. This polishing action can be enhanced by increasing the tailgate weight distance, thereby increasing back-pressure.

Increasing the gap increases residence time and results in increased capacity and cleaner flaking grits. While the manufacturer sells the Beall with half the outer cone as knobbie plates and the other half as screen, many dry millers operate with three-fourth screen and one-fourth knobbie plates. This setup has increased capacity and some reduction in performance.

#### **Impact**

This category includes a variety of horizontal and vertical disk pin type mills. Vertical disks with concentric rings of intermeshing pins with one or both of the disks driven are classically known as pin mills. If both disks are rotating, then they spin in opposite directions. The maize is fed into the center of the disks and pass between the rotating pins, where they are randomly impacted. The number of impacts is generally high and the magnitude and frequency of impact does not give good pericarp and germ separation from the endosperm as much as simply grinding the maize.

One type of pin mill is an Entoleter, which comprises two horizontal disks with two rows of pins on the rotating bottom disk and one row of intermeshing pins on the stationary upper disk. The maize is dropped into the center of the bottom disk (Figure 4), where it is accelerated by centrifugal force toward the rotating pins. The maize must pass between the

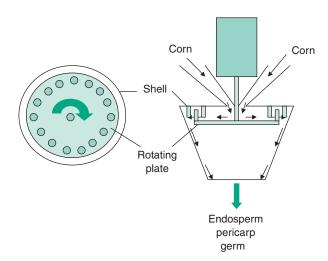


Figure 4 Diagram of the Entoleter degerminator.

three rows of pins before being collected. Because there is a limited number of impacts, the Entoleter recovers more whole germ and produces a larger germ fraction with equivalent oil content than the Beall. However, it produces more mid-size grits (-4 mm + 2 mm) and less flaking grits.

#### Multiple Impact/Shear

This is probably the most common type of degerminators worldwide (Figure 5). Tempered or untempered maize is fed into the annular region between two horizontal or vertical cylinders or near cylinders (with flat surfaces to connect impact attachments). The maize transverses the distance of the cylinder being impacted by the attachments to the inner cylinder. The impacting creates a shearing effect between the kernels as in the Beall degerminator. Small particles pass through the screen and large particles drop over a wire at the end of the cylinder. The attachments vary over a wide range of types, shapes or angles but all provide some degree of impact. Some models separate the flow through the screen into two or more sections and there are a wide variety of outlets. The screens can be made adjustable to increase or decrease the gap and some of the outer cylinder peripherals may be solid with or without rasp bars or other attachments to increase shear. The disadvantages of such machines, compared to the Beall, are that the impacts sustained by the kernels result in a reduction of flaking grits and the machines have reduced flexibility for making adjustments in gap and tailgate backpressure. These machines are acceptable and even preferable when producing prime products other than flaking grits because of higher prime product yields.

## **Compression (Cereal Technologies Inc.)**

A series of patents by Jim Giguere and owned by Cereal Enterprises, Inc. (now Cereal Technologies Inc.) describes mechanisms by which individual kernels are oriented and compressed lengthwise resulting in whole germ separation from the endosperm. The patents describe the mode of action but the actual design of the degerminator is guarded. Several plants in the US have been built using this proprietary technology. The process provides efficient clean germ recovery and high recovery of endosperm, primarily as brewers grit size material or smaller. Good separation has been found using tempered and untempered maize. Process yields are reported to be less sensitive to maize quality or hybrid type than other degerminators.

### **Roller Milling**

A series of roller mills, each followed by sifting and aspiration, can be used to recover a germ fraction from maize. A well-adjusted coarse break mill can crack the kernel, compress the germ so that it pops out and release the pericarp. Subsequent rolls and other mill separation equipment can be used to "clean up" the fractions. The use of roller mills for degermination is common in Africa, where two to three small roller mills with different corrugations can accomplish degermination and all needed particle size reduction.

The advantage of using a roller mill to degerminate maize is the availability and low cost of the roller mill since it is used in all types of dry milling (wheat, oats, etc.). There are several disadvantages of using a roller mill to degerminate. First, the system is not capable of producing large flaking grits. Second, the separation of germ and pericarp is not as clean as with other degerminators. Third, the roller mill has an adjustable fixed gap so the efficiency of degermination is a function of the range or spread of kernel size in the sample being tested. If the mill is adjusted to crack the smallest kernels, the larger kernel's germ is often damaged. If the mill is adjusted to optimally handle large kernels, a lot of smaller kernels will pass through unaffected. A series of break rolls can be utilized to handle the different size kernels or the maize can be presized and the mill adjusted for each size fraction. However, considering the requisite aspirators and sifters, any economic advantage over a dedicated degerminator is quickly lost as the system becomes more complicated.

#### **Products and Uses**

The products produced from dry milling can be simply classified as grits, meal, flour, germ, and hominy feed. The germ fraction ( $\sim 20\%$  oil) is usually pressed to recover oil that commands a premium over

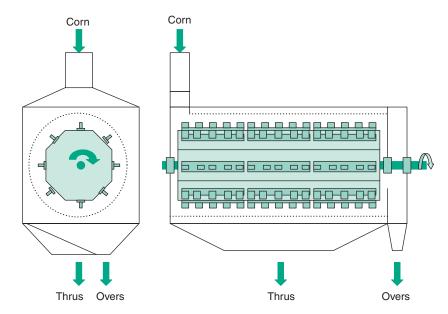


Figure 5 Diagram of a multiple impact/shear-type degerminator.

solvent-extracted, wet-milled oil. Solvent extraction is not practical for most dry mills due to the economy of scale in oil extraction and the high cost of shipping the dry-milled germ to an oil processor. In fact, some smaller mills will forgo pressing and add the germ fraction into the hominy feed. With pressing, oil yield is only 14.2 kg MT $^{-1}$  to 23.1 kg MT $^{-1}$  compared to 28.5 kg MT $^{-1}$  to 30.2 kg MT $^{-1}$  for wet-milled germ. The oil is valued at  $\sim\!5\%$  more than wet-milled oil because of lower refining losses and refining costs.

Hominy feed is the lowest valued product and is used almost exclusively as an animal feed. In practice, hominy feed, the trashcan of the process, comprises pericarp, degerminator fines, cracked maize, and foreign material and fines removed by the cleaners, pressed germ, out of spec product, and any endosperm fraction that cannot be marketed. There can be considerable variability in the fat and protein contents of hominy feed depending upon each component and the quantity added, although uniformity tends to improve as mill size increases.

Within each category of endosperm material (Table 1), there are nearly an infinite number of potential granulations, which can be marketed as distinct products. Table 2 lists granulation and representative fat content of some of the common products from a dry mill. Product specifications are usually more detailed than were shown (Table 2). Table 3 shows product specifications, in terms of granulation, for two different flaking grit products sold by one dry milling company. Also controlled (and typically specified by the customer) are the moisture and fat contents. One dry mill offers 38 different endosperm granulations and probably produces many

**Table 1** General product classification of endosperm material showing granulation

Product	US standar	d sieve size	Particle diameter (µm)			
classification	Less than	More than	Less than	More than		
Grit	3.5	28	5660	638		
Meal	28	75	638	194		
Flour	75	Pan	194	Pan		

Data from Johnson (1991) Corn: production, processing and utilization. In: Lorenz KJ and Kulp K (eds.) *Handbook of Cereal Science and Technology*, pp. 55–132. New York: Marcel Dekker and Brekke OL (1970) Corn dry milling industry. In: Inglett (ed.) *Corn Culture, Processing, Products*, pp. 262–291. Westport, CT: AVI Publishing.

more since most large customers provide their own specifications.

Degermed maize endosperm products are used in many processed foods and are processed directly into a variety of breakfast cereals and snack products. They are used in brewing and other fermentation industries to provide carbohydrates to the microorganisms. Industrial uses include hand cleaners, foundry core binders, explosives, adhesives, charcoal briquette binder, textiles, paper, gypsum board, and insulating material.

# Impact of Maize Quality and Kernel Characteristics

Probably the single largest variable affecting product yield and product quality is the genetics of the maize. There are considerable differences in the dry milling yields between yellow dent and hard endosperm hybrids as shown in **Table 4**. Desirable genetically

Table 2 Granulation for common maize dry milling endosperm products and associated fat content

Product	US standard sieve size		Particle dia	Fat (%, db)	
	Less than	More than	Less than	More than	
Flaking grits	3.5	6	5660	3360	0.6
Large grits	10	14	2000	1410	0.7
Brewers grits	12	30	1680	590	0.8
Regular grits	14	28	1410	638	0.7
Coarse meal	28	50	638	297	1.2
Dusted meal	50	75	297	194	1.0
Cones	40	80	297	177	0.6
Flour	75	325	194	45	2.0

Data from Johnson (1991) Corn: production, processing and utilization. In: Lorenz KJ and Kulp K (eds.) Handbook of Cereal Science and Technology, pp. 55—132. New York: Marcel Dekker; Watson SA and Ramstad PE (eds.) (1987) Corn: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists; and Brekke OL (1970) Corn dry milling industry. In: Inglett (ed.) Corn Culture, Processing, Products, pp. 262—291. Westport, CT: AVI Publishing.

**Table 3** Example of detailed granulation of two different-flaking grits products

Sieve	Range (% of sample)				
	Product 1	Product 2			
On 5.6 mm	0–2	0-5			
On 5 mm	20-55	28-32			
On 4 mm	45-80	68-72			
On 3.55 mm	0-6	0-28			
Thru 3.55 mm	0-1	0-2			

determined or genetically predisposed traits are a predominance of hard endosperm, kernel density  $> 1.30\,\mathrm{g\,ml^{-1}}$ , test weight  $> 0.77\,\mathrm{kg\,l^{-1}}$ , large blocky kernels, zero or only one stress crack per kernel, insect and mold free and free from mycotoxins.

Even though potential for these traits is controlled by genetics, some of the traits can be greatly altered by either environment and/or postharvest practices. Hot dry weather tends to increase protein deposition in the kernels and cool weather tends to favor starch production. Stress cracking will increase dramatically with forced-air drying. Stress cracks are formed when the rate of moisture removal from the surface of the kernel (external mass transfer) exceeds the rate of moisture transport from the kernel interior to the surface (diffusivity). This condition causes large moisture gradients to be established in the kernel. The drier side of this moisture gradient shrinks while the inner core of the kernel endosperm is still wet and the only way that the tremendous stresses created by the shrinking can be relieved is for the endosperm to crack. Field drydown or moderate to low drying rates can cause some single stress cracks to be formed, while higher drying rates create multiple nonintersecting cracks or checked (multiple intersecting stress cracks) endosperm. Stress cracks (more than one per kernel) reduces the yield of flaking grits and increases flour

yield. High drying temperatures (>70°C) can cause the starch to partially gelatinize, resulting in changes in the functional properties of the flour or other endosperm pieces.

There are other quality factors that are primarily influenced by environment. The production of mycotoxins in the field depends upon microbial load and weather conditions. It is desirable to have minimal mycotoxin levels in maize for dry milling since the maize components are often used directly in human food. Mold or insect damage is also primarily influenced by weather or other environmental conditions. Preharvest mold or insect infestation primarily affects kernel development leading to more small kernels as well as yield loss. Pericarp damage by microorganisms or insects can effect the distribution of water during tempering, resulting in germ and pericarp separation problems.

Meeting the criteria for quality described above is no guarantee that the maize will perform in the mill as desired or that the fractions will have the traits desired by the end users. Most mills purchase maize by measuring some combination of the traits listed above, although some mills have prescreened hybrids and offer an "approved hybrid list" to maize producers as a means of minimizing genetic variability. The mills either pay a direct premium to the producer or pay a hidden premium by setting their maize purchase price based upon the mills' need for maize. The result is that their purchase price for maize varies from 100% to 200% local price for US #1 Yellow, with an average of 120% local price. In either case, extensive laboratory testing of each load of maize is performed prior to acceptance.

Functional quality of prime products can also vary based on the mill streams combined to make the salable product. Functional quality is defined as the thermal, rheological, and organoleptic characteristics of the product. For example, coarse meal can be

**Table 4** Dry milling yields of nine commercial hard endosperm corn hybrids and two yellow dent samples using a Beall No. "0" Degerminator

Fraction	Hybrid										
	H1	H2	НЗ	H4	H5	H6	H7	H8	H9	Y1	Y2
+5.6 mm	0	0	0	0	0	9	5	3	0	3	na
-5.66  mm + 4  mm	29	26	17	33	39	47	49	52	38	18	6
$-4\mathrm{mm}+2\mathrm{mm}$	27	26	36	33	23	15	16	17	20	38	10
-2+520 micron	5	9	12	4	5	5	3	3	4	11	19
-520 micron $+$ Pan	16	14	15	12	12	13	13	11	17	10	23
Total endosperm	77	75	80	82	79	80	81	83	79	77	58
Pericarp	9	9	7	7	8	6	7	6	8	9	14
Germ	13	15	12	10	11	6	7	8	13	12	28
Oil in flaking grits	0.5%	0.3%	0.4%	0.4%	0.5%	0.3%	0.3%	0.3%	0.3%	na	na

na = Not available

Data from Mehra SK (1996) Factors Influencing Beall Degermination of Corn for Dry Milling. PhD thesis, University of Illinois, Urbana, IL, 317 pp.

produced using a Beall degerminator from four different streams or combinations there of: (1) sifted directly from the tail fraction, (2) sifted from the thru fraction, (3) produced from flaking grits or other grits in the tail fraction, and (4) produced from larger grits sifted from the thru fraction. Each of these methods of producing coarse meal potentially can meet the desired product granulation, fat, and moisture specifications. However, the functional characteristics of the meal is likely to be different for each method of production. Even when the hybrid variability is controlled, variability can exist due to selection of process streams and related "mill balance" issues.

#### **Future Trends**

- 1. Fastest growth in developing countries. The dry milling industry is a slow growing industry in the US, with a growth rate of ~2% per year and is most likely related to population growth. It is a mature industry in most industrialized countries and potential for growth beyond this level appears dim. Growth potential appears to be in less developed countries where maize products can be inexpensive ingredients for use in an expanding processed food industry. Dry-milled maize products can also be used to make ready-to-eat cereals and nutritious snacks.
- 2. Aging infrastructure in developed countries will lead to paradigm shift. A major proportion of the US degerminating dry mill capacity is over 50 years old. The basic infrastructure is expensive to maintain or restore. It may be time for a paradigm shift in the industry away from large centralized plants to smaller plants collocated by major end users. Collocation allows for sharing of

- utilities and infrastructure, minimizes bagged transportation of products, and maximizes technical communication between plants. The milling process can be designed to maximize the production of desired prime products and minimize capital costs.
- 3. Hybrid(s) specific processing will become the norm. The pressure to make traceable quality products for the food industry will continue to mount as consumers become more concerned about food security and food safety. Hybrid specific processing is one way to insure that no undesirable maize enters the processing system. Hybrid specific processing limits the acceptable maize hybrids and identity preserves them through the market channel. The millers can contract directly with the producers or work through various specialty maize merchandizers. This procedure is gaining in popularity and importance.

See also: Lupin: Agronomy. Maize: Breeding; Quality Protein Maize; Wet Milling; Foods from Maize. Wheat: Dry Milling.

#### **Further Reading**

Brekke OL (1970) Corn dry milling industry. In: Inglett (ed.) Corn Culture, Processing, Products, pp. 262–291. Westport, CT: AVI Publishing.

Duensing WJ, Roskens AB, and Alexander RJ (2004) Corn dry milling: processes, products and applications. In: White and Johnson (eds.) Corn: Chemistry and Technology, 2nd edn. Eagan, MN: AACC Press.

Eckhoff SR, Paulsen MR, and Yang Ping (2003) Maize. In: Caballero, Trugo, and Fingla (eds.) *Encyclopedia of Food Sciences and Nutrition*, vol. 4, pp. 3647–3653. London: Elsevier Science.

Giguere JR (1980) Method of degerminating a kernel of grain by simultaneously compressing the edges of the kernel. US Patent 4,189,503.

Giguere JR (1981) Method and apparatus for degerminating a grain kernel by impelling the kernels along a guide vane into an impact surface. US Patent 4,301,183.

Giguere JR (1982) Apparatus for degerminating a kernel by compressing the edges of the kernel. US Patent 4,365,546.

Giguere JR (1993) Grain milling and degermination process. US Patent 5,250,313.

Johnson LA (1991) Corn: production, processing and utilization. In: Lorenz KJ and Kulp K (eds.) Handbook of Cereal Science and Technology, pp. 55-132. New York: Marcel Dekker.

Johnson LA (1999) Corn: production, processing and utilization. In: Kulp and Ponte (eds.) Handbook of Cereal Science and Technology, 2nd edn., pp. 31-80. New York: Marcel Dekker.

Mehra SK (1996) Factors Influencing Beall Degermination of Corn for Dry Milling. PhD thesis, University of Illinois, Urbana, IL, 317pp.

Watson SA and Ramstad PE (eds.) (1987) Corn: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

#### **Relevant Websites**

http://www.grains.org - US Grains Council website with information on the various types of value enhanced corn, their growing location and the market channel contacts.

http://www.namamillers.org - North American Millers' Association site with information on the products produced by corn dry milling.

# Wet Milling

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#### Introduction

Worldwide production of maize (referred to as corn in the US) was nearly 600 million tons (Mt) in 2002 of which the United States produced ~40%. Maize is a starch crop, providing needed energy in animal diets and being converted into starch and co-products using the maize wet milling process. It is a near-perfect starch crop: it is readily transportable, easily dried and yields over 66% starch on a dry basis. Over 85% of the

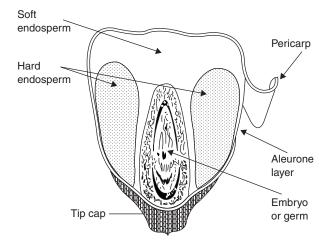


Figure 1 The mature corn kernel, showing component parts. (© Eckhoff SR, Savoy, IL.)

starch produced is derived from maize all over the world.

Wet milling is a complex process with a wide variety of unit operations and interdependence between process steps via recycling of process water. An understanding of wet milling begins with the maize kernel and the steeping process.

#### The Maize Kernel

The structure and composition of the maize kernel is covered in detail in Grain, Morphology of Internal Structure and so only a pragmatic illustration of the structure and composition of the maize kernel will be given here. The maize kernel is composed of four main parts: the pericarp, the tip cap, the endosperm, and the germ (Figure 1). The pericarp is the outer covering of the kernel and is composed of cellulose, hemicellulose, lignin, and various waxes, all unappetizing components to insects and microorganisms. The pericarp is essentially a plastic grocery bag. The opening in the top of the bag is analogous to the tip cap, the connecting tissue between the kernel and the cob, which is the only natural opening into the kernel. Pericarp consists of dense layers of cells near the surface and wideopen transport cells located next to the aluerone layer. The aluerone layer is the outer layer of the endosperm consisting of large, dense, highly proteinaceous cells, which acts as a semi-permeable membrane, restricting the flow of large molecules in or out of the endosperm. The purpose of the pericarp is exactly the same as the plastic bag: to protect the food material it contains.

The second component of the maize kernel is the germ or embryo, the living part of the maize kernel. It contains all the enzymes and building blocks needed

to quickly begin developing roots and shoots during germination. The germ contains a high percentage of the kernel's oil, water-soluble protein, water-soluble carbohydrate, ash, vitamins, and minerals. However, wet milling processors are primarily concerned with the fact that the germ is high in oil. In the analogy, visualize a sponge saturated with oil. Now put this oil-laden sponge into the bag.

The rest of the maize kernel is endosperm. Endosperm cells are storage cells containing starch granules encased in a protein matrix. The grocery bag is taken with the oily sponge and filled with marbles. A bottle of glue is opened, and the glue poured into the bag, filling the bag, and encapsulating the marbles. The glue sticks the marbles to the bag, the bag to the oily sponge, and the oily sponge to the marbles and glue mixture. It is assumed some of the glue trapped a lot of little air bubbles when it was being poured into the bag and the resulting glue matrix is thin and weak. This is the soft endosperm. Hard endosperm is where the glue matrix is thick and dense. When the glue is dry and a processor's mental picture of the maize kernel is obtained; a plastic bag containing an oily sponge filled with marbles encased in a glue matrix. The purpose of wet milling is to partially dissolve the glue to allow for mechanical separation of the water swollen plastic bag, the swollen oily sponge, the marbles, and the glue. Completed milling yields a pile of nearly empty bags (white fiber), oily sponges (germ), nearly pure marbles (starch granules), and partially degraded glue (maize gluten meal).

#### The Maize Wet Milling Process

Maize wet milling can be divided into five sections: steeping, germ recovery, fiber recovery, protein recovery, and starch washing (Figure 2). Each of these sections has unique equipment and objectives but all sections are interconnected by the flow of process water.

#### **Steeping**

Steeping is the heart of maize wet milling and will be discussed in more detail than the other sections. When steeping is done properly it may be possible to get a good recovery of starch, if downstream processing is performed well. If the maize is not steeped properly, no amount of downstream processing can correct for the poor steeping. Steeping is a process unto itself; a sequence of chemical and biochemical reactions, induced mechanical stresses, leaching, and kernel hydration.

Maize, which has been screened to remove broken pieces of kernels and foreign material is dropped into

an insulated hopper bottomed tank, cushioned on the bottom with a layer of water. The broken maize and foreign material are removed because they can plug the screen at the bottom of the tank, which interferes with the circulation of steepwater. The broken maize and foreign material is usually added into the gluten feed product, but may be sold separately to local livestock feeders. A battery of such tanks, interconnected to route steepwater through the tanks in a specific sequence, can vary from as few as 6 to over 50 tanks with sizes from 12.7 Mt to over 633 Mt. The tanks are equipped with a pump that runs continuously, either transferring water or recirculating the water back to the same tank. Recirculation serves the purpose of increasing temperature uniformity. The tanks are also equipped with heaters to maintain a uniform steep temperature of 52°C. Optimal temperature for lactic acid production is 52°C. If the steep temperature drops below ~47°C, yeast begins to propagate and produce alcohol. If the steep temperature gets above 56°C, acetic acid bacteria will begin to dominate the fermentation.

The steeping process takes anywhere from 20 to 48 h to complete. The difference in steep time depends upon the objectives of the wet miller, product mix, and the amount of horsepower used in the mill house. The average steep time in the US is  $\sim 30$  h, while the average steep time in Japan is over 42 h due to higher relative value of starch compared to the co-products and use of less hp per Mt of maize.

In the vast majority of maize wet milling plants worldwide, maize is steeped counter-currently using a pull system. Dry maize is exposed to the oldest steepwater and the longest steeped maize is exposed to the newest steepwater, process water to which sulfur dioxide has been added (Figure 3). As the steep water goes from the oldest maize to the newest maize in the steeps, the sulfur dioxide level drops significantly, from a high of 1500 ppm to 3000 ppm down to 30 ppm to 300 ppm. When the sulfur dioxide level drops to less than  $\sim$ 300 ppm, the ubiquitous lactic acid bacteria (carried in by the new maize) begin to propagate. This results in the fermentation of glucose to lactic acid at levels ranging from 1% to 3%. Because the lactic acid is high on one end of the process and the sulfur dioxide is high on the other end of the process, the pH changes little during the process and industrially is usually in the range of 3.5-4.2.

Industrial steeping sequences the water flow in order to progress the tanks through the process in a countercurrent manner. Water is pulled from the tank containing the newest maize, the rate determined by the steepwater evaporator capacity. When the steepwater evaporator feed tank is low, sensors open a valve to shunt steepwater from the tank with

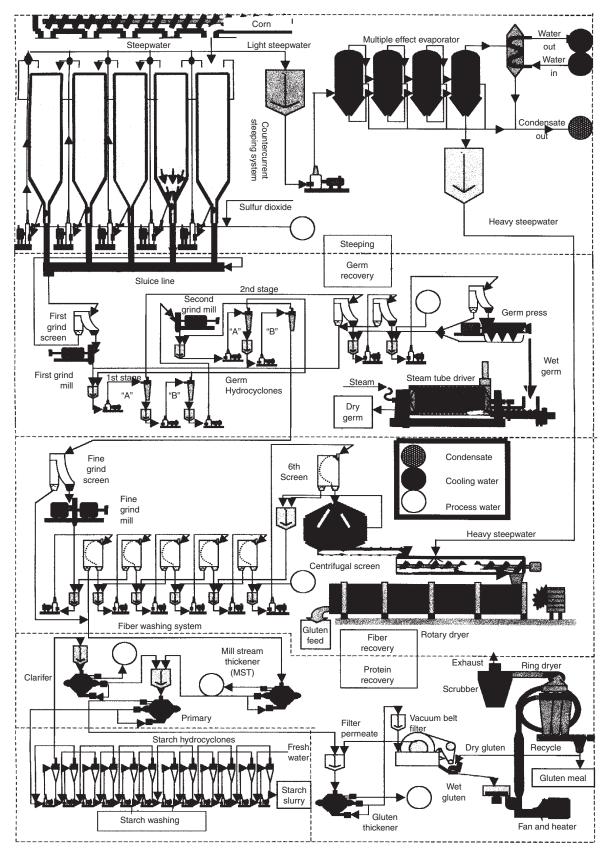


Figure 2 The wet milling process. (© SR Eckhoff, Savoy, IL.)

the oldest steepwater. When the lower limit switch is activated in the oldest steepwater tank, water is shunted from the tank with the second oldest steepwater. This transfer of water continues from tank to tank until the tank with the newest steepwater needs water. This tank is filled with fresh steepwater. When the steepwater evaporator feed tank is full, the tanks in time return to internal recycling of the steepwater.

Eventually the tank with the oldest maize needs to be drained and made ready for milling. Figure 4 illustrates the sequencing required as one tank of steeped maize is drained and the maize sent to the grind mill and the recently filled tank of dry maize is brought into the process. This pattern is continually repeated 24 h

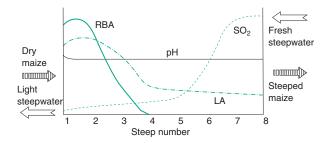


Figure 3 Profile for continuous counter-current steeping. (© SR Eckhoff, Savoy, IL.)

a day, 7 days a week. The figure shows the tanks in a circle for simplicity but usually the tanks are in rows.

The sulfur dioxide diffuses into the maize kernel during steeping and disrupts the endosperm protein matrix (the glue) by breaking inter- and intra-protein disulfide bonds. The sulfite and lactic acid, resulting from the fermentation, also lowers pH to where endogenous proteases can help solubilize part of the protein matrix.

The steeping process can be broken down into three stages: the lactic acid dominated stage, the sulfur dioxide diffusion stage, and the sulfur dioxide dominated stage (Figures 5 and 6). These three stages are each approximately one-third of the steep time in a properly operated system.

Lactic acid-dominated stage In the lactic aciddominated stage the key things which occur are: (1) rapid hydration of the kernel in the presence of 1-3% lactic acid to near equilibrium conditions (42-52%, wb), (2) leaching of soluble material from the germ into the steep water causing a concentrating of the oil from 35% to  $\sim$ 50%, (3) fermentation of the soluble carbohydrates coming from the germ and from recycled process water to produce lactic acid, and

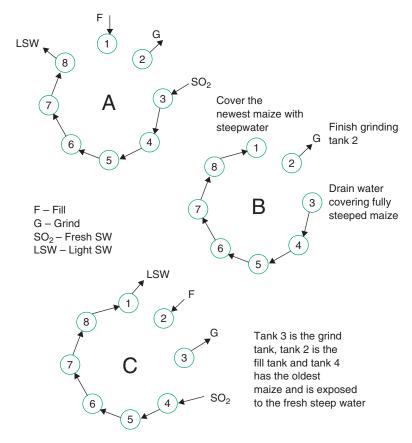


Figure 4 Sequencing of events required to change grind from one tank to another. (© SR Eckhoff, Savoy, IL.)

(4) creation of differential swelling stresses resulting from water uptake which loosens the connecting tissue between the component parts. The lactic acid-dominated stage starts with the introduction of the dry maize into the steep solution and ends when the sulfur dioxide level is high enough to inhibit fermentation.

Sulfur dioxide diffusion stage The sulfur dioxide diffusion stage is called so because the sulfur dioxide that diffuses into the maize kernel during this time is the sulfur dioxide that does the work of disrupting the protein matrix. This second stage is critical to steeping in that it stops the fermentation before producing too much lactic acid (LA levels greater than 1.5% can increase steepwater evaporator fouling) and indicates a point where the sulfur dioxide has a high enough flux to begin making significant inroads into penetrating the kernel. During the lactic acid-dominated stage the sulfur dioxide flux is too low to penetrate very far into the kernel because of the simultaneous reaction and

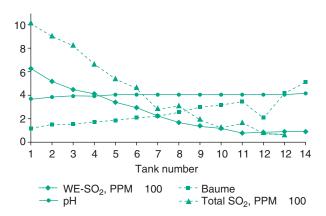
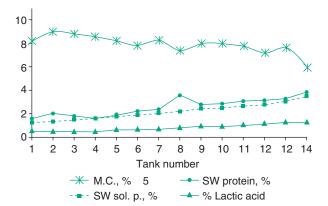


Figure 5 Industrial steep profiles showing pH, Baume, and two measures of sulfur dioxide level. (© SR Eckhoff, Savoy, IL.)



**Figure 6** Industrial steep profiles showing moisture content, steep water protein, steep water soluble protein, and lactic acid. (© SR Eckhoff, Savoy, IL.)

diffusion that occurs. The sulfur dioxide is a highly reactive chemical and in the kernel there are many opportunities to undergo oxidation, acid—base reactions, or become absorbed on the surface of the interstitial pores. Sulfur dioxide that enters during the third stage, the sulfur dioxide-dominated stage, essentially retraces the path through the maize kernel that has already been exposed to sulfur dioxide. During the second stage, there is also continued leaching of this from the germ and the sulfur dioxide begins to react with the protein matrix creating soluble protein, which diffuses from the endosperm into the steepwater. During this time equilibrium moisture levels are reached.

In the third stage of steeping, the sulfite absorbed during the second stage reacts with the protein matrix in the hard endosperm section of maize (the reactions take less than 4 h), so the rate-limiting part of steeping is the diffusion of sulfur dioxide into the kernel. The high level of sulfur dioxide that the maize is exposed to during this stage diffuses into the outer sections of the kernel and is carried downstream into the process to provide microbial control. The high level is also necessary to insure sufficient flux at the start of the second stage. Leaching continues to pull out more of the solubles created by steeping.

After the steep tank is drained, the swollen maize is discharged out of the bottom of the tank into a fast-moving stream of water (sluice water), which carries the kernels to the first grind mill or degerminating mill. The sluice water is recovered by screen and recirculated.

#### **Germ Recovery**

The first objective after steeping is to recover the swollen germ. If we visualize the germ as an oily sponge, it is clear that we want to remove the oily sponge from the system as quickly as possible to minimize germ damage, maximize oil recovery, and prevent the oil from getting all over the process equipment and gumming up operations. Mechanical sheer applied properly can separate the germ from the other components with little damage to the germ itself. To accomplish this, the industry uses 61 or 91 in diameter disk mills (224 kW) with specially designed intermeshing teeth (Figure 7) set at a gap setting which allows one or two kernels per handful not to be torn open. This objective operational parameter allows for the vast majority of the kernels to be broken open with minimal damage to the germ. A gap setting that gives 100% germ release would create excessive germ damage.

The milled maize slurry is then pumped through a two-stage hydrocyclone system to recover  $\sim 85\%$  of the germ. These germ hydrocyclones are

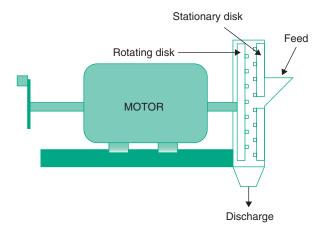


Figure 7 Single disk mill used in germ recovery. (© SR Eckhoff, Savoy, IL.)

15.25-22.86 cm diameter and 0.6-1.1 m tall. Operating pressures are generally 275–310 kPa with the specific density of the slurry adjusted to between 8 and 9 Baume. At these conditions the free germ (being 42–52% oil) floats and will be forced to the center of the hydrocyclone, away from the wall, where it goes out the vortex finder (Figure 8). The heavier material is forced to the wall of the hydrocyclone by centrifugal force and works its way by gravity to the bottom discharge. In the two-stage hydrocyclone system, stage 1 hydrocyclones are "A" type cyclones designed for a qualitative split between the germ and remaining slurry. Stage 2 hydrocyclones are designed for quantitative recovery of germ, with larger diameter feed and vortex finder connections and are designated "B" type hydrocyclones. Because of flow and capacity differences, there are generally two "A" cyclones for every "B" cyclone. The overflow of the "A" cyclone (light material i.e., germ) and the underflow of the "B" cyclone can be regulated by a flow control valve to achieve the desired split. The overflow of the "B" hydrocyclone is sent back to the feed tank of the "A" hydrocyclone (see Figure 2).

The slurry going out of the bottom of the "B" hydrocyclone is sent to a second single disk mill (known as the second grind mill), with the intermeshing plates set just close enough to make sure that all remaining kernels are properly ground. The reactions proceed at a relatively rapid rate (only minute kernels remain). The resulting slurry is pumped through a second twostage hydrocyclone system. Just as before, the second hydrocyclone overflow is sent back to the feed tank of the first hydrocyclone. The overflow of the second grind, first stage hydrocyclone is sent back to the feed tank of the first grind, first stage hydrocyclone. With this setup, the germ leaves the hydrocyclone system only from the first grind, first stage hydrocyclone,

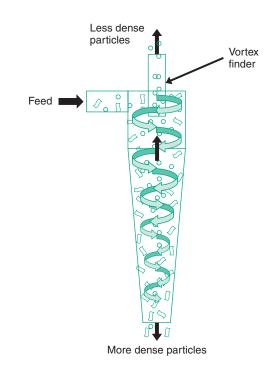


Figure 8 Operation of a germ hydrocyclone. (© SR Eckhoff, Savoy, IL.)

where it is sent to germ washing. Operation of the germ recovery system is a series of compromises. Attempts to increase the purity of the germ by restricting first grind "A" cyclone overflow increases the recycle and can result in plugged cyclones or a decreased grind. Approximate flow rates out of the overflow is 20% the feed rate for "A" cyclones, 30% feed rate for first grind "B" cyclones, and 30-45% feed rate for second grind "B" cyclones.

Most wet milling plants operate with this two grind, two-stage hydrocyclone system, which essentially gives a minimum of four chances to recover the germ. In-plant experimental data shows that  $\sim 85\%$ of the germ is recovered by the first grind hydrocyclones and the remainder by the second grind hydrocyclones. To reduce capital costs, some plants have only a single stage hydrocyclone system after the second grind. Unrecovered germ will primarily be recovered with the fiber or gluten meal. Quantitative recovery of the germ is an economic decision based on the relative values of maize oil and gluten feed and a quality decision based on the sensitivity of the plant's starch product mix.

The germ fraction is counter currently washed to remove residual starch usually in a three-stage process. The washed germ is the water in a germ press and then dried to less than 3% moisture content for shipment to a solvent extraction plant, unless the wet mill has its own extraction facility. The germ must be this dry to prevent oxidation of the oil during transport. The

germ fraction contains 42-55% oil depending on a number of factors including the amount of residual pericarp that floats with the germ, steep time, and maize hybrid or variety. Rotary steam tube dryers are commonly used to dry germ because the exposure time for any individual germ to be in contact with a steam tube is minimal. More recently, fluidized bed dryers are being used because they give more uniform moisture content although it is generally a less efficient drying method. Drier types which expose the germ to temperatures > 80°C for extended periods of time should be avoided because the oil will be burnt and extraction efficiencies and oil quality decreased.

## **Fiber Recovery**

The underflow of the second grind, "B" hydrocyclone is sprayed on a 50 μm 120° wedge bar screen (Figure 9) to dewater the mash and to allow starch and protein which has already been released from the fiber to go directly to the mill stream thickener (MST) in the protein recovery section of the process. Material retained by the screen is passed through a double disk refiner mill to release remaining starch from the weakened protein matrix. The double disk mill is similar to the single disk mill used for degermination except that the disks rotate in opposite directions with each disk driven by a separate large electric motor. This arrangement maximizes the sheer that is experienced by the particles. Most double disk refiners used in the US have 91 cm disks operating at 1800 rpm and are equipped with 187 kW electric motors on each disk. Recently some companies in place of the double disk mill have used a 132 cm diameter single disk mill, with 746 kW motor. When operated at 1250 rpm this large single disk mill has disk tip differential velocity similar to the double-disk refiner.

The finely ground slurry is now pumped under pressure onto a six-stage fiber-washing system. The pressure fed screens come in several configurations but most commonly used is the 120° screen (Figure 9). The finely ground fiber-starch-protein slurry is sprayed across the top of the screen at a pressure of  $\sim$ 275 kPa. The pressure and force of gravity causes the fiber to orient parallel to the flow where the wedge bar screen scrapes off residual starch and protein that is still attached to the fiber. The 120° arc of the screen insures contact between the fiber and the wedge bars. At the top of the screen, the pressure accelerates the fiber, pushing it along the surface of the screen. When the screen becomes more vertical near the center, the fiber has decelerated and the principle force on the fiber is gravity. By the time the fiber is on the lower portion of the screen, it has been dewatered

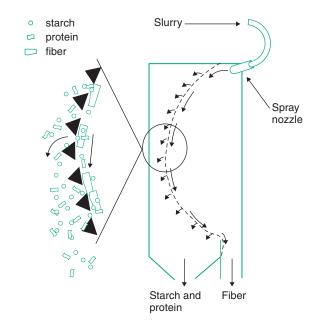


Figure 9 Pressure fed screen with exploded view of the wedge bars. (© SR Eckhoff, Savoy, IL.)

significantly and the fiber bunches up to allow for some more dewatering before it is forced off the screen. Moisture contents are 80–90%, wb.

The first stage screen is a 50 µm screen and is known as the fiber block. The other five screens are 75 µm screens. The theory is that fiber sized between 50 μm and 75 μm agglomerates with other fiber during the initial dewatering and is carried out to the feed house. The 75 µm screens are used because they have a 50% larger open area and less screen area is needed to achieve adequate dewatering in stages 2-6. There are some who consider this arrangement of screens to be a fine fiber generator because the 50-75 µm particles will recirculate between the 50 µm screen and the 75 µm screens until the particle is reduced in size enough to pass the 50 µm screen.

The fiber stream coming off the stage 6 screen is further dewatered using either a screening centrifuge, screw press or both in series. Screening centrifuges can decrease moisture to 65-75%, wb, while a screw press can decrease fiber moisture up to an additional 10 percentage points. This white fiber is blended with already dried fiber and heavy steepwater to form a mixture containing 35-40% moisture, wb, which can be dried to 10–12%, wb, using either a direct fired rotary dryer or steam maize tube dryer. Spent germ (germ meal left after solvent extraction of the oil), broken maize, or spent filter media from syrup production is added dry to the fiber when available. Adding germ meal or filter media to the wet fiber before drying results in excessive dryer smoke. The final product is known as gluten feed.

#### **Protein Recovery**

Protein separation depends upon the principle of density difference between the starch and protein. The density of the maize kernel components left at this point is highest for starch and lowest for any cell wall or fiber which passes through the pressure fed screens. (Table 1). The density difference between

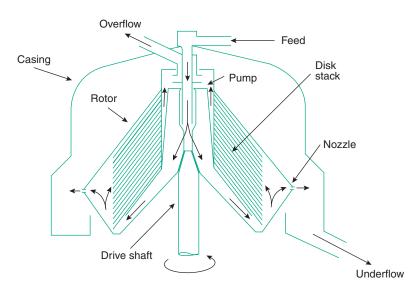
Table 1 Density differences in components found in starch slurries

Component	Density (g ml <sup>-1</sup> )
Sand, dirt	>2
Starch	1.55
Protein	1.10
Cell walls, fine fiber	1.05
Water	1.00

Sources: (1) Blanchard PH (1992) Technology of Corn Wet Milling. Amsterdam, The Netherlands: Elsevier Science. (2) International Starch Institute (2003) TM 18-2www - ISI Technical Memorandum on Production of Corn Starch, http://www.starch.dk/isi/starch/tm18www-corn.htm. Science Park Aarhus, Denmark. (3) US Grains Council (1996) 1995-1996 Value-Enhanced Grains Quality Report. US Grains Council, Washington DC, USA. (4) US Grains Council (1997) 1996-1997 Value-Enhanced Grains Quality Report. US Grains Council, Washington DC, USA. (5) US Grains Council (1998) 1997-1998 Value-Enhanced Grains Quality Report. US Grains Council, Washington DC, USA. (6) US Grains Council (1999) 1998-1999 Value-Enhanced Grains Quality Report. US Grains Council, Washington DC, USA. (7) US Grains Council (2000) 1999-2000 Value-Enhanced Grains Quality Report. US Grains Council, Washington DC, USA. (8) US Grains Council (2001) 2000-2001 Value-Enhanced Grains Quality Report. US Grains Council, Washington DC, USA. (9) Blanchard PH (1992) Technology of Corn Wet Milling, p. 73. Amsterdam, The Netherlands: Elsevier. (10) Radley JA (ed.) (1976) Starch Production Technology, p. 123. London: Applied Science. (11) May JB (1987) Wet milling. In: Watson SA and Ramstad PE (eds.) Corn: Chemistry and Technology, p. 390. St. Paul, Minnesota: American Association of Cereal Chemists. (12) Johnson LA (1991) Corn: production, processing and utilization, In: Lorenz KJ and Kulp K (eds.) Handbook of Cereal Science and Technology, p. 58. New York: Marcel Dekker. © SR Eckhoff, Savoy, IL.

good starch and protein is sufficient to be able to make a good separation. Seventy years ago, all maize starch, as well as other types of starch was purified by pouring the starch-protein mixture onto tables that were 30-100 m long, about 1 m wide and sloped  $\sim 1.04$  cm m<sup>-1</sup>. The starch would settle on the table, while the protein mixture was carried along by the water and ultimately off the end of the table. This technology is still used today in laboratory milling (with much smaller tables) because the starch purity recovered from the starch table is nearly as good as from a wet milling plant. The large amount of space needed for the tables eventually dictated that new technology be developed. The development of the disk-nozzle centrifuge systems for starch-protein separation greatly reduced space requirements and allowed wet millers to grow in size.

The principal piece of equipment in any wet milling centrifuge system is what is known as the primary centrifuge. A disk-nozzle primary centrifuge is a continuous centrifuge where a light fraction is separated from a heavier fraction as it is being forced to flow in between rotating disks in a disk stack, with the lighter phase going up between the disks and being discharged as overflow. The heavier phase flows back down the disks and is ultimately forced out of the centrifuge through nozzles located at the periphery (Figure 10). The mill starch is pumped into the centrifuge, at a constant rate, to a spot near the underside of the disk stack. Approximately 1/4 of the way up the disk there are a series of holes, 2.5-5 cm diameter, located every  $\sim 30^{\circ}$  radially. These holes are lined up in the disk stack to provide an entry point for the feed material. As mill starch enters the space



**Figure 10** Disk nozzle centrifuge. (Reproduced with permission from Blanchard PH (1992) *Technology of Corn Wet Milling*, p. 393. Amsterdam, The Netherlands: Elsevier Science).

between the disks, the starch, protein, and fiber particles in the slurry are subjected to the centrifugal force caused by the rotating action of the disk stack, the force of gravity and the hydraulic force resulting from pumping (Figure 11). The acceleration of gravity can be neglected at high centrifugal forces. The separating ability of the disk-nozzle centrifuge depends on the relative acceleration of the particle due to centrifugal forces and due to the hydraulic force. For a given particle, the hydraulic force depends upon its position in the flow field. The velocity of the fluid is greatest at the center between the two disks and drops off to zero at the surface of each disk. The force experienced by the particles due to centrifugal action depends upon the mass of the particle, the angular velocity ( $\omega$ ) of the centrifuge, and the settling rate of the particle based on Stokes law.

Figure 11 illustrates the paths taken by light and dense particles. The dense particles are accelerated toward the top surface of the disk by the centrifugal force that is larger than the hydraulic forces even at the center of the channel. As they approach the upper surface of the channel the hydraulic forces continue to decrease due to the decreasing velocity near the top surface of the channel. The centrifugal forces dominate and pull the dense particles toward the outer edge of the disk stack channel. Eventually the dense particles are forced toward the nozzle where it is discharged from the centrifuge. The lighter particles do not have as large a centrifugal force acting on them but the hydraulic force is just as large as for the dense particles. As the lighter particles move toward the center of the channel, the hydraulic forces dominate and the lighter particles are pulled up toward the discharge. Figure 11

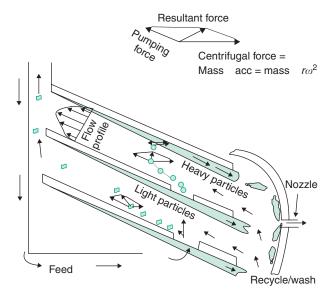


Figure 11 Illustration of the movement of particles between disks in a disk nozzle centrifuge. (© SR Eckhoff, Savoy, IL.)

shows lighter particles and denser particles in different stack channels for illustrative purposes but in actuality their movement is occurring simultaneously in all channels of the disk stack. The primary centrifuge's disk stack is designed to maximize separation between starch and protein at a specified feed rate. Disk stack diameters range from 23 cm pilot plant size to 91 cm commercial size. A 91 cm diameter primary centrifuge can process over 1000 Mt day<sup>-1</sup> when driven with a 150 kW motor.

If the disk stack configuration and/or pump volume is changed to lower the hydraulic forces relative to the centrifugal forces, the centrifuge can be used to recover all nonsoluble solids. It essentially becomes a dewatering centrifuge. Disk-nozzle centrifuge systems for separating starch and protein can have 2–4 centrifuges. One is the primary while the other 1-3 centrifuges are dewatering centrifuges. The most common centrifuge system used in wet milling is the high-density four-centrifuge system shown in Figure 1. To increase the capacity of the primary centrifuge, MST is used to remove water from the starchprotein slurry coming from the fiber recovery system. The density is increased from  $\sim$ 8 to  $\sim$ 12 Baume. The overflow from the MST is used as the process water going into the steeping system. The overflow from the primary centrifuge is high in protein, nearly 70%, db, but is only 1.5-3.0% solids. Prior to dewatering to 40% solids using a vacuum belt filter, the protein-rich stream is dewatered using a centrifuge known as the gluten thickener, where the gluten stream is dewatered up to 16.5% solids. The overflow from the gluten thickener is used as the process water for germ and fiber washing. The protein cake from the vacuum-belt filter is mixed with dry gluten meal to reduced tackiness and increase the handling characteristics of the wet gluten. It is then dried to ~11% moisture using a ring dryer. The protein content of the resulting gluten meal is established at the primary centrifuge.

The underflow of the primary centrifuge still contains 1.5-3% protein and is sent to the starch washing system, where the starch is washed repeatedly to flushback more of the remaining protein and any fine fiber particles. The overflow of the starch washing system is sent to a dewatering centrifuge known as the clarifier. The overflow to the clarifier is fairly clean water and is used as germ and/or fiber washing process water. The underflow from the clarifier is mixed with the underflow of the MST and is fed to the primary centrifuge. In the four-centrifuge system, the MST, gluten thickener, and clarifier are all designed for dewatering. There are various 2 and 3 centrifuge systems that take out 1 or more of the dewatering centrifuges and have application primarily in smaller plants where capacity is less than can be handled by one primary centrifuge.

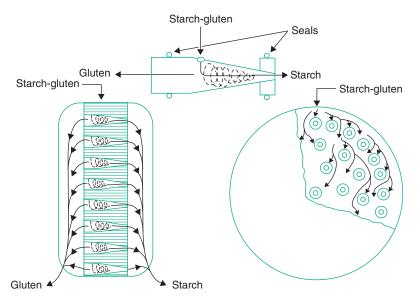


Figure 12 The flow of starch and protein in a 10 mm (Clamshell type) hydrocyclone system for starch washing. (© SR Eckhoff, Savoy, IL.)

#### **Starch Washing**

Starch washing is the last step of the wet milling process and is the only place in the whole process that fresh water is added into the system. Starch washing is performed counter currently using 10-14 stages of 10 mm diameter hydrocyclones (Figure 2). Each stage of the starch washing system contains enough hydrocyclones to handle the flow rate of the starch slurry at that point. Approximately 1200–15001 of fresh water per Mt of maize (2.1–2.6 lbs water per lb of dry starch) is used to counter currently wash the starch to a protein level of 0.25-0.5%. There are several different styles of containers for the numerous parallel 10 mm cyclonettes needed for starch washing. The most popular style still in use in the USA is a clamshell arrangement shown in Figure 12 in which up to 480 individual cyclonettes can be packed into one clamshell and can handle  $\sim$ 890 Mt day<sup>-1</sup>. Newer units are designed for rapid changing of cyclonettes to minimize downtime of the unit. The underflow of the last starch washing unit is at ~23 Baume. From this point, the slurry can be dried to make unmodified starch, chemically modified and dried or hydrolyzed to make a variety of maize syrups, dextrins or glucose syrups. Drying is usually done with a ring dryer after dewatering the starch using basket centrifuges or vacuum belt filters.

# **Capital Requirements and Energy Use**

Maize wet milling is a capital and energy intensive process (Table 2). The values shown are the purchased equipment and energy for a 2667 Mt day<sup>-1</sup> wet mill,

which produces only dry starch. To calculate the total fixed capital costs, on-site costs need to be known, which can be calculated as a percentage of the purchase equipment costs, based on historical data for processing plants of this type. Fixed capital cost is  $1.81 \times$  the on-site costs and as shown in Table 3 is  $\sim$ \$127 million (\$US).

Starch dewatering and drying is the largest single user of energy and capital in the wet milling process. However, most maize wet millers do not produce just dry starch but have a wide variety of starch hydrolysate products, as well as modified starches. A similar sized plant dedicated to making fructose, would have a total fixed capital cost of ~\$206 million (\$US) and a wet mill dedicated to producing ethanol has a total fixed capital cost of nearly \$171 million (\$US). These values will vary depending upon plant location, local labor costs, and product mix, yet because the equipment suppliers market internationally, the equipment costs will be similar.

## **Water Flow and Mass Balance**

Balancing water flow in a wet mill is critical to profitable operation. In general, any problem can be made a little bit easier by adding more water. The downside of running a dilute wet mill is that you have to get rid of all the water when you are done using it. Wet mills have been bottled up for  $\sim 80$  years, with all of the water leaving in the products or being evaporated into the air. The average wet mill will spend  $\sim \$0.35$  per bushel in energy, more than at any single operating expense except the maize itself.

**Table 2** Equipment cost and energy use for a 2668 Mt day<sup>-1</sup> maize wet mill

Item	kWh Mt <sup>−1</sup>	% of total	\$ (1000s)	% of total
Corn receiving	4.66	0.54	3500	9.45
Steeping	38.97	4.62	4332	11.69
Steepwater evaporation	230.86	27.41	2037.5	5.50
Germ recovery (first grind)	7.88	0.94	911.5	2.46
Germ recovery (second grind)	3.92	0.52	640.5	1.73
Germ recovery (germ washing)	0.27	0.03	251	0.68
Germ dewatering and drying	44.34	5.26	1069	2.89
Fiber recovery	23.60	2.80	2304.5	6.22
Fiber dewatering	0.42	0.49	1923	5.19
Protein recovery	10.96	1.30	3071.5	8.29
Gluten thickening and drying	75.76	9.00	2740	7.39
Starch washing	5.26	0.62	1302.5	3.52
Starch dewatering and drying	243.76	28.94	9030	24.38
Gluten feed dryer	147.73	17.53	3940	10.65
	841.09	100.00	37 053	100.00

Assumptions: (1) 2668 Mt d<sup>-1</sup> grind. (2) Modern plant. (3) Feed house integrated to maximize energy efficiency. (4) Incoming freshwater to starch washing heated with waste heat.

Sources: Blanchard PH (1992) Technology of Corn Wet Milling. Amsterdam, The Netherlands: Elsevier Science and Wideman J (2003) Handout at AACC Wet Milling Short Course, Urbana, IL, 27–30 May 2003. © SR Eckhoff, Savoy, IL.

Table 3 Calculating on-site and fixed capital cost

Category	Cost (\$)
Purchased equipment cost (PEC)     Purchased equipment installation (35% of PEC)	37 053 000 12 968 550
3. Instrumentation and controls (10% of PEC)	3 705 300
4. Piping and material handling (30% of PEC)	11 115 900
5. Electrical equipment and material (15% of PEC)	5 557 950

On-site costs = 70 400 700

Fixed capital costs (1.81 × on-site costs) 127 425 267

Source: Douglas JM (1988) Conceptual Design of Chemical Processes, New York, NY: McGraw-Hill.

Fresh water enters the process only at the last stage of starch washing and works its way up through the process, where it washes the primary centrifuge, fiber, and germ. The water, now laden with solubles and some insolubles, ends up as "fresh" steepwater (process water with sulfur dioxide added) moving through the steep battery and finally evaporated to heavy steepwater. Some seal water and rinse water

enters the process but usually amounts to less than 8% of the total water added (shown on Figure 13 as extra water). Of the 1200 to 1500 l water per Mt maize, which enters starch washing,  $\sim 700 \, l \, Mt^{-1}$  is needed just to hydrate the maize during steeping. Another  $420 \, l \, Mt^{-1}$  is needed to fill the void space in the tank so that the maize is not left uncovered. While  $1200-1500 \, l$  water per Mt maize is a lot of water,  $1120 \, l \, Mt^{-1}$  is needed just to be able to run the process.

The mass balance (Figure 13 and Table 4) shows how much water and solids are recycled in the process. Even though the inflow rate of maize and water combined is over 10 000 lbs min<sup>-1</sup>, individual streams are in excess of 17 500 lbs min<sup>-1</sup>. Variability in maize characteristics (fraction yields, water-holding capacity, millability, etc.) can cause great swings in process flows. For example, switching from maize with an equilibrium moisture after steeping of maize at 44% to 52%, causes a swing in the water going to the steepwater evaporator of 255 l Mt<sup>-1</sup> or ~40% of the flow.

# **Wet Milling Products**

Wet milling results in four main products: starch, oil, gluten meal, and gluten feed. Compositional data for these products as well as for specific components of gluten feed is shown in Table 5. Starch is the prime product but co-product value can greatly influence overall plant economics. Co-products make up 30-40% of the total product yield, yet 20-25% of the kernel is processed without increasing value, even though oil and gluten meal have higher value than starch in the US market. There is a wide range of products that can be made from starch (Figure 14), while nonanimal food uses of the co-products are more limited. However, there is an increasing research and commercial interest in improving the existing coproducts, finding new industrial uses or extracting nutraceuticals from co-products.

# **Factors Affecting Starch Yield**

# **Kernel Composition**

Based upon \$ kg<sup>-1</sup>, maize oil is the most valuable component of the maize kernel (\$0.66 kg<sup>-1</sup>), followed by high protein gluten meal (\$0.30 kg<sup>-1</sup>), starch (\$0.22 kg<sup>-1</sup>), and gluten feed (\$0.07 kg<sup>-1</sup>). These simple facts often lead to an assumption that maize with enhanced quantities of oil or protein would be desirable in a wet mill. This is generally not true because the change in composition effects mill balance. The mill is designed to process maize of average composition. To handle more germ or more protein the plant often has

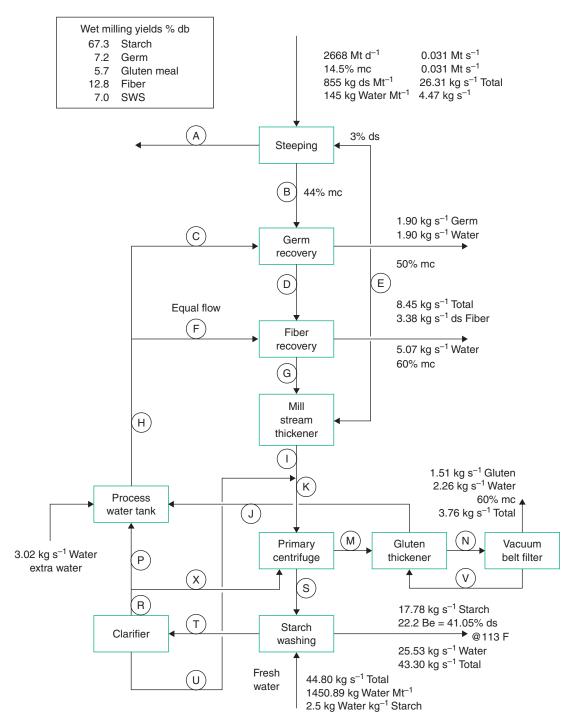


Figure 13 Mass balance on maize wet mill. (© SR Eckhoff, Savoy, IL.)

to decrease its grind rate (the number of bushels processed per day) which increases the processing cost per bushel. It is also possible to expand or redesign wetmilling plants to handle maize with altered compositional characteristics. However, these redesigned mills need to insure themselves of sufficient quantities of reasonably priced enhanced composition maize to justify the added capital.

There are situations where specific mills have economic reasons to desire enhanced compositional characteristics. For example, most of the specialty food and industrial starch plants are smaller, older, land-locked facilities, which cannot economically expand their maize-handling or by-product-handling facilities. High extractable starch maize will allow them to expand production of high-value specialty starches

Table 4 Stream composition for wet mill mass balance shown in Figure 13

Letter	Location	$kg s^{-1} total$	$kg s^{-1} ds$	$kg s^{-1} H_2O$	Baume <sup>a</sup>	%ds
A	Light steepwater	19.38	1.89	17.74	5.00	9.50
В	Steeped corn	45.70	25.90	20.35	NA	56.00
С	Germ wash water	50.85	0.19	51.26	$\cong$ 0	0.37
D	Mill slurry	93.85	24.16	53.73	14.49	25.75
E	Fresh steepwater	34.61	1.04	33.57	1.69	3.00
F	Fiber wash water	50.85	0.19	51.26	$\cong$ 0	0.37
G	Defibered mill stream	136.75	20.93	115.82	8.61	15.31
Н	Germ and fiber wash	102.91	0.38	102.53	$\cong$ 0	0.37
1	MST underflow	102.14	19.89	82.25	10.96	19.48
J	Gluten thickener overflow	65.76	0.23	60.97	$\cong$ 0	0.35
K	Primary feed	126.56	24.84	100.95	11.04	19.6
M	Primary overflow	69.57	1.75	63.26	1.42	2.52
N	Feed to vacuum belt filter	9.89	0.01	8.37	8.67	15.40
Р	Clarifier overflow extra	34.09	0.15	33.94	$\cong$ 0	0.44
R	Clarifier overflow	45.91	0.19	45.72	$\cong$ 0	0.41
S	Primary underflow	68.81	23.12	45.69	18.91	33.61
T	1st Stage starch washing	70.33	5.14	65.19	4.11	7.30
U	Clarifier underflow	24.41	4.95	19.47	11.40	20.26
V	Belt filter water	6.08	0.00	6.08	0	0
Χ	Primary wash water	11.82	0.04	11.78	$\cong$ 0	0.32

<sup>&</sup>lt;sup>a</sup>%ds (dry solids) = 1.777 × Baume; light steepwater used relationship in Blanchard PH (1992) *Technology of Corn Wet Milling*. Amsterdam, The Netherlands: Elsevier Science, © SR Eckhoff, Savoy, IL.

Table 5 Range of compositional values for wet milling co-products

	Starch	Condensed steepwater	Gluten feed	Gluten meal	Germ meal	Oil
Moisture (% wb)	10-12	45–55	10-2	10-12	10-12	0
Protein (% db)	0.30	40-50	21-24	66-70	20-28	0
Oil (% db)	0.02	0	2-3	2	1-2	100
Starch (% db)	99	NA	15	NA	NA	0
NFE (% db <sup>a</sup> )	NA	30-40	50	22-30	50-56	0
Crude fiber (% db)	0.03	0	8-9	1	10-12	0
Ash (% db)	0.10	6–8	8-9	2-3	3-5	0

<sup>&</sup>lt;sup>a</sup>NFE is nitrogen free extract.

Sources: Blanchard PH (1992) *Technology of Corn Wet Milling*. Amsterdam, The Netherlands: Elsevier Science. Johnson LA (1991) Corn: production, processing and utilization, In: Lorenz KJ and Kulp K (eds.) *Handbook of Cereal Science and Technology*, pp. 55–132. New York: Marcel Dekker and Wright KN (1987) Nutritional properties and feeding values of corn and its by-products. In: Watson SA and Ramstad PE (eds.) *Corn: Chemistry and Technology*. Minnesota: American Association of Cereal Chemists, St. Paul. © SR Eckhoff, Savoy, IL.

without expanding their handling capabilities. Japanese wet millers also desire high extractable starch maize due to low by-product values.

Changes in the "quality" of the components, i.e., better amino acid balance in the endosperm glutelin protein or more desirable fatty acid composition, would not affect the mill balance and thus are attractive to wet millers if: (1) any premium they pay for the maize can be more than offset by the increased by-product value, and (2) they can be assured of sufficient quantity of maize to effectively run their facility. In general, wet-milling companies want yellow dent maize with average compositional characteristics.

## **Test Weight**

Higher test weight as controlled by genetics is most often related to the percentage of hard or vitreous endosperm found in the kernel. Hard endosperm takes longer to steep and generally results in less starch release due to the reduced diffusional characteristics of hard endosperm and increased disulfite bonding. Wet millers desire sound, solid kernels, but test weights above 1036 kg Mt<sup>-1</sup> probably indicated kernels which will require longer steep times and potentially will have lower starch yields. Flint type maize is undesirable, not only for the higher percentage of hard endosperm it contains, but also because it has been

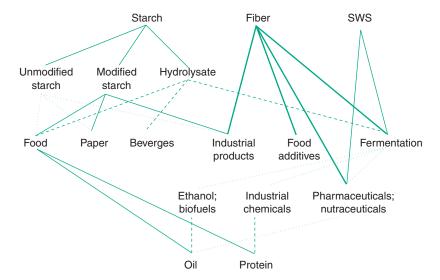


Figure 14 Diagram showing the use of various wet milling co-products in food and industrial products. (© SR Eckhoff, Savoy, IL.)

found to take considerably longer to steep than dent maize, even when diffusional barriers are removed.

The effect of environment on test weight is varied. Hot, dry weather will increase test weight by inducing the formation of a higher percentage of hard or vitreous endosperm. Cool, wet weather has the opposite effect. A recent study found no difference in the yield of starch, even though test weights varied from 700-800 kg Mt<sup>-1</sup> range to over 1075 kg Mt<sup>-1</sup> due to weather. Low test weight can also be caused by an early frost causing the maize to never "fill out" or physiologically mature, which has a negative effect on starch yield.

Although test weight differences caused by weather does not affect starch yield, it can affect wet mill economics. Steeping is a volume limited process so that lower test weight means less maize in the steep tank, and if the steep time is held constant; the mill's grind rate is decreased. Test weights below 1000 l Mt<sup>-1</sup> should be discounted at a rate of \$0.03 per 18 l Mt<sup>-1</sup> below 1000 l Mt<sup>-1</sup> to compensate for the loss of grind capacity. Because much of the rest of the wet-milling process is dry solids limited, there is no advantage to having higher test weight maize.

# Millability

Millability is defined as the ease of component separation. Genetically some hybrids are easier to process than others. Such hybrids do not contain more starch but milling them results in higher starch yields. The germ floats more readily, the fiber dewaters easier, the bound starch in the fiber is lower, starch and protein are easier to recover and other qualitative as well as quantitative characteristics have been observed. Figure 15 shows the variability in starch yields for

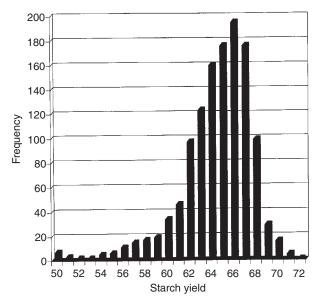


Figure 15 The frequency distribution of maize hybrid starch yields. (© SR Eckhoff, Savoy, IL.)

1244 commercially available hybrids (breeder samples) in the Midwest over a 6-year period. Starch yields varied from less than 50-72% with an average near 63.5% and a peak frequency at 66%.

# **Mechanical Damage**

Mechanical damage to the kernel resulting in broken maize is not desired by wet millers. The broken maize plugs the screens at the bottom of the steep tanks resulting in nonuniform steepwater flow and poor steeping. The broken maize also results in excessive loss of solids into the steepwater and can cause evaporator fouling problems.

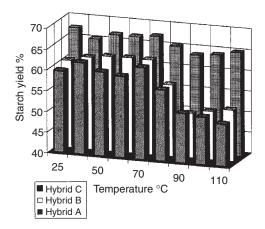


Figure 16 The effect of drying temperature on starch extractability from three maize hybrids. (© SR Eckhoff, Savoy, IL.)

#### **Drying**

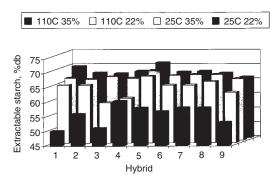
Rapid high-temperature drying is the single most detrimental thing that can be done to maize to reduce its starch yield. Numerous studies have shown that drying air temperatures above 70°C can result in partial gelatinization of the starch, protein denaturation and endogenous proteolytic enzyme denaturation.

Figure 16 shows the effect drying air temperature had on the starch yield of three different hybrids. Starch yields decreased as drying air temperature increased above 70°C. As much as 10% yield loss of starch can be observed. Figure 17 shows the wetmilling yields for nine commercial hybrids representing a range of endosperm hardnesses, harvested at two different moisture contents and dried by either ambient air or 110°C drying air. Drying from a higher initial moisture content (>28%) has a severe effect on starch yield. Hybrid variability in sensitivity to drying temperature can also be observed in the data.

#### **Storage**

Wet millers have reported over the years that the milling quality of maize diminishes over the course of the season, with considerable difficulty in the summer months. It was perceived that there was some intrinsic loss in quality associated with storage time; possibly due to loss or gain of some enzymatic activity. Wet millers also reported difficulty in the fall in processing new crop maize. They complained of foaming problems and reduced starch yield. These problems generally disappeared after 1–2 months.

Recent studies indicate that there is no intrinsic long-term degradable loss in starch yield due to storage at either ambient conditions or at 4°C until after 4 years. It is likely that the effect of storage observed



**Figure 17** The effect of drying temperature and harvest moisture on starch extractability for nine commercial hybrids. (⊚ SR Eckhoff, Savoy, IL. Reproduced with permission from Singh V, Haken AE, Paulsen MR, and Eckhoff SR (1998). Starch yield sensitivity of maize hybrids to drying temperature and harvest moisture content. *Starke* 49(10): 181−183.)

by the industry in the same crop year is due to the blending of low-quality maize with higher-quality maize to meet minimum grade standards. The amount of microbial and insect damaged maize increases after the spring thaw and accounts for the lower starch yields and greater processing difficulties. The excessive foaming observed with fresh maize is probably related to natural proteolytic activity, which decreases with time.

# Milling Unique Hybrid Maize

#### **High Amylose**

High-amylose maize is a genetic mutant that has higher levels of the linear form of starch than the 27% normally found in dent maize. Two classes of high amylose are generally grown: Amy 5, which is a group of hybrids containing ~50% amylose and Amy 7, which is a group of hybrids containing  $\sim$ 70% amylose. High amylose starch is produced for a variety of food application and industrially is used in the production of biodegradable packing peanuts. Starch granules of high-amylose maize are generally smaller and more irregularly shaped than normal dent maize starch granules. As a result, high-amylose maize is difficult to mill and recover quantitative amounts of starch. When steeping highamylose maize, care must be taken to account for the high degree of swelling which occurs. Regular dent maize will swell 60-65% by volume but high-amylose maize will swell 105–128%. In swelling this amount, the maize also absorbs a proportionally larger amount of water. It is usually stated that because amylose has a higher gelatinization temperature, it should be steeped 3°C hotter than normal dent maize. However,

in practice industry does not adjust the temperature. This may be due to the optimal temperature for lactic acid fermentation being 52°C. High-amylose maize requires a longer steep time, has greater difficulty in achieving starch-protein separation and yields only 80-90% as much starch as dent maize.

#### Waxy

Waxy maize is a genetic mutant that has  $\sim 100\%$ amylopectin starch. Amylopectin is the branched form of starch and has a lower pasting temperature than normal dent maize starch. Waxy starch is produced for a variety of food applications. Nearly all of the anticipated demand for waxy maize by wet millers is produced under contract. However, the yield drag associated with waxy maize has disappeared and now that a lot of speculative waxy is being produced, in a year when the contracted acreage does not yield sufficiently to meet demand, speculative waxy is sought out by the wet millers at a sizable premium.

Starch-protein separation is much easier in waxy maize than dent maize, primarily due to the larger granule size of the starch. However, starch yields 5-10 percentage points lower than dent maize. It appears to be an issue with the density of the starch. Even though the starch granules are larger, the absence of amylose in the starch granule apparently does not allow the granule density to be as large. Because waxy starch will gelatinize a lower temperature than dent maize, it is often recommended that it be steeped 3°C cooler (49°C). In practice, the temperature is not adjusted.

## **High Oil**

High oil maize contains  $\sim$ 7% oil primarily by increasing the size of the germ, although, most hybrids have some degree of elevated germ oil concentration as well. Development of high oil maize was primarily for livestock feeding but considerable interest was generated by the wet-milling companies because of the high value of oil. Most wet milling companies had trouble adapting high oil maize into their system because they did not have sufficient germ processing capacity to handle the large influx of germ.

High oil maize hybrids produced by the male sterile pollinator method generally have wet-milling characteristics that are comparable to normal dent maize, with the exception that there was considerably more germ. Starch yields vary from  $\sim$ 55–61%, db. Germs tend to float better primarily because they are larger in size and have higher oil content than normal dent maize. Starch-protein separation does not seem to be affected in the hybrids tested.

## **Genetically Modified**

Genetically modified is a large, diverse, and growing group of hybrids. Most of the commercially available genetically modified maize hybrids have been genetically modified for some agronomic characteristic. These hybrids, to date, have improved or comparable wet-milling characteristics to their nongenetically modified counter parts (Table 6). The generally accepted rationale for improvement of the wet milling characteristics is based on the improved agronomic conditions, i.e., less insect or disease stress on the plants. Hybrids will eventually become available that have been genetically modified to produce nutraceuticals, pharmaceuticals, specialty chemicals, hormones, or to contain enhanced nutrient or functional characteristics. Even if the wet milling characteristics of these hybrids are not altered, alternative fractionation procedures are going to need to be developed to maximize recovery of the high-value product or to

Table 6 Yield of wet mill fractions for different maize phenotypes and genotypes

Fraction (% db)	Normal dent	High extract <sup>a</sup>	L	Dent	Waxy	High amy <sup>b</sup>	High oil	Hard endo <sup>c</sup>	White	Industry dent
			Bt	Non Bt						
Starch	65.5	68.5	64.2	63.7	61.3	40.1	57.4	64.3	63.9	66.8
Germ	5.5	5.4	6.3	6.5	5.4	5.5	8.7	5.7	5.7	7.6
Fiber	14.2	12.7	14.3	13.8	15.4	18.3	14.5	16.0	14.7	11.8
Gluten meal	10.0	9.3	10.3	11.1	12.7	32.7	13.9	9.4	10.9	5.9
Steepwater	4.2	3.5	4.3	4.1	4.0	3.5	4.2	3.9	3.8	7.0
# Samples	110	49	8	8	90	9	5	45	90	4

<sup>&</sup>lt;sup>a</sup> High extractable maize.

All data except "Industry" are from 100 g Laboratory Milling, © SR Eckhoff, Savoy, IL.

Sources: US Grains Council (1996) 1995 - 1996 Value-Enhanced Grains Quality Report. Washington DC, USA: US Grains Council and Eckhoff SR (2003) Unpublished data. University of Illinois, Urbana, IL.

<sup>&</sup>lt;sup>b</sup>High amylose.

<sup>&</sup>lt;sup>c</sup>Hard endosperm.

retain the enhanced nutrient or functional characteristics through the milling operation.

#### **Future Trends**

There are four basic trends, which emerge when examining the maize wet-milling industry. They are given as follows.

- 1. The large commodity markets of the recent past have reached maturity and there does not seem to be any new large-scale markets in the near future. The result is that many wet milling companies are looking to higher valued products with much smaller markets and to bio-based products for the industrial markets.
- 2. There is considerable interest in developing a new technology for wet milling, which does not use sulfite. "E-milling," where specifically designed proteases (enzymes) are used to release the starch from the protein matrix in the endosperm, is one example. Because of government regulations on sulfur dioxide emissions, taste and odor problems associated with co-products exposed to sulfites and potential for new product development, the industry is more willing than they have been in the past (due to the large capital investment they have in place with current technology) to embrace new fractionation technology.
- 3. The wet-milling industry is undergoing considerable worldwide consolidation. This trend is likely to continue, although probably at a slower pace than in the past three years. Consolidation provides access to worldwide markets, buys capacity, and expertise to diverse product lines and leverages expertise.
- 4. The industry seems to be slowly moving toward hybrid specific processing, which is the processing of selected maize hybrids with similar characteristics in order to enhance processing throughput, improve quality, and increase food safety via traceability.

See also: Maize: Genetics; Breeding; Quality Protein Maize; Dry Milling; Foods from Maize.

# **Further Reading**

Blanchard PH (1992) Technology of Corn Wet Milling. Amsterdam, The Netherlands: Elsevier Science.

Douglas JM (1988) Conceptual Design of Chemical Processes. New York: McGraw-Hill.

- Eckhoff SR, Paulsen MR, and Yang Ping (2003) Maize. In: Caballero, Trugo, and Fingla (eds.) Encyclopedia of Food Sciences and Nutrition, vol. 4, pp. 3647-3653. London: Elsevier Science.
- Jackson DS and Shandera DL, Jr (1995) Corn wet milling: separation chemistry and technology. In: Kinsella JE and Taylor SL (eds.) Advances in Food and Nutrition Research, vol. 38, pp. 271-299. San Diego, CA: Academic Press.
- Johnson LA (1991) Corn: production, processing and utilization. In: Lorenz KJ and Kulp K (eds.) Handbook of Cereal Science and Technology, pp. 55-132. New York: Marcel Dekker.
- Radley JA (ed.) (1976) Starch Production Technology. London: Applied Science.
- Singh V, Eckhoff SR, and Tumbleson ME (eds.) (2001) Proceedings of the Second Annual International Starch Technology Conference. Urbana, IL: University of Illinois.
- Watson SA and Eckhoff SR (2003) Corn and sorghum starches: production. In: Whistler RL, BeMiller JN, and Paschall EF (eds.) Starch Chemistry and Technology. Amsterdam, The Netherlands: Elsevier Science (in press).
- Watson SA and Ramstad PE (eds.) (1987) Corn: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.
- Whistler RL, Bemiller JN, and Paschall EF (eds.) (1984) Starch: Chemistry and Technology. Orlando, FL: Academic Press.

# **Relevant Websites**

http://www.grains.org - US Grains Council website with information on the various types of value enhanced corn, their growing location and the market channel contacts.

http://www.corn.org - Corn Refiners Association website with general information on the wet milling process, member company, products from wet milling and wet milling utilization statistics.

http://www.ncga.com - National Corn Growers Association website with up to date information on corn and biotechnology, ethanol, developments in value-added research and http://lepton.marz.com has an on-line corn-based product database with product information and supplier contacts.

http://www.starch.dk - International Starch Institute (Denmark) website, which has information on engineering, research, production and application of modified and unmodified starch. Many useful tables and information. Also has information on processing some downstream products like glucose and high fructose syrups.

# **Foods from Maize**

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## Introduction

Maize (Zea mays L.) is the leading grain in the world and staple for large groups of people in Latin America, North America, Asia, and Africa. In contrast to rice and wheat, maize is mainly utilized for animal feeding although direct and industrial food uses are increasing. According to the FAO (2003, http://apps.fao.org) in the year 2000, 387, 111, and 53 million ton (Mt) were used for feed, food, and food manufacture, respectively. Maize is a crop with a remarkable genetic variability; therefore, many specialty genotypes are available. The main specialty corns are popcorn, waxy, high amylose or amylo-maize, sweet, blue, Cuzco, and quality protein maize (QPM). Maize foods are characterized by their unique distinctive flavor not duplicated by any other cereal. The grain is used for production of numerous indigenous foods: maize meal, flour, grits, starches, sweeteners, cooking oil, breads, tortillas, breakfast foods, snacks, industrial alcohol, and alcoholic beverages (see Beverages: Distilled. Extrusion Technologies. Fermentation: Origins and Applications; Foods and Nonalcoholic Beverages. Maize: Dry Milling; Wet Milling. Snack Foods, Processing. Tortillas). Processed maize products are manufactured from raw materials obtained from three major milling industries: dry milling (see Maize: Dry Milling), wet milling (see Maize: Wet Milling), and nixtamalization (see Tortillas) (Figure 1). The dry milling industry produces an array of refined products widely used by the baking, brewing, snack, and breakfast cereals industries. Most of the starch obtained from the wet milling industry is bioenzymatically converted into maltodextrin, maltose, glucose, and high-fructose syrups. The alkaline cooking or nixtamalization process of whole maize has become important in the United States and other parts of the world due to the increase in popularity of Mexican foods.

# **Food Uses of Specialty Corns**

Specialty corns have been selected due to their unique properties, the most important being popcorn, sweet, high amylose, waxy, blue, and quality protein (see Maize: Quality Protein Maize) (Table 1). Popcorn has been a favorite traditional snack worldwide for more than a century, whereas sweet maize is one of the most popular canned or frozen vegetables in the USA and Canada. Large quantities of waxy maize that contains more than 95% amylopectin are channeled to the wet milling industry with the aim of obtaining starch that has unique functionality (e.g., low retrogradation).

QPM was developed from the mutant opaque-2 corn discovered in 1963 and contains almost twice

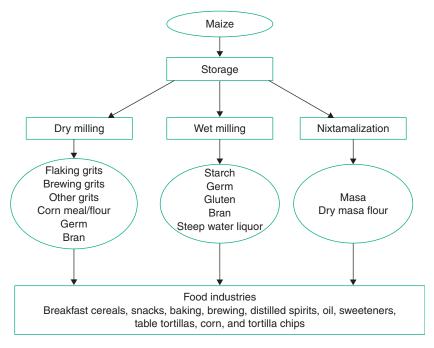


Figure 1 Flowchart of the postharvest management and processing of maize.

as much lysine and tryptophan than regular corn. Thus, QPM-based foods can upgrade the nutritional status of infants, who consume cereals daily in marginal areas around the world. Blue maize has a pigmented aleurone that imparts an intense blue color and a unique flavor. It has been traditionally dry milled for production of flours or meals and also used for tortillas and chips. Blue maize tortilla chips are often served in specialty restaurants and are also sold as organic food. It has high levels of anthocyanins and other phenolics that may have nutraceutical properties (see Nutraceuticals from Grains).

Table 1 Characteristics and food uses of specialty maizes

Specialty maize	Food uses
Popcorn	Special kind of flint corn. Hybrids or varieties with high proportions of translucent, flinty, or vitreous endosperm give higher expansion rates. Expansion volume is the most critical quality factor for popcorn. Most commercial popcorn has a 30- to 40-fold expansion. Popping occurs at ~177°C (350°F), which is equivalent to a steam pressure of 2.5 t cm <sup>-2</sup> (135 psi) inside the kernel. The water in the kernel is superheated and at the moment of popping converts to steam, which provides the driving force for expanding the thermoplastic endosperm after the kernel ruptures. The pericarp and outer layers of the kernel participate directly in the popping action by serving as a pressure vessel enclosing the endosperm. Popped corn with a spherical shape is called mushroom or ball type and is preferred in the confection industry. The butterfly-type popcorn has a higher expansion, lower apparent bulk density, and better mouth feel; it is preferred for on premises popping.
Food grade yellow and white maize	Mainly developed for alkaline cooking and dry milling. These types of maizes have improved processing efficiency in dry milling and snack food processing plants. These maizes should be hard, medium to large kernels, have high test weight, high density, and a pericarp that is easily removed during processing. In addition, the kernels should come preferably from white cobs. Kernels from pink- or red-colored cobs are high in phenols and yield undesirable off-colors in processed foods.
Quality protein maize	QPM has the opaque 2 gene that is combined with modifier genes that significantly improve the hardness and agronomic performance of the crop. The QPM material has been incorporated into high-yielding hybrids in Brazil, Mexico, and other countries, while open pollinated varieties are grown in Africa and Central America. Harder QPM corns are suited for dry milling and alkaline cooking, while soft hybrids for use in wet milling to produce sweeteners, starches, and alcohol would be desirable since the co-products would be more valuable.
Blue corn	Blue corn is a floury or soft endosperm type that generally grows in long ears (8–12 rows). The aleurone layer contains the anthocyanins that imparts the blue appearance. Blue corn is especially prized as a ceremonial corn by the North American Indian tribes and is currently being used to produce organic flours and foods such as tortilla chips. The blue corn contains higher levels of flavonoids that are currently thought to be excellent source of antioxidants for nutraceutical foods.
Sweet corn	Sweet corns have recessive genes (sugary 1 or su1, sugary 2 or su2) that causes an alteration in the endosperm that results in higher levels of soluble sugars and reduced levels of starch in the kernel. Sweet corn hybrids have been developed specifically to produce corn with desirable color, sweetness, and tenderness.
Baby corn	Special corn varieties are grown and shucked immediately after pollination when the ears are 1–2 in long. These small ears are used as pickles and other tasty snacks in salad bars. Most of the baby corn used is produced in Thailand and exported to Europe and North America.
Waxy maize	Waxy maize is named for the somewhat waxy appearance of the kernel. Waxy maize starch is composed entirely of amylopectin. It is utilized mainly by the wet milling industry. Waxy starch has a higher hot viscosity and produce softer, more stable, and clearer gels due to its lower retrogradation. It also has a higher freeze—thaw stability. Waxy maize is currently being utilized to produce snacks with different textures.
High amylose maize	Also named amylomaize. Expresses high quantities of linear amylose because of the recessive <i>ae</i> gene located in chromosome 5. Most genotypes contain amylose from 37% to 65%. Although it is not commercially planted, the amylomaize has potential for the paper, textile, corrugating, and adhesive industries. The high-amylose starch produces rigid opaque gels with potential for the confectionery industry and as a thickener in various puddings and processed foods. It can be used as a binding agent for dehydrated potatoes or as a coating to reduce oil absorption of deep fat fried potatoes. One of the best potential uses of it is for production of biodegradable packaging materials that resembles polystyrene foam used for "plastic peanuts."
Cuzco/cacahuacintle maize	The Cuzco maize comes from an eight-rowed ears that produces the largest known kernels. Cuzco corn grows at high altitudes and produces white kernels with soft endosperm texture and bland flavor. Cuzco corn is mainly used to manufacture Cornnuts <sup>TM</sup> and Cacahuacintle kernels for hominy and pozole production.

Data from: Johnson LA (2000) Corn: the major cereal of the Americas. In: Kulp K and Ponte JG, Jr (eds.) Handbook of Cereal Science and Technology, 2nd edn. New York: Marcel Dekker; Hallauer AR (2000) Specialty Corns. Boca Raton, FL: CRC Press; and Rooney LW and Serna-Saldivar SO (2003) Food uses of whole corn and dry milled fractions. In: White P and Johnson L (eds.) Corn Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

# **Indigenous Maize Foods**

Regular and specialty corns are widely consumed in numerous forms in Latin America, Asia, Africa, and the Balkans (Table 2). White maize is generally preferred for foods because it favors the final color of processed foods, but yellow maize is preferred in Brazil, China, Argentina, and some other areas. Most traditional foods are produced from grain that is milled into a meal or flour.

The use of fresh or immature maize on the cob is practiced worldwide. Maize cobs with or without husks are boiled in water or cooked over a fire and then flavored with salt, cream, butter, margarine, and other sauces. In most areas, regular maize is used as green maize.

In Latin America, corn is processed into tortillas (see Tortillas), "arepas," "couscous," "polenta," porridges), and various meals and gruels (Table 2). The traditional method to process maize into tortillas was developed by ancient Mesoamericans. In the traditional process maize is lime-cooked in clay pots over a fire, followed by overnight steeping. The cooking liquor, called "nejayote," is discarded and then the "nixtamal" is hand-washed and ground into a "masa" with a stone grinder called "metate." Masa is handmolded or pressed into disks which are baked on a hot griddle or "comal." Tortillas are mainly used as wraps to prepare tacos that hold different types of fillings (beans, various meats, cheeses, and vegetables). Tacos are the symbol of the Mexican cuisine and are known and consumed worldwide. The Mexicans also inherited many other nixtamalized-derived foods such as "tamales," "atole," "pinole," "pozol," and others (Table 2).

Arepas, the national maize bread of Venezuela and Colombia, are traditionally produced from white grits or meal that is moistened, cooked in water, and ground to a dough. The dough is hand-shaped into flat disks that are browned on each side and baked in an oven. Arepas are cut in half and stuffed with meat, cheese, butter, jellies, and other fillings. Stuffed arepas are also fried to produce "hallaquitas," "empanadas," and other foods.

"Chicha" is a prehispanic sacred fermented beverage prepared in Peru and Bolivia in which ground maize is first treated with amylases from germinated maize or saliva and then naturally fermented. Other alcoholic beverages such as "Tesguino" are consumed in Mexico and "Pito," "Talla," and other related opaque beers in Africa (Table 2).

In Africa and Asia, maize is generally dry-milled into grits, meals, and flours for production of flat breads, unfermented and fermented porridges, steamed foods (couscous), and alcoholic or nonalcoholic beverages.

Many of the traditional foods are produced from fermented or germinated maize which increases the vitamin content, mineral bioavailabilty, and protein quality. "To," "Pap," and "Sadza" are popular porridges consumed in Africa (Table 2).

The early settlers of North America cooked maize in lye or wood ashes to produce hominy. The alkali effectively removes the pericarp, enhances the palatability and nutritional value of the kernels (Table 3), and transforms the hard raw kernels into a soft, chewable product which could be stored relatively safely. In Mexico, lime-cooked hominy is commonly used for the preparation of "pozole" and "menudo;" both are soups in which the hominy is mixed with condiments and shredded meats (Table 2).

# Industrial Processing of Maize for Foods

Most of the maize is first industrially processed by wet milling, dry milling, or nixtamalization (Figure 1). Wet milling produces relatively pure starch, protein, fiber, and germ. Dry milling produces refined endosperm fractions of varying particle size (grits, meal, flour), germ for oil, and sometimes dietary fiber ingredients. Starch and refined dry-milled products are mainly used for the production of snacks, breakfast cereals, syrups, lager beer, and alcohol. Nixtamalization produces dry masa flours, tortillas, and related snack foods that are increasing rapidly in popularity in the USA and other areas.

#### **Food Uses of Starch**

More than 90% of the commercial starch is from maize due to the low cost of the grain, high starch content, and high value of the co-products. The refined starch is channeled to various food industries to be used as additive or it is enzymatically hydrolyzed into various types of syrups (*see Maize:* Wet Milling). In addition, the co-products are highly valued by the feed and vegetable oil industries. The bran is generally used to feed ruminants, although some is finely ground to produce high dietary fiber foods. The germ is demanded by oil mills to produce the added value refined oil and defatted protein meal. Corn oil is widely used as cooking oil because of its stability and high levels of polyunsaturated fatty acids.

# **Corn Syrups**

In the USA more than 90% of the starch is transformed into syrups (Figure 2). The production and utilization of maize syrups has increased during the past decades because the soft drink industries prefer to use sweeteners instead of crystallized cane or beet

Table 2 Major uses of maize for preparation of indigenous foods

Food	Description of product and process
	Description of product and process
Tortillas	Maize is lime-cooked for 5–30 min and steeped overnight. The cooking liquor is discarded and the resulting "nixtamal" rubbed between the hands to remove the bran. The washed nixtamal is hand-ground with a cylindrical stone and a "metate" or flat stone slab to produce a dough or masa. Pieces of masa are patted into thin round disks that are baked for 30–60 s onto a hot clay circular hot griddle or "comal" to produce tortillas.
Cornbread	Commeal alone or blended with wheat flour is processed into dough with water and/or milk and baked. Chemical leavening agents and flavorings (i.e., sugar, salt) are used in some formulations to obtain better texture, volume, and flavor. Many types of combread exist around the world.
Arepas	National bread of Venezuela and Colombia. Moistened corn is de-hulled and partially de-germinated in a wooden mortar using a pestle. The meal is cooked in boiling water and stone-ground into masa. Arepas are manually formed into "7.5 cm diameter × 1 cm thick" flat disks and baked for 2 min on each side on a clay or metal griddle. Arepas are stuffed with meats, cheese, butter, jellies, or other fillings.
To, Sadza, and pap	Staple foods of Africa. Corn meal is cooked in water until completely gelatinized. The porridge is placed in a gourd, cooled for 1 h, and eaten with the fingers and a sauce. Acid (tamarind, lemon), fermentation, or alkali (wood ashes) is added to cooking water to produce acid and alkali porridge. Granulation of the flour, composition, type of sauce, and final consistency vary among countries and tribes.
Canjica	Dish widely consumed in Brazil. Canjica is de-germed corn kernels cooked with sugar and milk, and generally consumed as a dessert or breakfast cereal.
Pozole or menudo	Prehispanic dishes in which one of the main ingredients is whole nixtamal generally prepared from <i>Cacahuacintle</i> (white and large kernels with floury endosperm) corn. It is a spicy soup that generally contains peppers, shredded meats, and other spices. The lime or lye cooked corn is mixed with beef stomach, peppers, spices, and onions to produce a soup called Menudo or with shredded pork meat to yield Pozole.
Piki	Traditional dish of American Indians of the southwest prepared from a thin batter of blue corn meal, ash, and water. The batter is cooked on a hot flat stone to form a parchment-like product. Piki can be crumbled, salted, roasted, and eaten-like chips.
Atole and champurrado	Prehispanic breakfast gruels or porridges made out of masa that is diluted in water, sweetened with sugar, and flavored with cinnamon, vanilla, orange leaves, and others. <i>Champurrado</i> is produced from cocoa beans, brown sugar, and cinnamon.
Mingau	Brazilian porridge. Corn grits are mashed or immature kernels are cooked in water to produce porridges similar to atole.
Pinole	Corn kernels are toasted on a hot griddle until attaining a brown-golden color, dry milled into a meal, and blended with cinnamon, anise, brown sugar, and other flavorings. The resulting shelf stable mix is diluted with water or milk boiled for ~4 min and consumed as a breakfast gruel.
Pozol	Prehispanic beverage made out of fermented masa. Fresh masa is wrapped in banana leaves and allowed to naturally ferment for 3–5 days. The fermented masa is diluted in water and consumed as beverage. It is considered the major source of nutrients and the "job beverage" for some indigenous groups in south Mexico.
Tamales	Prehispanic dish widely consumed during festivities, Christmas holidays, and weekends. Consists of masa surrounding a filling that has been placed in a wrapper and steamed. In tropical areas tamales are wrapped in banana leaves; hydrated corn husks are used in the rest of Mexico. The coarse masa is blended with lard, salt, chicken or beef broth, and baking powder. Tamales are steamed in a large pot equipped with a rack in the bottom so as to avoid their contact with the boiling water. Before cooking, tamales are packed or stacked in an organized way, because they increase in volume upon cooking. The pot should be tightly covered with the lid to prevent vapor loss. Cooking times vary from 1 to 3 h or until the masa does not stick to the wrapper. The most common tamales contain spicy beans, shredded chicken, beef, pork, fish, seafood, cheese, or sweets (fruit pastes, jelly, nuts, and raisins). Tamales or TV dinners are available in the frozen shelves of the major food markets.
Tacos	This is the most popular way of consuming table tortillas. Soft tortillas are filled with refried beans, shredded meat or poultry, eggs, guacamole, and vegetables. Quesadillas are tacos filled with cheese, while Sincronizadas contain a combination of cheese and ham. Tacos are generally rolled or simply folded.
Flautas	Tortillas are filled with shredded meat, rolled into small cylinders, and fried until crisp. Guacamole, sour cream, and/or cheeses with vegetables are usually spread on top of the flautas.
Enchiladas	Masa for table tortillas is blended with coloring agents (mild red pepper extract, paprika, or artificial), molded, and baked into a red tortilla. Tortillas are fried for few seconds and immediately filled with cheese/raw onions. Some people also called enchiladas the red-colored tortillas filled with shredded chicken or other fillings (mashed potatoe, various types of meats). Shredded fresh cheese and sauces are spread on top of the filled tortillas prior to serving.

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Food	Description of product and process
Tortillas de manteca or gorditas	Masa is mixed with salt, lard, and tallow. After blending the masa is formed into balls which are allowed to rest for 30 min. Balls are hand-pressed or patted into thick disks which are baked on a hot griddle. These tortillas are usually furnished with beans/cheese and different fillings. Another type called "beany gorditias" are the ones produced by mixing masa with shredded cheese, ground peppers, and refried beans.
Panuchos or papusas	Thick tortillas are filled with refried beans, shredded meats, or poultry with the filling placed underneath the tortilla skin. The product can also be fried. Baked or fried panuchos are generally served with sliced tomato and cooked or cured onions.
Sopes, chalupas, or garnachas	Masa, sometimes blended with lard, is formed into a flat thick disk 1 cm thick and $\sim$ 10 cm diameter and, while baking, a 1 cm high peripheral wall is formed by pinching. The sope is filled with a combination of refried beans, cheese, shredded meats, poultry, and vegetables (lettuce, tomato, radishes, onions).
Tlacoyos	Masa is flattened into thick disks and refried beans placed in the middle of the formed cake. Both sides are folded to cover the beans and to impart the typical tlacoyo configuration (oval-elongated shape). Tlacoyos are baked on both sides onto a hot griddle and usually served with green sauce and dairy cream. Blue corn tlacoyos are very popular in central and south Mexico.
Empanadas	Masa, sometimes mixed with baking powder, is formed into small balls and pressed into thin disks. The preformed masa is filled with shredded meats, beans, cheeses, and folded. The rim of the folded (half-moon) tortilla is sealed by pressing with a fork and then fried.
Tostadas and taco shells	Tostadas are tortillas fried in the flat form and widely used as the base to hold different fillings. For their preparation, nixtamal and red-paprika peppers or annatto is stone-ground into a coarse masa. Today coloring agents are also used. The red- or orange-colored masa is baked into a tortilla and then fried into a tostada. Tostadas are the base for the preparation of wide array of meals. It is very common to consume tostadas with refried beans, cheese, shredded meats, and vegetables such as lettuce, tomato, onions, peppers, and radish. Taco shells are the American version of tostadas with the only difference that they are usually fried bent (U form) and rarely colored. Regular and low-fat tostadas and taco shells are available in grocery stores in Mexico and USA.
Pimes	Mayan dish. Corn masa is blended with salt and lard and sometimes ground peppers. The masa is formed into thick disks that are baked on a hot griddle previously greased with lard.
Pemoles	Mayan dish. Black beans are smashed, fried, and sun-dried. Then they are mixed with masa to form a thick black tortilla disk. The disks are fried and served with picante sauce.
Joroch	Mayan dish. Small masa balls are cooked with smashed beans and the resulting blend served with ground squash seeds.
Tobi holoch	Mayan dish. Annato seeds are fried in lard until release their color. The resulting colored lard is mixed with masa and salt. Alongside, ground meat is cooked with finely ground onion, tomato, and sweet peppers. Masa is hand-formed into an oval shape and the filling placed in the center. The masa is folded, wrapped in banana leaves, and steam-cooked.
Salbutes	Corn masa is blended with salt and wheat flour. Hand-made tortilla disks are fried and then spread on top lettuce or cabbage, shredded poultry, cured onions, and sliced tomato.
Tortilla soup	One of the most popular Mexican soups. Contains up to 15 different ingredients in which the main one is fried tortilla strips. Garlic, onion, and tomato are ground into a sauce and diluted and simmered in chicken broth. Salt, peppers, and other seasonings are added. Few minutes before serving, the soup is reheated, and fried tortilla strips, cheese cubes, and avocado incorporated onto the top of the mix.
Chilaquiles	Prehispanic dish very popular in all Mexico. The nahuatl word derives from <i>chilli</i> (= peppers) and <i>quilitl</i> (= edible) herbs. Chilaquiles are prepared by first producing a sauce based on tomato, onion, peppers, garlic, and salt. Then stale or leftover tortillas cut into pieces and fried until crisp and incorporated into the sauce. Shredded chicken, cream, and cheese are usually placed on top of the chilaquiles.
Chicha morada	Prehispanic sacred beverage prepared in Peru and Bolivia. Salivated or germinated corn flour and water is heated to 75°C, thoroughly mixed for 1 h, and cooled. Upon sedimentation the upper layer called "Upi" is placed in another pot and simmered for several hours until it is caramelized. This product called "misqui kheta" is allowed to cool, combined with more upi, and fermented for 48–144 h to produce a clear, yellowish effervescent beverage. Chicha morada is made from blue corn that is cooked in water with sugar for several hours. The mixture is filtered and the purple liquor is blended with fruit juice (i.e., pineapple) and consumed as a beverage.
Tesguino	Alcoholic beverage produced in Mexico. Corn is soaked in water for several days, drained, germinated, ground, and steeped in water until the mixture turns yellow (8 h). Then, the liquid is filtered, ground leaves, beans, and legumes are added. Fermentation produces an opaque, sour beer with 3–4% alcohol.

Table 2 Continued

Food	Description of product and process
Pito	Nigerian alcoholic beverage. Corn is soaked in water, drained, and held in a moist chamber for 5 days germination. The corn malt is mashed for 6–10 h, cooled, and sieved. The filtrate sours due to microbial fermentation. Then it is concentrated and inoculated with starter from a previous batch and allowed to ferment overnight. Pito is a light brown, slightly bitter, sweet–sour alcoholic beverage.
Talla	Ethiopian alcoholic beverage. A slurry of toasted, ground, and cooked maize flour is mixed with flavorings, pieces of freshly baked flat bread, and wheat or barley malt. After a day, the mixture is diluted with water, fermented for 5–7 days, and filtered. Talla has a smoky flavor and a tan to dark brown color.
Ogi and ugali	Fermented porridges widely consumed in Africa. Corn kernels are steeped and fermented for several days. Steeped grain is wet milled, slurried with water, and sieved. The slurry is allowed to ferment longer. The fermented sediment is separated and boiled in water to yield ogi porridge that is consumed warm or cooled to form a gel or pudding.
Polenta	Popular dish in Italy and South America. De-germinated corn grits are cooked in water until gelatinized, mixed with tomato sauce, cheese, meat, etc., and baked.
Couscous	Ground corn is kneaded with water until the flour particles agglomerate when forced through a coarse sieve. Then the particles are steamed. At intervals the couscous is removed, resieved, and returned to the steamer. The cooked product is consumed with a sauce. In Africa, ground baobab leaves, peanut butter, okra, etc. are mixed with the couscous during the final stage of steaming when it is to be dried and used as a convenient food.

Data from: Steinkraus KH (1983) Handbook of Indigenous Fermented Foods, vol. 9. New York: Marcel Dekker; Serna-Saldivar SO, Gomez MH, and Rooney LW (1990) The technology, chemistry, and nutritional value of alkaline cooked corn products. In: Pomeranz Y (ed.) Advances of Cereal Science and Technology, vol. 10. St. Paul, MN: American Association of Cereal Chemists; and Wacher MC and Lappe P (1993) Alimentos Fermentados Indigenas de Mexico. Mexico, DF: Universidad Autonoma de Mexico.

sugar. Maize sweeteners are preferred over sucrose because they readily dissolve in water and are easier to incorporate into soft drinks, are easily flavored, and impart a fruit flavor to beverages and foods. The high-fructose corn syrup (HFCS) containing 90% fructose impart 1.7 times more sweetness than sucrose at equivalent concentrations.

The various types of syrups are manufactured from acid hydrolysis or enzymatic conversion or a combination of these two processes. The industrial production of amylolytic enzymes allowed the production of better-quality low DE syrups and the production of glucose syrups with high DE or sweetness. The industry evolved with the commercial availability of heat-stable  $\alpha$ -amylase and amyloglucosidase that could transform practically all the starch into glucose. The greatest technological developments were the utilization of immobilized glucose isomerase and the continuous separation of glucose and fructose by moving bed chromatography. With these developments, the industry is capable of producing the popular HFCSs.

Low DE syrups (maltodextrins) The low DE or maltodextrin-rich syrups are industrially produced by acid hydrolysis or by  $\alpha$ -amylase conversion. These syrups are the easiest to manufacture and are the first step for the production of maltose, glucose, and HFCS. The acid hydrolysis is performed in 35–40% starch suspensions using a 0.02–0.2 N hydrochloric solution in a pressurized reactor. A higher acid concentration or

longer process time can yield undesirable compounds such as methylfurfural, formic acid, and/or off-flavors and off-colors. That is the reason why use of this technology is now limited. The most popular way to convert starch into maltodextrins is via the utilization of α-amylases or liquefying enzymes. Today, heat-stable α-amylases are used because they require shorter incubation. Their optimum activity is achieved at pH of 6.5 and temperatures of 90–100°C. These syrups with 10–20 DE are very viscous, rich in maltodextrins and low in sweetness; therefore, they are mainly used as thickening agents. Regular cornstarch produces haze formation, whereas utilization of waxy starch, more stable syrups. A high dextrin syrup adjusted to contain 15% solids, and pH 5-6 is used as substrate for CGTáse for cyclodextrin production. These cyclic oligosaccharides are composed of six, seven, or eight glucose units linked by  $\alpha$ -(1-4) linkages to form  $\alpha$ -,  $\beta$ -, or  $\gamma$ -cyclodextrins, respectively. Cyclodextrins have several useful properties. They have good chemical stability to bases and weak acids and good thermal stability because they melt at temperatures of 270°C. Their hygroscopicity is low and are resistant to amylolytic enzymes. In addition, they are used to enhance chemical stability and reduce volatility of complexed molecules, and mask unpleasant odors and flavors.

**Maltose syrups** Regular and high maltose corn syrups are manufactured starting with a low DE syrup that is treated with  $\beta$ -amylase or a combination of

Table 3 Nutritional value of different types of corn-based foods (100 g)

Food	Moisture	Energy	Protein	Fat	СНО	Dietary	ı	Mineral	s		B-vi	tamins	
	(g)	(kcal)	(g)	(g)	(g)	fiber (g)	Ca (mg)	P (mg)	Fe (mg)	B1 (mg)	B2 (mg)	B6 (mg)	Niacin (mg)
Miscellaneous products													
Corn on the cob with butter	72.1	106	3.1	2.4	21.9		3	74	0.6	0.17	0.07	0.22	1.49
Yellow sweet corn, boiled and drained	76.7	80	2.8	0.4	19.6	2.4	4	57	0.4	0.08	0.07	0.13	1.30
Cooked corn grits	85.3	60	1.4	0.2	13.0	0.2	0	12	0.6	0.10	0.06	0.02	0.81
White hominy, canned	82.5	72	1.5	0.9	14.2	2.5	10	35	0.6	0.00	0.01	0.01	0.30
Corn pudding	76.3	109	4.4	5.3	12.7		40	57	0.6	0.41	0.13	0.12	0.99
High-fructose corn syrup	24.0	281	0	0	76.0	0	0	0	0	0	0.02	0	0
Bourbon whiskey, 86° proof	63.9	250	0	0	0.1	0	0	5	0	0	0	0	0.1
Flat and leavened breads													
Tortillas	44.1	222	5.7	2.5	46.6	5.2	175	314	1.4	0.11	0.07	0.22	1.50
Corn bread	39.1	266	6.7	7.1	43.5		249	169	2.5	0.29	0.29	0.11	2.25
Corn muffins	32.6	305	5.9	8.4	50.9	3.4	74	284	2.8	0.27	0.33	0.08	2.04
Hush puppies	29.0	337	7.7	13.5	46.0	2.8	278	189	3.0	0.35	0.33	0.10	2.78
Arepas	55.9	176	4.2	1.6	37.0		29	32	0.5	0.02	0.03		0.10
Hallaquitas	70.0	126	3.0	1.3	25.2		39	14	2.3	0.01	0.02		0.10
Breakfast cereals													
Corn flakes	3.0	361	7.0	8.0	86.0	3.5	7	50	30	1.30	1.53	1.80	17.9
Corn chex	2.2	373	7.0	0.9	86.0	2.0	333	72	30	1.25	1.42	1.7	16.7
Corn pops	3.0	380	3.7	0.7	90.0	8.0	17	31	6.2	1.20	1.40	1.60	16.1
Kix	3.2	389	9.0	2.3	82.6	1.4	125	138	28.6	1.3	1.5	1.8	17.6
Snack foods													
Extruded cones	2.0	510	5.8	26.9	62.9	1.1	3	44	2.5	0.32	0.24	0.04	1.41
Extruded puffs, cheese flavored	1.5	554	7.6	34.4	53.8	1.1	58	108	2.4	0.26	0.35	0.13	3.23
Cornnuts, plain	1.3	439	8.5	14.1	73.3	6.9	9	275	1.7	0.04	0.13	0.23	1.69
Corn chips, plain	1.0	539	6.6	33.4	56.9	4.9	127	185	1.3	0.03	0.14	0.24	1.18
Tortilla chips	1.8	501	7.0	26.2	62.9	6.5	154	205	1.5	0.08	0.18	0.29	1.28
Light tortilla chips, nacho flavor	1.3	445	8.7	15.2	71.6	4.8	159	318	1.6	0.22	0.27	0.23	0.41
Popcorn, oil popped	2.8	500	9.0	28.1	57.2	10.0	10	250	2.8	0.13	0.14	0.21	1.55
Popcorn, caramel coated	2.8	431	3.8	12.8	79.1	5.2	43	83	1.7	0.07	0.07	0.03	2.20

Data from: Fontana Nieves H and Gonzalez Narvaez C (2000) El Maiz en Venezuela. Caracas, Venezuela: Fundación Polar and USDA (2003) http://www.nal.usda.gov/fnic.

pullulanase and  $\beta$ -amylase (Figure 2).  $\beta$ -Amylase works best at pH 5 and 55°C. The utilization of only  $\beta$ -amylase yields a syrup with 50–55% maltose, while the use of de-branching pullulanase and  $\beta$ amylase syrups results in syrups with  $\sim 80\%$  maltose. Regular and high maltose syrups are widely used as flavorings for breakfast cereals, beverages, and other food products.

Glucose syrups For production of 90 DE glucose syrups, low DE syrups are treated with amyloglucosidase or saccharifying enzyme at pH 4.6-5.2 and a temperature of  $55-60^{\circ}$ C (Figure 2). The syrup is refined and clarified through columns of activated carbon and ionic resins with the aim of removing minerals, pigments, soluble protein, fat, and enzyme resistant starch. Glucose syrups are utilized as sweeteners for soft drinks, baking formulations, and

as a source of fermentable carbohydrates for light beer, alcohol production, and yeast-leavened baking goods. Crystallized dextrose can be produced by concentrating the syrup to 75% solids, adding glucose, and gradually cooling to drop the temperature to 20−30°C for several days.

High-fructose corn syrups HFCSs are manufactured starting from a 90 DE glucose syrup that is further treated with immobilized glucose isomerase (Figure 2). The refined glucose syrup is de-aerated and treated with magnesium sulfate so as to assure oxygen removal and the sequestration of calcium that lowers enzyme activity and half-life. There are three major types of HFCSs: 42, 55, and 90. The 55 and 90 HFCSs are produced from the 42 HFCS. The 42 HFCS is industrially produced by passing glucose syrup through a reactor with glucose isomerase. The

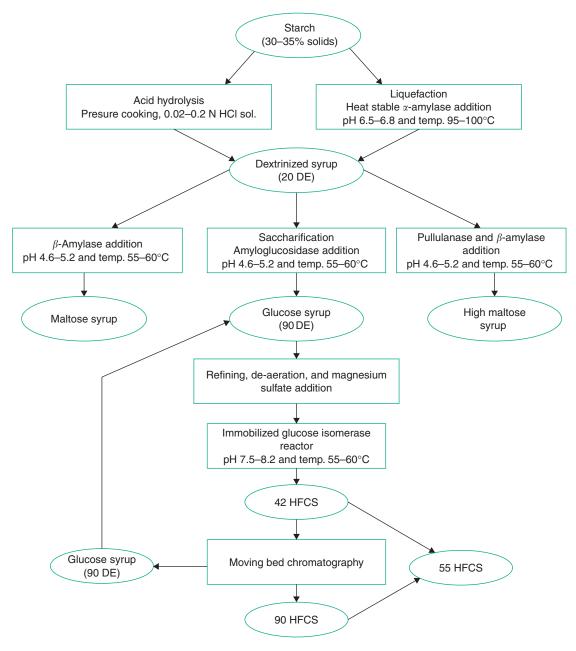


Figure 2 Flowchart of industrial processes to produce corn syrups.

optimum operating conditions are pH 7.5-8.2 and 55–60°C. The substrate flow rate is controlled so as to convert 42–45% of the glucose into fructose. The 90 HFCS is obtained after separating fructose and glucose in a moving bed chromatography system that produces two syrups with ~90% of each type of sugar (Figure 2). If desired, the glucose-rich syrup can be converted into 42 and 90 HFCS as explained before. The 55 HFCS is manufactured by mixing 42 and 90 HFCSs. The 90 HFCS has ~1.7 times more sweetness than the other HFCSs and crystallized

sugar. HFCSs are used as substitutes of table sugar especially in soft drinks. The main advantage of using HFCS is that they readily dissolve in water, are easily flavored, and impart a sweet fruit flavor to beverages and foods.

#### **Food Uses of Dry-Milled Fractions**

Endosperm products from corn dry milling, ranging from large grits to flour, are widely used by brewers, snack food, and breakfast cereal processors.

Flaking grits Flaking grits are large (US 3.5–6 mesh sieves) endosperm particles obtained after maize degerming (see Maize: Dry Milling). These large pieces of endosperm are almost exclusively used to manufacture cornflakes by the traditional way, the most popular ready-to-eat breakfast cereal. The fundamental process has remained relatively unchanged over the past century (see Cereals: Breakfast Cereals). Yellow maize is preferred because of its stronger flavor and rich golden color after toasting. The flaking grits are pressure-cooked in rotary cookers for 1–2 h with syrup, sugar, nondiastatic malt, salt, and water. The cooked grits are conveyed to a delumping equipment and dried at 66°C to lower its moisture to 20%. Then, the grits are equilibrated for 6-24 h in a bin and flaked through a pair of counter-rotating rollers. The resulting soft flakes are toasted in a gas-fired oven in order to develop the crisp texture, brown color, and the characteristic flavor. The flakes are sprayed with nutrients and equilibrated. The typical nutritional value of cornflakes is presented in Table 3. Sugarcoated flakes are made by the same process, but the sweetener is sprayed onto the flakes after toasting. Cornflakes can also be made from meal or grits that are extruded to produce pellets (see Extrusion Technologies).

Corn grits Corn grits are low in fiber and contain less than 1% oil. Grits of various granulations are widely utilized by the snack, breakfast cereal, and brewing industries. In the USA, maize grits are consumed as a side dish for breakfast (Table 3). The grits are cooked in boiling water for 10–25 min and then seasoned with butter or margarine. Instant or precooked maize grits which require only 5 min cooking are popular. In southern states, white maize grits are preferred over the yellow counterpart because they possess a bland and sweeter flavor.

Traditionally, the brewing industry has been the largest user of corn grits. Grits are used as a source of inexpensive fermentable carbohydrates. Brewer grits have a size range which facilitates filtration of the wort. The most desirable grits contain less than 1% oil, ash, and fiber, have optimum particle size distribution, and high malt extracts.

Whole ground maize and refined grits are used to produce alcoholic beverages. Straight bourbon whiskey is obtained from distillation of a fermented mash containing at least 51% maize in the United States. The distillate is aged for 2–10 years in wooden barrels, blended, and bottled.

Maize alone or in combination with other cereals and ingredients is often used in ready-to-eat breakfast foods (see Cereals: Breakfast Cereals). Grits, meals, or flours are cooked to gelatinize the starch, denature

the protein, and produce a dough, which can be processed into flakes, shreds, granules, puffs, or collets. The nutritional value of several maize-based breakfast cereals is summarized in Table 3. Desirable flavor, aroma, and texture are usually obtained by controlled toasting. Currently, most new breakfast cereals are prepared by continuous extrusion and puffing which has economic advantages. Maize flour or meal is moisturized, combined with starches, flavorings, and coloring agents to prepare a wide array of products. Extruded gun-puffed cereals are still one of the most popular types of ready-to-eat breakfast cereals. The puffing gun is charged with equilibrated collets, sealed, and heated to increase the pressure to 150-200 psi. The pressure is suddenly released and the material explodes from the gun. The degree of puffing varies according to temperature and pressure. The expansion varies from 10 to 16 times.

Products that require less expansion are generally oven puffed. Puffing is achieved by exposing the product to radiant heat on a belt or by tumbling it in a rotating cylinder. This process produces a three to fourfold expansion. The oven puffing operation requires the correct balance of moisture content and oven temperature to achieve the desired puffing.

Extruded snacks are a growing segment of the maize-based snack market (see Extrusion Technologies. Snack Foods, Processing). Corn meal or grits are processed through extrusion cooking and puffing to produce maize curls, puffs, and balls (Table 3). The shape of the puffed extrudate is determined by the die, operational temperature, cut-off knife speed, and other factors. Extrudate expansion is closely related to product texture and is affected by the viscoelastic nature of the material and the amount of moisture in the material flowing through the extruder die assembly. The extrudates are baked or fried, flavored, and packaged to produce the final ready-to-eat product. Mouth feel is affected by oil content of the products.

Collets or half products can be produced by conventional methods, i.e., macaroni presses, or by using a combination of two extruders. The first extruder cooks the raw ingredients while the second cools, forms, and sizes the extrudate into dense collets, which are dried, stored, and then baked or fried into the final product called third generation snacks. Baked collets are crisp with a light, crunchy texture. Deep fat fried collets are crisp with smoother texture due to the uptake of the oil. Pre-extrusion moisture content of the meal is critical in determining the characteristics and texture of the product. As the moisture content is increased, extrusion cooking temperatures generally drop, and a dense, less expanded product is obtained. High moisture meals produce hard, dense

extrudates that are generally fried. Meals with low moisture produce expanded extrudates that are generally baked to form a light textured puffed snack.

Large, state-of-the-art plants commercially produce instant arepa flours that require only hot water to produce the dough. Maize grits are cooked and then passed through flaking rolls to gelatinize the starch. The flakes are dried and ground into flour with acceptable granulation. With precooked maize flour, are pascan be prepared in  $\sim 30$  min instead of 12-24 h required by the traditional process.

Maize meal and flour Corn meals and flours have smaller granulation than grits and are popular products because of their long shelf-life, freedom from black specks, and bright color. Maize meal is often enriched with thiamin, riboflavin, niacin, and iron. It is used to produce an assortment of chemically leavened baked and fried products such as corn bread, muffins, pancakes, cornsticks, fritters, hush puppies, and spoon bread (Table 3). Most maize bread formulations contain wheat flour, chemical leavening agents, sugar, salt, milk powder, and other ingredients. Maize does not have a functional gluten so wheat

flour is included to give the dough more elasticity and hence produce a more aerated lighter product. Hush puppies are produced from a chemically leavened dough which contains maize meal, wheat flour, eggs, milk, salt, onions, and tomato. Pieces of dough are deep fat fried for 2–3 min. Maize flour is also widely used as an ingredient in many formulations, for breadings and batters and as a binder in processed meats.

#### **Food Uses of Nixtamalized Products**

Three basic types of products are industrially produced from lime-cooked maize: table or soft tortillas, corn chips, and tortilla chips (Figure 3). Corn and tortilla chips are primarily produced and consumed in developed countries, where they have an important share within the salted snack food market.

Table tortillas Tortillas and masa products constitute the staple food for large population in Mexico and Central America. Tortillas are produced using traditional and industrial processes. Tortillas are the main source of energy, protein, calcium, and other important nutrients in Mexico and Central America

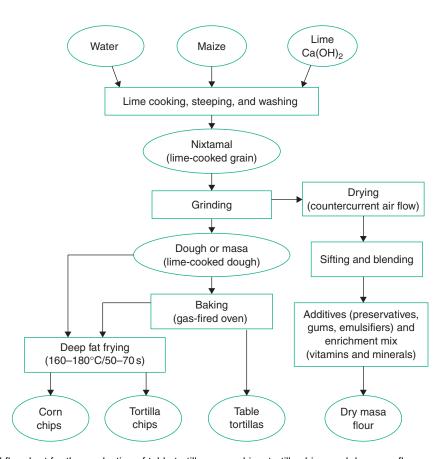


Figure 3 General flowchart for the production of table tortillas, corn chips, tortilla chips, and dry masa flour.

(Table 3). Lime cooking considerably increases calcium and the bioavailability of niacin, and significantly decreases the potential for aflatoxins in contaminated maize.

The industrial production of maize tortillas is labor intensive and requires considerable equipment (see Tortillas). It starts when the grain is lime-cooked in agitated open baths, vertical cookers, or steam kettles. The grain is generally mixed with 3 parts water and 1% lime, based on grain weight, and cooked for 15– 45 min at temperatures ranging from 85°C to 100°C. The nixtamal is then steeped for 8–16 h in the hot lime solution. The cooking liquor is drained and the nixtamal washed with pressurized water. Most of the pericarp and excess lime is removed during this step. The cleaned nixtamal is discharged into a stone grinder, where it is disrupted into a plastic and cohesive dough or masa. Masa is then kneaded by mixers or extruders that feed the forming machine or sheeter rolls. During forming, the masa is rolled into a sheet, which is cut by a rotating cutter positioned underneath the rolls. The formed pieces of masa are fed into a three-tier, gasfired oven for baking (temperature ranging from 280°C to 302°C for 30–45 s), then cooled through a series of open tiers and packaged. Tortillas are generally treated with gums, emulsifiers, and acidulants and antimycotics (e.g., sorbates and/or propionates) to improve textural and microbial shelf life.

Nixtamalized dry masa flours The use of dry masa flour is rapidly growing because of its convenience. Dry masa flour is produced by drying and grinding lime-cooked, coarsely ground masa (Figure 3). The masa is dried in large dryers in which warm air flows countercurrently to the pieces of masa. A wide array of products can be manufactured by selecting and blending streams with different particle size and color. In most cases, resulting flours are enriched with B-vitamins and trace minerals. Coarser flours with lighter colors are required for snacks. The flour with less than 10% moisture is shelf-stable and only requires water (1.1-1.21) water per kg flour to form masa for further processing. Many manufactures use dry masa flour because it does not require much labor, equipment, or space, and processors do not have to worry about effluent disposal and control of scheduling and manufacturing practices.

Fried/snack products Frying has expanded the market for masa-based foods because the final product has excellent organoleptic properties and a long shelf life. The two most popular snacks, tortilla and corn chips, are usually made from coarsely ground fresh masa or masa flours. Corn chips are produced directly from masa (Figure 3) and contain more oil than tortilla

chips (Table 3). Tortilla chips are baked similarly to tortillas before frying. Tortillas chips absorb less oil, have a firmer texture, and a stronger corn flavor than corn chips. Nixtamal for these snacks is generally cooked less than for table tortillas, and it is ground into coarse masa, which allows steam to escape through the many small pores during baking and frying. This prevents the formation of serious quality defects, such as oily appearance and blistering. Masa for corn chips is extruded through a die, and cut by rotating knives before frying. Masa for tortilla chips is formed into triangles, strips, or circles before baking, equilibrating, and frying. Frying temperatures and times range from 165°C to 195°C and from 50 to 90 s. Corn and tortilla chips are often salted and flavored immediately after frying. Most popular flavorings include nacho-cheese, hot/spicy, barbecue, lemon-salt, and jalapeño. Special processes to produce baked low-fat tortilla chips that combine air impingement, infrared, and microwaves have been developed.

See also: Fortification of Grain-Based Foods. Labeling of Grain-Based Foods. Nutrition: Guidelines for Grain-Based Foods.

# **Further Reading**

Fontana Nieves H and Gonzalez Narvaez C (2000) El Maiz en Venezuela. Caracas, Venezuela: Fundación Polar. Hallauer AR (2000) Specialty Corns. Boca Raton, FL: CRC

Johnson LA (2000) Corn: the major cereal of the Americas. In: Kulp K and Ponte JG, Jr (eds.) Handbook of Cereal Science and Technology, 2nd edn. New York: Marcel Dekker.

Kearsley MW and Dziedzic SZ (1995) Handbook of Starch Hydrolysis Products and Their Derivatives, 1st edn. Cambridge, Great Britain: Blackie Academic and Professional.

McDonough CM, Gomez MH, Rooney LW, and Serna-Saldivar SO (2001) Alkaline cooked corn products. In: Lusas E and Rooney LW (eds.) *Snack Foods Processing*, 1st edn. Lancaster, PA: Technomic Publishing.

Rooney LW and Serna-Saldivar SO (2003) Food uses of whole corn and dry milled fractions. In: White P and Johnson L (eds.) Corn Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Serna-Saldivar SO, Gomez MH, and Rooney LW (1990)
The technology, chemistry and nutritional value of alkaline cooked corn products. In: Pomeranz Y (ed.)
Advances of Cereal Science and Technology, vol. 10.
St. Paul, MN: American Association of Cereal Chemists.

Steinkraus KH (1983) Handbook of Indigenous Fermented Foods, vol. 9. New York: Marcel Dekker.

Wacher MC and Lappe P (1993) Alimentos Fermentados Indigenas de Mexico. Mexico, DF: Universidad Autonoma de Mexico.

Whistler RL, Bemiller JN, and Paschall EF (1984) *Starch: Chemistry and Technology*, 2nd edn. Orlando, FL: Academic Press.

#### **Relevant Website**

http://www.nal.usda.gov - USDA (2003).

# MILLET

Contents

**Pearl** 

Minor

# **Pearl**

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#### Introduction

Pearl millet, commonly known as bulrush millet (*Pennisetum glaucum* (L.) R. Br.), also classified as *P. typhoides*, *P. americanum*, or *P. spicatum*, is a cultivated, small-grain, tropical cereal grass. Vernacular names include: "bajra" (India), "gero" (Nigeria, Hausa language), "hegni" (Niger, Djerma language), "sanyo" (Mali), "dukhon" (Sudan, Arabic), and "mahangu" (Namibia). Pearl millet is quantitatively the most important millet, with world annual production ~14 million tons (Mt). It is cultivated mainly in the semiarid tropics, almost exclusively by subsistence and small-scale commercial farmers.

This article will examine pearl millet production and agronomic issues, grain structure, chemistry, and nutritional aspects, primary processing technologies, important foods and beverages produced from pearl millet, pearl millet's future prospects, and research and development challenges.

# **Production and Agronomic Issues**

Pearl millet's great advantage over other cereals is the fact that it can be and is cultivated in areas of very low rainfall  $(300-500 \, \text{mm})$  per year) and very high temperatures (day time temperatures above  $30^{\circ}$ C). Its importance in food security in dry, marginal agricultural areas is immense. It is estimated that some 500 million people in the semiarid tropics depend on pearl millet as a food.

The major areas of pearl millet cultivation are India and northern Africa. It is also cultivated in eastern and southern Africa. In northern Africa there is a broad band of cultivation from Senegal in the west, through Nigeria and Niger to Sudan in the east. Similarly, but on a smaller scale in southern Africa, there is a band of cultivation from Angola and northern Namibia, through Zimbabwe and northern South Africa to Mozambique.

Figures for pearl millet production are only approximate. Some of the available data do not distinguish between the different types of millets and even between millets and sorghum. Also as pearl millet is generally a subsistence crop and not traded, figures are not always available. Pearl millet accounts for about half of the world millet crop, which is  $\sim$ 28 Mt per year. The major pearl millet producing countries are: India ~6.2 Mt per year, Nigeria 4.5 Mt, Niger 1.9 Mt, Burkina Faso 0.8 Mt, Mali 0.7 Mt, and Senegal 0.6 Mt. However, these data belie its importance in some other countries. For example, in Namibia pearl millet is the staple food in the northern, most populous part of the country. The average production is only 65 000 t, but this has to be seen in perspective as Namibia's population is less than 2 million.

Yields are in general very low, on average  $\sim$ 750 kg ha<sup>-1</sup>. This is because pearl millet is grown in areas of low-rainfall, high-temperature, on light, well-drained soils. Generally traditional farming practices are used with very low inputs, i.e., no biocides or inorganic fertilizers and little application of organic fertilizer, and use of traditional landraces. Pearl millet has, however, much greater potential. In areas where new varieties, some irrigation, and higher input agriculture are used, yields can be well in excess of 2 t ha<sup>-1</sup>.

Postharvest practices also remain backward in many areas, e.g., manual threshing of the grain (Figure 1). Pearl millet can also suffer severe storage losses, particularly as a result of insects such as the rice moth (Corcyra cephalonica). The larvae contaminate the grain by producing webbing (silk) and consume the protein- and fat-rich grain germs. Control is traditionally effected by putting wood ash in the storage bin. Today, application of the fumigant phostoxin (active ingredient phosphine gas) is becoming more widespread.

# Grain Structure, Chemical Composition, and Nutritional Value

#### **Grain Structure**

Pearl millet grains are tear shaped to ovoid (Figure 2). They are up to 2 mm in length and the 1000 kernel weight is in the range  $3-15\,\mathrm{g}$  (typically  $\sim\!8\,\mathrm{g}$ ), about one-quarter that of a wheat grain. The overall grain color varies from pearly white (hence the name) to yellow, slate gray, brown, or purple. Individual grains are often not uniformly colored.

The general grain structure is essentially the same as the other major tropical cereals, maize and sorghum (Figure 3). The kernel is naked, i.e., it generally threshes free of the hull. A feature characterizing pearl millet grain structure is the proportionally very large germ, and hence relatively smaller endosperm. This impacts on the chemical composition of the grain. The endosperm comprises two major components: the outer corneous (also referred to as vitreous or hard) endosperm and the inner floury (also referred to a soft) endosperm. The cells of the corneous endosperm are filled with a continuous matrix, without airspaces of proteins comprising protein bodies and matrix protein. It contains few starch granules. In contrast, the cells of the floury endosperm have airspaces in them and contain relatively many more starch granules and less protein. The pericarp is variable in thickness, dependent on variety, and comprised of three layers: the epicarp, mesocarp, and endocarp. The mesocarp apparently may contain starch granules such as sorghum grain. Beneath the pericarp is a seedcoat, which may be pigmented and beneath this is the aleurone layer, part of the endosperm, which is one cell thick. The kernel is enveloped in a waxy cutin layer, which protects the grain against weathering.

#### **Chemical Composition and Nutritional Value**

The general chemical composition and major nutrient contents of pearl millet grain are given in Table 1. It should be noted that these values refer to the whole, unprocessed grain. Processing by milling and malting will affect both the content and availability of many nutrients.



Figure 1 Manual threshing of pearl millet in northern Namibia.

Energy Whole pearl millet grain has a high energy content, in the range ~1646-1691 kJ per 100 g dry basis (db), compared to all other cereal grains except maize. This is due to the high fat content of these two grains.

Carbohydrates As with all other cereals, the predominant carbohydrate in pearl millet is starch. The starch content, typically  $\sim 71-72\%$  (db), is perhaps slightly lower than most other cereals, due to the fact that the germ of pearl millet grain is large and hence the endosperm is smaller. The percentage amylose in pearl millet starch is in the range  $\sim 17.0-21.5\%$ , which is relatively low for normal starches. However, waxy, 100% amylopectin (amylose-free) pearl millet mutants have not been found as yet. The starch

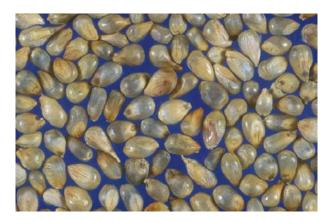


Figure 2 Pearl millet grain. (Courtesy of L A M Pelembe.)

gelatinization temperature is in the range  $61-69^{\circ}$ C, typical of tropical cereal starches.

Soluble sugars in pearl millet grain, as in all sound cereal grains, are low, in the range  $\sim 1.4-2.8\%$ , with the major sugars being sucrose and raffinose.

The dietary fiber content of pearl millet grain,  $\sim 8-$ 9% (db), surprisingly appears to be lower than most

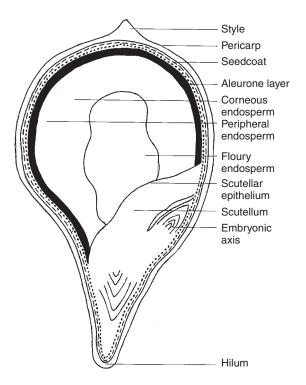


Figure 3 Diagrammatic longitudinal section through a pearl millet grain. (Courtesy of S C Barrion.)

Table 1 Proximate chemical and nutrient composition of pearl millet grain

Proximate composition (db) (g per 100 g) <sup>a</sup>		Minerals (db) (mg per 100 g) <sup>b</sup>		Essential amino acids (g per 16 g N) <sup>c</sup>	
Food energy (kJ)	1646-1691 (1733)	Ca	41	Isoleucine	3.9-4.6 [4.6] <sup>d</sup>
Protein	8.6-19.4 (14.5)	CI	47	Leucine	9.5-12.4 [9.3]
Starch	63.1-78.5 (71.6)	Cu	0.5	Lysine	2.8-3.2 [6.6]
Fat	1.5-6.8 (5.1)	Fe	10.8	Phenylalanine $+$ tyrosine	7.1-9.3 [7.2]
Dietary fiber	8.0-9.0 (8.5)	Mg	125	Threonine	3.3-4.1 [4.3]
Ash	1.6-3.6 (2.0)	Mn	0.8	Tryptophan	1.4-1.5 [1.7]
Vitamins (mg per 100 g) <sup>b</sup>		Р	373	Valine	4.9-6.0 [5.5]
Vitamin A (RE) <sup>e</sup>	24	K	460	Cystine + methionine	3.4-4.4 [4.2]
Thiamin	0.3	Na	17	•	
Riboflavin	0.2	Zn	2.4		
Niacin	2.9				
Vitamin E	1.9				

<sup>&</sup>lt;sup>a</sup>Range and typical values.

<sup>&</sup>lt;sup>b</sup>Typical values.

 $<sup>^</sup>c$ Typical range.

dIdeal pattern for infants.

<sup>&</sup>lt;sup>e</sup>Retinol equivalents.

other cereals, despite the fact that the grain is relatively small. This should mean that the proportion of bran is higher. However, this dietary fiber value may be an underestimate since dietary fiber determination is notoriously complex. The dietary fiber of pearl millet, as with sorghum, is mainly of the insoluble (water-unextractable) type. Thus, it has benefits in terms of preventing constipation, but probably does not have the cholesterol lowering effect of soluble fiber.

Proteins and amino acids Pearl millet grain protein content, typically 14.5% (db), is high for cereal grains. This is because the grain has a large germ. This, in turn, also affects the balance of Osborne protein groups and the overall amino acid composition of the pearl millet protein. Considering the protein fractions, the prolamins (aqueous alcohol soluble) proteins, 31–41% of total protein, are as in most other cereals the major fraction in pearl millet. However, this level is lower than most other cereals as prolamins are endosperm-specific proteins. In contrast, the amount of albumins and globulins (saline soluble) proteins, 25–26% of total proteins, is probably somewhat higher than in most other cereals.

Concerning the prolamin proteins themselves, in pearl millet they are called pennisetins and are similar to the prolamins of maize and sorghum, with high contents of glutamate+glutamine (23–24 wt.%), alanine (8-9 wt.%), and leucine  $(\sim 14 \text{ wt.}\%)$ , but relatively low in proline (~8 wt.%) and cystine and methionine (~1 wt.% each). Sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions shows a major band of  $M_r \sim 22$  kDa and minor bands of  $M_r \sim 20$  and 10 kDa. Research indicates that, unlike the situation with sorghum, wet cooking, as occurs in porridge making, does not reduce the digestibility of pearl millet protein. The reason for this is not known, since the reduction in sorghum protein digestibility is believed to be due to cross-linking of the prolamin proteins, which, as stated, are very similar to those in pearl millet.

The amino acid composition of pearl millet protein (Table 1) is characterized by a relatively high content (compared to most cereal grains) of the essential amino acid lysine, in the range 2.8–3.2g per 16 g N. This is on account of the relatively high proportion of lysine-rich albumin and globulins, due to pearl millet's large germ. However, the lysine content of pearl millet by no means meets the requirements of infants (or for that matter school age children), although it is satisfactory for adults. This emphasizes the importance of supplementation of cereals in the diet with meat, dairy, or legumes, as sources of lysine. Pearl millet protein is, as shown, a satisfactory source of the other essential amino acids.

Fats The fat content of pearl millet grain, typically 5.1% (db), is high compared to all other cereal grains, except maize. In the case of both pearl millet and maize the high fat content is due to the large germ. As with all other cereals, the major fatty acids are linoleic acid (C18:2), typically 43–45% of the total, oleic acid (C18:1), 26–27% of the total, and palmitic acid (C16:0) 20–21% of the total. The high content of fat and in particular of polyunsaturated fatty acids means that whole grain pearl millet when milled is highly subject to deterioration through oxidative rancidity.

Vitamins Pearl millet, like other cereal grains, is an important source of vitamin B (Table 1). These are concentrated in the aleurone layer and germ. Hence, their content can be adversely affected by milling. Niacin occurs partially bound to carbohydrate. To make it fully available, the flour has to be treated with alkali. This is done by treatment with wood ash in the preparation of the West African porridge called "tô." Because of its high fat content, pearl millet is a good source of tocopherols (Vitamin E), located mainly in the germ.

Minerals Pearl millet grain, like other grains, is a good source of most dietary minerals (Table 1), with the exception of calcium. Quantitatively the highest contents are of phosphorus and potassium. However, the availability of the former and of the multivalent metal ions is negatively affected by the presence of phytate (see below). The minerals are concentrated in the pericarp, aleurone layer, and germ. Hence, their content can be adversely affected by milling.

#### **Antinutrients**

There is evidence that pearl millet contains goitrogens. It appears that the goitrogens are primarily phenolic flavonoid-type compounds, the C-glycosyl flavones: vitexin, glucosyl vitexin, and glucosyl orientin. Other phenolic compounds, such as phloroglucinol, resorcinol, and p-hydroxybenzoic acid, could also be involved. These compounds apparently inhibit the deiodination of the hormone thyroxine to its more active form triiodothyronine. They are concentrated in the outer layers of the grain and are considerably reduced when the grain is de-hulled during milling. In fact, the nutritional significance of the goitrogens in pearl millet should not be overstated. Although some rural people, e.g., in the Sudan, who consume pearl millet as a staple have been found to suffer from goiter, it is probable that this is because their diet is very restricted and hence deficient in iodine.

The C-glycosyl flavones contribute to the brown/gray color of pearl millet and also appear to be responsible for the characteristic musty flavor of damp pearl millet flour, which is probably of greater nutritional significance. Some people describe the flavor as being "mousy" or "mouse-dropping" like. In pearl millet foods, such as porridges, it can be rather disagreeable to those not familiar with the food.

Pearl millet, like all grains, contains the phosphorus-containing compound phytate, myo-inositol hexaphosphate. Phytate is believed to act as the main phosphorus store in seeds. In grain foods, it has the undesirable property of binding multivalent metal cations such as iron and calcium and rendering them biologically unavailable. The level of phytate in pearl millet grain, in the range  $\sim 172-327$  mg per  $100\,\mathrm{g}$ , is typical of cereal grains. Phytate in pearl millet, as in other cereal grains, is located in the aleurone layer and germ. Hence, de-hulling the grain during milling substantially reduces the level of phytate (but also the minerals) in flour. Malting specifically reduces the level of phytate by enzymatically degrading it, freeing the minerals.

It should be noted that contrary to what is written in some texts, pearl millet, unlike some varieties of sorghum, does not contain the antinutritional polyphenolic compounds, condensed tannins.

# **Primary Processing**

## Milling

Pearl millet is milled into flour for porridge and bread making. The milling process used in northern Namibia is shown in Figure 4 and will be described in detail as it has some unique features.

**De-hulling** To improve the palatability and storage quality of the flour, the bran (pericarp and germ) is generally removed from the grain first. This process of bran removal is referred to as de-hulling. Strictly speaking, decortication is the correct term since the pearl millet grain does not have a hull.

Figure 5 shows the equipment used in a small-scale commercial pearl millet mill in Namibia. In the background is a Prairie Research Laboratory (PRL) dehuller, named after the place where this equipment was first developed. Today, PRL-type de-hullers are manufactured in a number of countries in southern Africa. The PRL de-huller comprises an axle with ~12 carborundum disks mounted on it, within a cylindrical box. The axle revolves at high speed and the disks abrade off the bran from the grain. A suction fan removes the bran. The amount of de-hulling is determined by the duration of the de-hulling

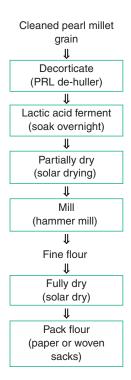


Figure 4 Pearl millet milling process used in northern Namibia.



**Figure 5** Small-scale commercial pearl millet mill in northern Namibia. Foreground – hammer mill; background – PRL de-huller.

process. Anything between 10% and 30% of the grain is removed, depending on the desired final color and fat content of the flour. De-hulling has the effect of reducing the fiber, fat, and protein content of the grain due to removal of pericarp and germ material. The quality of the protein will also be adversely affected as the quantity of the lysine-rich germ proteins is reduced. The starch content is increased slightly.

**Steeping** The de-hulled grain is steeped in ambient temperature water overnight. This steeping process is

actually a lactic acid fermentation. A lactic acid bacteria "culture," maintained by the process of "backslopping," is used to inoculate the steep water. The culture comprises a portion of the previous steep, which has developed a strong, clean acidic taste due to a high load of suitable lactic acid bacteria. The purpose of the lactic acid steep seems to be several fold. It lightens and brightens the flour. The low pH of the steep water is responsible for lightening the color and partially leaching out the brown/gray polyphenolic pigments of the grain. Goitrogens may also be leached out of the grain. However, it is probable that water-soluble proteins, vitamins, and minerals are lost in the steeping process. The steep softens the grain, facilitating its reduction into a fine flour. The resulting flour has an acidic flavor, which consumers prefer.

Solar drying The de-hulled, steeped grain is partially dried before milling, and after milling the flour must be fully dried to a shelf-stable moisture content of  $\sim 10\%$ . Solar drying is used for both steps. The grain or flour is dried on polythene sheets, either outdoors or under cover, on either directly on a concrete floor or on long tables made of wooden slats.

Milling The partially dried, de-hulled steeped grain is milled into a fine flour using a powerful hammer mill, fitted with an  $\sim$ 1 mm screen. The hammer mill has to be powerful on account of the high moisture content (30–40%) of the grain. Thus, the process is actually semiwet milling. This apparently rather inefficient process seems to be necessary in order to mill the grain into a suitably fine flour.

#### **Malting**

In sub-Saharan Africa, pearl millet is malted to produce malt for brewing traditional opaque beer and



**Figure 6** Pearl millet malt before drying. (Courtesy of L A M Pelembe.)

nonalcoholic beverages. Pearl millet malting is almost exclusively done in the home, although one large-scale commercial malting in Zimbabwe malts pearl millet together with sorghum. Figure 6 shows pearl millet malt prior to drying. It can be seen that there is extensive root and shoot growth. The optimum temperature conditions of pearl millet malting are in the range 24–28°C, identical to that for sorghum. The malting process involves three steps: steeping, germination, and drying.

The steeping time for pearl millet is short, up to 8 h, as the grain germinates rapidly. Germination is for 4–5 days. Like sorghum, pearl millet must be watered during germination. Drying is at ambient temperature or up to 50°C in order to conserve malt enzyme activity.

The pearl millet malt quality seems to be somewhat higher than that of sorghum malt. Diastatic power (total amylase activity) is similar,  $\sim 30-50$  sorghum diastatic units (SDU)  $g^{-1}$ . However, the activity of the  $\beta$ -amylase component is higher.  $\beta$ -Amylase is the enzyme that produces the fermentable sugar maltose. The higher  $\beta$ -amylase activity indicates that pearl millet malt could have potential as partial substitute for barley malt for brewing lager beer. Free amino nitrogen and extract are also higher in pearl millet malt than sorghum malt. Of particular significance is the fact that malting almost completely eliminates the mousy flavor associated with pearl millet flour. Some nutritional improvements also occur, in particular improvement in carbohydrate and protein availability. However, against this there is a substantial overall loss in mass of grain, 10–15%, due to respiration during germination.

## **Food and Beverage Production**

Worldwide, pearl millet is processed into many different food and beverages. Foods include: "rice" and flatbreads ("roti") in India, "couscous" in Mali and Senegal, thick porridge such as "to" in West Africa and "bogobe" in southern Africa, and thin fermented porridges ("uji") in eastern Africa, respectively. Beverages can be nonalcoholic like "oshikundu" in Namibia or alcoholic like the opaque beer of southern Africa.

#### **Foods**

**Porridge** In sub-Saharan Africa, pearl millet is often consumed as a porridge. A traditional Pedi (Limpopo Province, South Africa) recipe to make "bogobe bia bupi bja leotsa" (literally porridge of pearl millet) involves cooking ~1 kg of whole grain pearl millet meal in 1.9 l water. The porridge has greenish-brown

color, and a thick, smooth viscous consistency. Because of the high ratio of starch to water the porridge gels on cooling. The flavor is musty, sweet, and bitter. The meal ration size is  $\sim$ 1 kg, which provides some 525 kJ, 20–25% of an adult's "recommended dietary allowance." The porridge is traditionally eaten cool or cold with the hand. It is served with vegetable relish.

Flatbread In India, pearl millet is generally consumed as a crisp flatbread, called roti or "chapati." Figure 7 shows a flowchart for making roti. A problem in making breads from nongluten containing grains such as pearl millet is that the product tends to break into pieces. It is notable that in the milling process the flour is ground to fine particle size,  $\sim\!43\%$  having a particle size  $<\!75\,\mu m$ . This increases the surface area of the flour particles and damages the starch granules, increasing their water absorption. Both factors increase the cohesiveness of the dough. Variations on the roti-making process which also alleviate the problem can involve cooking part of the flour in water and mixing it together with the uncooked

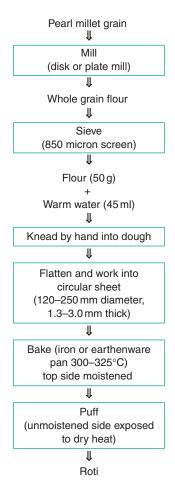


Figure 7 Roti-making process.

flour. Cooking gelatinizes the starch making it into a binder which improves the cohesiveness and elasticity of the dough. Alternatively, pearl millet flour can be composited with flours that give better cohesiveness such as legume flours or wheat flour which gives elasticity.

Because of the absence of gluten in pearl millet, breads made from pearl millet flour are unleavened. However, in the roti-making process, as shown in Figure 7, the flatbread is exposed on one side to high heat, which puffs it giving the bread some "leavened" texture. Puffing takes place as result of the fact that the moistening water added during baking is very rapidly turned into steam. The resulting increase in volume creates an air sac as the steam escapes.

Roti is served with a small amount of hot pickle, "dhal," or vegetable sauce.

#### **Beverages**

Throughout Africa, sorghum, maize, and millets are used to produce nonalcoholic and alcoholic beverages. Invariably, these beverages have undergone a lactic acid fermentation, giving them a refreshing sour taste. The low pH of the beverages renders them free from food-borne pathogenic bacteria and helps protect them against microbial spoilage.

Nonalcoholic A very popular nonalcoholic fermented beverage in Namibia is oshikundu made from pearl millet and sorghum malt flour. Some 200 g of pearl millet flour are thoroughly mixed into 500 ml of boiling water. Then  $\sim 100\,\mathrm{g}$  of sorghum malt flour is added. Sorghum malt provides amylase enzymes and lactic acid bacteria. α-Amylase thins the porridge into gruel by hydrolyzing the starch into dextrins and  $\beta$ -amylase further hydrolyzes the dextrins into maltose. The thinning and hydrolysis actions of the amylases improve food palatability and carbohydrate availability. The lactic acid bacteria use the maltose and other fermentable sugars for respiration, producing lactic acid and other flavor compounds. After the mixture has cooled to room temperature, 1-1.51 of cold water is mixed in. The container is closed and the mixture is allowed to ferment overnight before consumption. Oshikundu is greenish brown in color and has the consistency of drinking yogurt. It is slightly effervescent with a buttery sour taste. A lactic acid content of 0.6% has been measured.

Opaque beer In several southern African countries pearl millet malt is used to brew traditional, opaquetype beer. Figure 8 shows a flowchart for a Pedi recipe for making "bjalwa bja leotsa" (literally beer of pearl millet). The process has some interesting

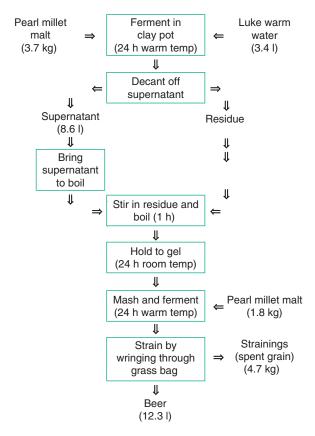


Figure 8 Pearl millet opaque beer-making process.

features. It is noteworthy that only malt is used in the recipe. In contrast to brewing traditional sorghum beer, no unmalted grain adjunct is used. In fact, part of the malt is used as adjunct by cooking it after the first fermentation process. The first fermentation process is probably primarily a lactic acid fermentation but some fermentable sugar production will also take place. As there is no unmalted adjunct in the recipe, it means that there is a proportionally higher ratio of amylase enzymes to starch, producing more fermentable sugars. Coupled with the fact that pearl millet malt intrinsically contains a higher ratio of  $\beta$ amylase, this probably accounts for the fact the pearl millet beer is claimed to be more intoxicating than sorghum beer. Typical of traditional recipes more than one process takes place during a particular step. This is unlike modern food and beverage manufacturing processes where there is a system of unit operations. In the mashing and fermentation stage, gelatinized starch is hydrolyzed by the malt amylase enzymes into fermentable sugars, which are fermented by both yeast and lactic acid bacteria to produce ethanol, carbon dioxide, and lactic acid as the main products. The beer is described as turbid, greenish-brown in color, with a milk-like consistency. The flavor is musty and bitter sour, with a lactic acid content of  $\sim$ 1.8%. Pearl millet beer could be much thinner and more acidic than sorghum beer.

# **Future Prospects and Challenges**

With the world faced by the threat of global warming and its resulting climatic changes, pearl millet with its unique ability to grow under hot and dry conditions should become a far more globally important food grain. In the developing world the need for food security demands that pearl millet increasingly becomes a commercially traded grain and is processed into food products, instead of just being a subsistence crop.

ICRISAT, the International Crops Research Institute for the Semi Arid Tropics, has helped develop pearl millet varieties with improved agronomic and processing qualities. In Namibia, for example, the "Okashana" improved varieties that are early maturing, higher yielding, and large grained have found wide acceptance among farmers, resulting a substantially increased pearl millet crop. This has resulted in a surplus over and above immediate household requirements, which is available for trade and processing. In turn, this has led to the growth of small-scale commercial milling operations, as shown in Figure 5.

Such developments can, however, only take place if governments create an enabling environment. In Namibia, the Ministry of Higher Education, with the assistance of the Food and Agriculture Organization of the United Nations (FAO), is implementing a very interesting local project for the development and promotion of new pearl millet products. In 2002, the Council for Scientific and Industrial Research in South Africa produced sample quantities of a range of concept products, such as instant porridges and snack foods. These were consumers evaluated in Namibia to determine the preferred concept products. At the same time a pearl millet food product manufacturing training facility has been constructed in northern Namibia. Food technologists at the facility will train entrepreneurs to manufacture these products. This project could serve as a model for other millet and sorghum-producing countries to follow.

Pearl millet scientific and technological problems still requiring further research and development include: finding a more efficient way of milling the grain to reduce losses, elimination of the "mousy aroma" in pearl millet foods, and determining whether there is really a significant goitrogen problem.

See also: Fermentation: Foods and Nonalcoholic Beverages. Grain, Morphology of Internal Structure. Grain and Plants, Morphology. Millet: Minor. Nutrition: Effects of Food Processing. Taxonomic Classification of Grain Species. Teff.

# **Further Reading**

Dendy DAV (ed.) (1995) Sorghum and Millets: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

FAO (1995) Sorghum and Millets for Human Nutrition. Rome: Food and Agriculture Organization of the United

ICRISAT and FAO (1996) The World Sorghum and Millet Economies. Patancheru, India: International Crops Research Institute for the Semi-Arid Tropics, Rome: Food and Agriculture Organization of the United Nations.

National Research Council (1996) Lost Crops of Africa, vol. I, Grains, pp. 77-125. Washington, DC: National Academy Press.

Obilana AB and Manyasa E (2002) Millets. In: Belton PS and Taylor JRN (eds.) Pseudocereals and Less Common Cereals, pp. 177-217. Berlin: Springer.

Quin PJ (1959) Food and Feeding Habits of the Pedi. Johannesburg: Witwatersrand University Press.

#### **Relevant Websites**

http://www.icrisat.org - Website of the International Crops Research Institute for the Semi Arid Tropics, one of the 16 CGIAR (Consultative Group for International Agricultural Research) centers. ICRISAT carries out science-based agricultural development in sorghum, pearl millet, finger millet, chickpea, pigeonpea and groundnut.

http://www.intsormil.org - Website of the USAID (United States Agency for International Development) supported International Sorghum and Millet Collaborative Research Support Program (INTSORMIL). The program works with host country scientists in developing new technologies to improve sorghum and pearl millet production and utilization worldwide.

## Minor

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Millet is a generic term implying "small seeded grain;" thus the variations in millet are quite large. The millets are all within the grass family (Poaceae or Gramineae), but include two tribes, Paniceae and Chlorideae (Table 1). The most widely grown millets include pearl millet (Pennisetum glaucum), proso millet (Panicum miliaceum), and foxtail millet (Setaria italica). However, smaller areas have meaningful production of brown top millet (Brachiaria ramosum), Japanese (Echinocloa frumentaceae), Shama (Echinocloa colonum), Australian (Echinocloa decompositum), and barnyard millet (Echinocloa frumentaceae). Finger millet (Eleusine coracana) is the most economically important member of the Chlorideae tribe, but teff (Eragrostis tef) plays a major role in Ethiopia. Black and white fonio millet (Digitaria iburua and Digitaria exilis, respectively), while grown in limited areas, are known for their good taste, short growing season and ability to produce on very poor soils. The variation represented by millets as a group, ranges from plants that grow to well over 4 m in height and take more than 150 days to reach maturity, to plants that seldom reach a height of more than 1 m and mature in less than 75 days. Production of millets can be found from the tropics, with rainfall averaging over 1200 mm per year, to the steppes of Siberia with rainfall averaging less than 300 mm. While all millets can be classified as cereals, in that they are grown for their edible starchy seeds, many are also important for annual forage production. Primary uses for millet grain vary as much as the species themselves, but include various forms of porridge for human consumption.

# Millet Importance and Production Areas

According to 2002 FAO reports, millet ranks fifteenth among all crops in terms of calories produced for human consumption. Of the minor millets, foxtail, proso, and finger millet account for the bulk of this production, but teff and fonio millets are regionally important. For example, a high portion of the cereal production of Ethiopia is dedicated to teff, and in West Africa, fonio is extremely important for the highly degraded soils (see Teff). Little millet (Panicum miliare or sumatrense) and kodo millet (Paspalum scrobiculatum) are primarily restricted to limited production in India and appear to be related to proso millet. Brown top millet (Brachiaria ramosum) has been used to a limited extent in India, but has played a role in the southeastern US as a cover crop and for wild bird hunting areas. Millet production data are elusive and frequently confusing because these sometimes include sorghum and the figures from subsistence agriculture production regions are often crude estimates. However, the total millet production probably provides a primary calorie source for more than 500 million people in the world. The bulk of this production is in China and India, with the next largest areas including West Africa and the former Soviet Union.

Table 1 Classification of millets, family Poaceae

Tribe	Genus	Species	Common names	Potential center of origin
Chlorideae	Eleusine	coracana (L.) Gaertn	Finger millet	Africa
Paniceae	Brachiaria	ramosum (L.) Stapf	Brown top millet	Africa
	Digitaria	exilis (Kipp.) Stapf	Fonio millet	Africa
	Echinocloa	colona (L.) Link	Shama millet	India
		frumentacea Link	Japanese, barnyard millet	China
	Panicum	miliaceum L.	Proso, common, hog millet	China
		<i>miliare</i> Lam.	Little millet	India
	Paspalum	scrobiculatum L.	Kodo, ditch millet	India
	Pennisetum	glaucum R. Br.	Pearl millet	Africa
	Setaria	italica (L.) P. Beauv.	Foxtail, Italian millet	China

Adapted from Rachie KO (1975) The Millets Importance, Utilization and Outlook. Hyderabad, India: International Crops Research Institute for the Semi-Arid Tropics.

# Millet Biology

Millets initially develop from seminal roots. These roots arise directly from the hypocotyl of the seedling. Further plant growth includes the development of a second set of roots, called the adventitious or crown roots, which form at a point on the plant just below the surface of the ground called the crown. Millet roots are characteristically fibrous rather than tap rooted like alfalfa or sunflower. Soil erosion is a limited problem with millet production because of this root system.

Alternate two-ranked leaves are the identifying characteristic of cereals in general, but the millets are differentiated, by most taxonomists primarily, on the basis of their inflorescence. The stems are composed of nodes and internodes which elongate to varying degrees as the crop matures. The lower nodes on a plant have the potential to develop additional stems, which are referred to as tillers. Millets have wide variation in their ability to tiller, and the ability to regrow when harvested as forage. This is a primary selection criteria once a particular end use is determined.

Millet seeds (correctly described as caryopses) are composed of three main parts, the endosperm, embryo (germ), and seedcoat (pericarp). Starch is the primary constituent of the whole grain, but is especially concentrated in the endosperm. Protein is found primarily in the embryo and to a lesser extent in the rest of the seed. Most of the oil is located in the embryo as well. The seedcoat consists mainly of cellulose and hemicellulose. Typical crude protein levels for the millets average  $\sim$ 12% on a dry matter basis.

#### **Proso**

Proso is the most widely grown millet grain crop in the US. It is well adapted to short-season production with both quick maturity and a low water requirement. It is

more cold-tolerant than either foxtail or pearl millet. Proso is considered a short-day plant and most US plantings are of an upright growth type with a relatively dense head compared with wild types. Recent studies suggest that proso plant development and maturity are heat-unit-driven across a wide range of day lengths.

Proso is considered to be diploid by some, but 2n = 4x = 36 is a more appropriate discussion. While pairing is relatively normal during cell division, many alleles follow a tetraploid inheritance pattern. Self-pollination dominates in proso, but natural crosspollination may exceed 10%. Proso seeds are smaller than grain sorghum (Sorghum bicolor L.), generally oval in shape and about 3 mm long and 2 mm wide. Seed size selection has led to an increase in seed size in popular varieties along with most varieties being a light cream color (referred to as white proso). Some niche markets exists for red types and the germplasm base covers the breadth of seed colors. Little millet may be related closely enough to proso millet to be used as a germplasm source, but carries few traits of known economic importance, being smaller seeded and less productive.

Most proso is swathed prior to harvest, allowed to dry and then combined. Selection has decreased seed shatter loss and increased uniformity of maturity so that increasing areas are harvested each year directly. Stripper headers and combines, better able to separate the green plant parts from the seed have, also helped to increase the amount of direct harvested proso millet. More primitive harvest techniques are still utilized in regions where proso is grown as a subsistence crop.

## **Pearl Millet**

Pearl millet (*see* Millet: Pearl) breeding has led to the development of some grain type hybrids adapted to US conditions, but most of the pearl millet produced in the

Table 2 Main uses of different millets

Millets	Food use	Feed use	Other use
Proso millet	Steamed buns, cake, dumpling, oil pudding, sour meal, and popping food (Chinese traditional millet foods), snack foods	Pet and livestock feed	Therapeutic/healthy use, brewing Chinese white or yellow millet wines and vinegar
Foxtail millet	Thin or thick porridges (sargati, sankati, etc.), boiled rice-like products, snack foods	Forage or grain feed	Therapeutic/healthy use, brewing material of Chinese five-grain vinegar
Finger millet	Porridges or sweet gruel, "chapati" and "soda" (Indian unfermented and fermented breads), popping meal, snack foods, sweetmeats, weaning foods	Animal feed	Brewing traditional African beer
Teff	Porridges, fermented flat bread (e.g., "injera" in Ethiopia and "kisra" in Sudan), unleavened flat bread ("kitta" in Ethiopia), cookies, muffins, waffles, soups, and pudding in Ethiopia	Forage or grain feed	
Fonio	Porridge, composite flour bread, popping food	Forage or grain feed	Brewing African local beer
Barnyard/Japanese millet	Rice substitute, Japanese traditional food		
Kodo millet	Food uses are the same as for foxtail millet in India		

Sources: Murty and Kumar (1995) Traditional uses of sorghum and millets. In: Dendy DAV (ed.) Sorghum and Millets: Chemistry and Technology, pp. 185–221. St. Paul, MN: American Association of Cereal Chemists; Corke and Lin (1998) Proceedings of the 1st International Conference on Asian Fooduct Development – Focus on Specialty Grains and Grain Products (September 6–10, 1998, Taiyuan, China). Beijing and New York: Science Press; Lin et al. (1998) Spotlight on shanxi province China: its minor crops and specialty foods. Cereal Foods World 43: 189–192; McDonough et al. (2000) The millets. In: Kulp K and Ponte JG Jr (eds.) Handbook of Cereal Science and Technology, 2nd edn., pp. 177–217. New York: Marcel Dekker; Dendy (2001) Sorghum and millets. In: Dendy DAV and Dobraszczyk BJ (eds.) Cereals and Cereal Products: Chemistry and Technology, pp. 341–366. Gaithersburg, Maryland: Aspen Publishers; and Obilana and Manyasa (2002) Millets. In: Beton PS and Taylor JRN (eds.) Pseudocereals and Less Common Cereals, pp. 177–217. Berlin: Springer; and authors.

US is for forage production. This is in stark contrast to areas of Africa where it is a primary grain crop. Pearl millet is adapted to more acid soils, higher temperatures, and higher humidity than proso. It is more susceptible to injury by cold temperatures and most genotypes have a relatively long growing season compared to proso.

## **Foxtail**

Foxtail (*Setaria italica*) is one of the world's oldest cultivated crops. Foxtail was the most important plant food in the neolithic culture in China, and its domestication and cultivation was the earliest identifiable manifestation of this culture. The US patent office introduced foxtail millet as a forage crop in 1849. It has since become well adapted to the western Great Plains. Nearly all foxtail millet cultivars grown in the US are the result of selections from land races rather than designed crosses and selections. Foxtail is recognized as a diploid (2n = 2x = 18), but is closely related to many tetraploid and higher ploidy level species.

Foxtail millet grown in the US is typically less than 1.5 m in height with a stem intermediate in size between proso and pearl millet. Head length is variable like pearl millet, but shorter and more lax. Foxtail

is self-pollinated for the most part and, with a compact panicle and small florets, it is extremely difficult to cross. Improved techniques have been developed by Melicio Siles and others, but limited directed genetic improvement has been made due to this constraint.

## Finger, Teff, and Browntop

Finger millet (*Eleusine coracana*) and browntop millet (*Brachiaria ramosum*) have been utilized on a small regional basis and have characteristics that offer potential for further development. Browntop is grown in the southeastern US primarily as a cover crop and for wild game feed. Finger millet has been explored as a grazing and forage crop, but is currently used on only a limited basis.

#### **Millet Uses**

Table 2 summarizes major uses of different millets. Millets have long been utilized as traditional staple foods for a large amount of the world's poor, especially in Asia and Africa. Currently, millets are consumed in northern China, India, Africa, and southern Russia, with ~80% of the world's millet production directly consumed as human food. Other utilization of

millets includes brewing use, therapeutic or health-food use, feed use (bird feed, livestock feed, forage, etc.), and mushroom production. Different millets have been used to process numerous food products, such as thick or thin porridges, steamed food products, cakes, fermented and unfermented breads, snacks, weaning foods, and alcoholic and nonalcoholic beverages, etc. These millet foods originated from various places of the world with unique local flavors and rich nutritional profiles. Moreover, there are differences in the details of recipes and preparation for each kind of millet-based food between communities and regions in different countries. Frequently, the same millet food may have several different names because of language and custom differences.

Foxtail millet is the most widely grown millet in China and millions of people depend on it as a primary calorie source. It is grown, to a lesser extent, in India, and throughout Europe and Asia.

In northern China, minor crops including millets and their specialty foods are preferred. Major millets in China include proso millet, foxtail millet, and finger millet. There are many local traditional foods made from proso millet and foxtail millet with a long history and special flavor, such as proso millet oil pudding, sour meal, steaming buns, sweet cakes, pyramidshaped dumpling, foxtail millet porridge, cooked rice-like foods, etc. These traditional millet foods have been and are still popular throughout the rural areas and towns of northern China and also in many major cities of northern China (especially in Shanxi, Shaaxi, Ninxia, Inner Mongolia, and Gansu). Popped yellow millet grains are used as a major component of local milk tea or butter tea (famous in Inner Mongolia). Waxy proso millet has been used to brew local white millet wine and yellow millet wine in various regions of northern China for  $\sim$ 2000 years. Millet grains are the brewing material base of Chinese five-grain vinegar (rice, buckwheat, millet, sorghum, and mungbean). Proso production is very important in China and the former Soviet Union, but is also used in Eastern Europe, the Middle East, India, and Southeast Asia.

India and some African countries (Nigeria, Niger, Sudan, Ethiopia, etc.) are important areas of world millet production. In India, most millets (e.g., proso millet, finger millet, barnyard millet, kodo millet, little millet, foxtail millet, etc.) are used for many kinds of foods, including porridges, boiled rice-like products, steam-cooked products, baking foods, snacks, weaning foods, composite flours mixed with other cereals, and pulses for making common foods. In Africa, many millets (e.g., pearl millet, teff, finger millet, fonio, etc.) are consumed in the form of thick and thin porridges, fermented or unfermented

flat breads, steamed or boiled cooked millet foods, snacks, and composite flours mixed with other cereals for breads, cookies, noodles, etc.

In India and Africa, millets are used or mixed with other cereals to make different local traditional foods. For example, pearl millet and finger millet are used to prepare "chapati" or "roti" (unfermented bread) and "soda" (fermented bread) in India and "ndaleyi" (local traditional food) in Nigeria. In Sudan and Ethiopia, fermented flat breads ("kisra" and "injera") can be prepared using both pearl millet and teff. Unleavened flat bread ("kitta") are made using teff in Ethiopia. These breads can be eaten in different ways for breakfast, lunch, and dinner. Also, they can be consumed with vegetables, sauces, milk, meat, curd, etc. Foxtail millet is used to make "sargati" (porridge) and pearl millet is blended with baobab flour to prepare "bulum mardam" (gruel) in India. Teff is employed to prepare "genfo" (stiff porridge) and "atmit" (thin porridge/ gruel) in central Ethiopia. Additionally, pearl millet, fonio, and finger millet are usually used or mixed with other cereals to brew traditional African alcoholic and nonalcoholic beverages with different local flavors, such as "chibuku" in Zimbabwe, "tchapalo" in Togo and "burujuto" or "pito" in Nigeria. Malting and brewing local beers using millets is significant in many countries of Africa, especially in eastern and southern Africa. Nonalcoholic beverages are also made from millets in West Africa.

Proso is produced throughout the central and northern Great Plains of the US with more than 170 000 ha produced annually. Nebraska, Colorado, North Dakota, South Dakota, Kansas, Wyoming, and Minnesota account for more than 90% of all proso production. Proso is the primary millet traded across national borders and most world trade figures for millet consist primarily of proso.

Interestingly, millets can be used for therapeutic purposes. Most millets are highly nutritious, for example, rich in protein, lipid, vitamins, and minerals compared to some other cereals. They also have a unique balance of amino acids that complements other cereals. In China and Japan, some food products made from proso millet, foxtail millet, and Japanese millet are considered to be functional or therapeutic foods to prevent and reduce incidence of certain human diseases. Regularly eating millets as a dietary and nutritional component in foods can reduce incidence of chronic human diseases. It is said that regularly drinking Chinese millet wine may improve human health, and yellow millet wine made in Shandong and Shanxi provinces of China is used for recovery of malaria patients. Special local millet foods (e.g., millet sour meal or porridges) are helpful in case of sunstroke. Japanese barnyard millet grains have

been used as basic food materials for patients with allergic disease, including atopic dermatitis in Japan. Also, African, Indian, and Russian reports indicated that millets have a higher glycemic index than wheat breads, rice, potato, maize, and cassava. Russian millet is promising for use by diabetics because of moderating influence on blood glucose level. It has been observed that millet consumption is related to a lower incidence of pellagra (a niacindeficiency disease).

Also, millet grains are commonly used as feed for pets and other animals. It is also extensively used for poultry feed and to a more limited extent as feed for other livestock. US proso is primarily consumed by the birdseed market, but poultry and other livestock are also major users. Human consumption, while prevalent elsewhere, accounts for a small proportion of US production. Foxtail millet has been playing an increasingly large role in wild bird feeding mixes in developed countries.

Many reports have shown that animals fed pearl millet, finger millet, proso millet, and foxtail millet grains generally had better performance in bodyweight and body-condition scores than those fed corn or sorghum. It has been particularly valuable in the poultry industry. Since millet usually has a premium price for human use and the bird seed trade, it typically only finds its way to livestock rations in years of excess production. Extensive research supports the use of all millet grains as livestock feed from a nutritional standpoint, but corn is frequently less costly.

One of the largest uses for millets is as forage. When green forage is harvested at plant heading up to the initiation of grain fill, the quality for livestock is excellent. With most millets, total energy per unit land area is maximized around heading. The millets are harvested for hay, green chop, silage, or grazed directly. Foxtail and pearl millet are primarily used as forages in the US, with foxtail millet being preferred as a hay crop and pearl millet preferred for grazing, but proso millet is also used as an emergency hay crop. Foxtail is easier to harvest for hay than pearl millet due to finer stems that cut and dry more readily. Under marginal conditions, due to limited rain or short growing season, it becomes the hay crop of choice. It is not as readily utilized for grazing with limited regrowth and a tendency to be pulled up by grazing animals. It is typically more productive as a forage than proso varieties, but has similar uses. Protein and energy values for millets have a very wide range with maturity and growing conditions, but protein levels of 12–15% with TDN levels greater than 65% are common when hay is put up at the heading stage of plant development. Crop residues or straw of proso, teff, fonio, finger millet, and foxtail millet are fed to livestock, and are considered to be valuable forage with protein and energy levels higher than wheat or rice straw.

# Millet Processing and Development

Because millets are still a "poor man's crop" in the developing countries, both milling and food processing of millets are considered to be mainly at traditional, manual, or household levels without industrial standardization compared to other important cereals. However, some small-scale mechanical operations have been used in modern millet food processing.

Traditional milling methods, while labor-intensive and time-consuming, are still widely used in many developing countries. Millet grains are usually decorticated and ground with mortar and pestle or stone grinders by hand. Millet grains have been decorticated with mechanical de-hulling equipment and ground into flour with similar mechanical attrition or hammer mills in some villages and urban areas in India, northern China, and some African countries. Milling time and flour yields are normally dependent on millet grain size, shape, hardness, and thickness of pericarp. It is also reported that modern milling equipment for wheat flour has been used for milling proso millet into flour.

Currently, most traditional millet foods are handmade. Germination (malting) and fermentation are major processing steps of millets, widely used for production of traditional millet foods. Malted and fermented millets (e.g., pearl millet, proso millet, finger millet, fonio, teff) can be used in the preparation of porridges, flat breads, and weaning foods and in the brewing of various traditional African beers and Chinese traditional millet wines. For instance, injera is a traditional Ethiopian fermented flat bread. Teff is considered to produce injera with better quality than sorghum. Figure 1 describes details of traditional processing procedures of injera made from teff. African opaque beer is brewed from sorghum and/or millets. The opaque beer production takes  $\sim 5-7$  days, depending on ambient temperature. Its processing procedure mainly includes grain malting, souring (lactic acid fermentation), cooking, mashing, straining, and alcoholic fermentation. The important stages are lactic acid fermentation, mashing, and alcoholic fermentation. African opaque beer usually has high levels of suspended solids, and has a sour taste and light pink color.

Most common, of the many hand-made traditional millet foods in China, are the various types of foxtail millet porridges. Proso millet oil pudding is a famous local traditional food in Shanxi province. Detail of its

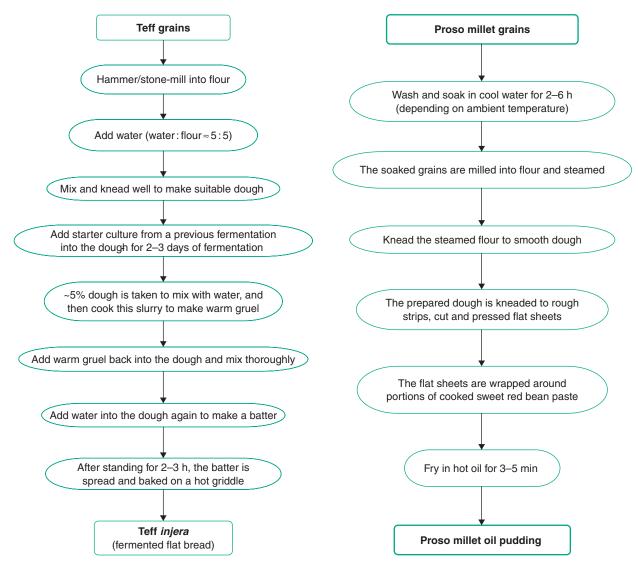


Figure 1 Processing procedure of *injera* made from teff millet – Ethiopian traditional fermented flat bread. (Adapted from Obilana AB, and Manyasa E (2002) Millets. In: Belton PS and Taylor JRN (eds.) Pseudocereals and Less Common Cereals, pp. 177-217. Berlin: Springer.)

Figure 2 Preparation method of proso millet oil pudding -Chinese local traditional food. (Adapted from Lin RF, Li WD, and Corke H (1998) Spotlight on Shanxi province China: its minor crops and specialty foods. Cereal Foods World 43: 189-192.)

preparation is shown in Figure 2. The millet oil pudding is immediately eaten after frying. Color of its outer surface is normally pale. It has a delicate texture inside and has a sweet aroma. Proso/broomcorn millet sour meal is another traditional local food in Shanxi province for 400 years. Millet grains are poured into the prepared special sour soup (made from fermented soybean flour) with sweet-sour flavor, stirred, and soaked overnight. The soaked sour millet grains are used to cook final sour meals (sour porridge or sour millet rice). Sweet potato or yam is added into the soaked sour millet grains to cook the best sour porridge.

Indian chapati or roti (unfermented bread) is prepared from finger/pearl millet flour or composite flour

mixed with others (e.g., sorghum). The processing procedure is traditional and simple, as shown in Figure 3, although there are some minor regional variations in the procedures throughout India. Indian "dosa" is fermented bread prepared from millets or mixed with other cereals and pulses. Dosa preparation is different from chapati preparation and takes more time, mainly due to the addition of a fermentation operation. For instance, millet grains and black gram are mixed in a ratio of 3:1 by volume and wet-ground. The mixed batter needs to be fermented overnight. Additionally, popping of finger millet is common in India on a cottage-industry level. Some popped meal is packed in polythene pouches for marketing.

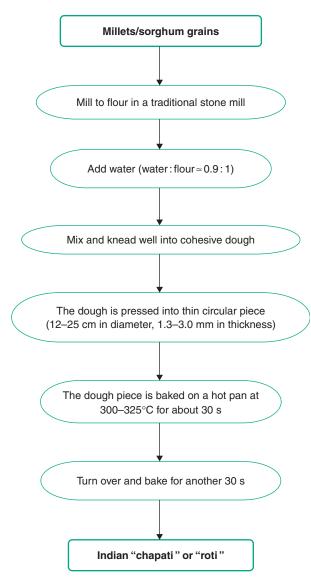


Figure 3 Traditional preparation of "chapati" or "roti" made from finger/pearl millets or mixed with sorghum - Indian unfermented bread. (Adapted from Murty DS and Kumar KA (1995) Traditional uses of sorghum and millets. In: Dendy DAV (ed.) Sorghum and Millets: Chemistry and Technology, pp. 185-221. St. Paul, MN: American Association of Cereal Chemists.)

Most millets, like wheat, contain a similar level of protein and a large quantity of starch, but the physiochemical properties of millet flour are different from wheat flour. Because millet flour does not have gluten, it is difficult to use 100% millet flour to produce wheat flour-like food products. However, millet flour can be mixed with wheat flour and other cereal and pulse flours into composite flours to process baking foods, noodles, cookies, weaning foods, extruding foods, instant powders, healthy foods, etc. These milletbased foods are, to a certain extent, acceptable and their processing techniques are available in some millet production countries, especially in India and

China. Their production can be carried out using modern baking, extruding or expansion technology and other ordinary processing methods. For example, both commercial and experimental weaning foods have been successfully made from millet flour mixed with other cereals and pulses in India. The millet-based weaning foods have good quality with desired nutrient compositions within the range prescribed by the India Standard Institute for processed weaning foods. In northern China, some village/town-owned and small state-owned food factories have commercially or experimentally produced a wide range of milletbased composite flour dry noodles and other local/ traditional food products containing various types of small grains.

More research work is needed on minor millets to enhance large-scale industrial utilization and commercialization of traditional millet foods and development of millet-based specialty food markets. This work will be useful for alleviating food shortage of the poor and also helpful for increasing the value of millets and accelerating agricultural improvement in developing countries.

See also: Cereals: Overview; Grain-Quality Attributes. Grain Production and Consumption: Cereal Grains in North America. Millet: Pearl. Teff. Variety Identification of Cereal Grains.

### **Further Reading**

Andrews DJ, Hanna WW, Rajewski JF, and Collins VP (1996) Advances in grain pearl millet: utilization and production research. In: Janick J (ed.) Progress in New Crops, pp. 170–177. Proceedings of the Third National Symposium, Indianapolis, Indiana, 10/22–25/96.

Baltensperger DD (1996) Foxtail and proso millet. In: Janick J (ed.) Progress in New Crops, pp. 182-190. Proceedings of the Third National Symposium, Indianapolis, Indiana, 10/22-25/96.

Baltensperger DD (2002) Progress with proso, pearl and other millets. In: Janick J and Whipkey A (eds.) Trends in New Crops and New Uses, pp. 100-103. Proceedings of New Crops and New Uses Strength in Diversity, 5th. Atlanta, GA. Alexandria VA: ASHS Press.

Corke H and Lin RF (eds.) Proceedings of the 1st International Conference on Asian Food Product Development - Focus on Specialty Grains and Grain Products (September 6-10, 1998, Taiyuan, China). Beijing and New York: Science Press.

Dendy DAV (1995) Sorghum and Millets: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Dendy DAV (2001) Sorghum and millets. In: Dendy DAV and Dobraszczyk BJ (eds.) Cereals and Cereal Products: Chemistry and Technology, pp. 341–366. Gaithersburg, Maryland: Aspen Publishers.

Lin RF, Li WD, and Corke H (1998) Spotlight on Shanxi province China: its minor crops and specialty foods. *Cereal Foods World* 43: 189–192.

Malm NR and Rachie KO (1971) Setaria Millets – A Review of The World Literature. Experiment Station University of Nebraska College of Agriculture S.B. 513.

McDonough CM, Rooney LW, and Serna-Saldivar SO (2000) The millets. In: Kulp K and Ponte JG, Jr (eds.) *Handbook of Cereal Science and Technology*, 2nd edn., pp. 177–201. New York: Marcel Dekker.

Murty DS and Kumar KA (1995) Traditional uses of sorghum and millets. In: Dendy DAV (ed.) *Sorghum and Millets: Chemistry and Technology*, pp. 185–221. St. Paul, MN: American Association of Cereal Chemists.

National Research Council (1996) Lost crops of Africa, vol. I *Grains*, pp. 77–125. Washington DC: National Academy Press.

Obilana AB and Manyasa E (2002) Millets. In: Belton PS and Taylor JRN (eds.) *Pseudocereals and Less Common Cereals*, pp. 177–217. Berlin: Springer.

Rachie KO (1975) *The Millets Importance, Utilization and Outlook.* Hyderabad, India: International Crops Research Institute for the Semi-Arid Tropics.

Wietgrefe GW (1989) Proso Millet: A Trade Summary. Pierre, SD.

Wietgrefe GW (1990) How to Produce Proso Millet (A Farmer's Guide). Pierre, SD.

### **Relevant Websites**

http://www.hort.purdue.edu/newcrop/ — This website gives an overview of alternative crops and areas of adaptation. It includes descriptions of the millets. http://www.jeffersoninstitute.org/ — This website includes information on alternative crop markets, production practices and updates on millets.

http://www.ars-grin.gov/npgs/ — This website offers descriptions of millet germplasm available through the National Plant Introduction System.

http://www.ecoport.org/ — This website is an international directory of crop descriptions, germplasm etc.

http://apps.fao.org/ – This website contains statistics on world crop production.

# **MILLING AND BAKING, HISTORY**

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### Introduction

The histories of milling and baking parallel each other, as have developments in their technologies. Mutually dependent upon each other, they originated together and remain inextricably linked, even though the two fields have specialized and separated to the point that many people no longer consider them together.

### Flour Milling

Volumes have been written on the history of milling and how it parallels the development of civilization. So much has been written that we can only hit the high points without much detail. It has been said that if we compare mankind's time on earth to a 60 min period,

milling started 55 min after his appearance. Agriculture started 4 min later and recorded history 30 s after agriculture. These observations lead one to believe that grain milling may be the oldest manufacturing process in the world.

Man probably used stones to break up nuts, berries, grains, and bones to produce food that was easier to chew. The basic process of pounding or rubbing between two stones went on for thousands of years. A saddle stone was found in an Egyptian tomb built around 3660–80 BC. Milling and baking scenes have been found on walls of Egyptian tombs dating from 2600 BC. The drawings show grinding with saddlestones and separating the meal with sieves made of papyrus or horsehair. Saddlestones produce a rubbing or attrition action, making a better separation of bran from endosperm as compared to pounding, which pulverizes the entire kernel. The use of sieves with saddlestones became a popular means to separate the meal from the bran particles. Many versions of the saddlestone have appeared but the back and forth

motion required hours of back-breaking labor to produce enough meal to feed a small family. Saddlestones are still used in many parts of the world.

### **Rotary Motion**

Around 800 BC, rotary motion was applied to a device called a quern, which is made up of two horizontal circular or conical stones with one on top of the other. A device called a rynd supported the upper stone, allowing a slight gap between the two grinding surfaces. The upper stone was turned while the lower stone remained stationary. Going from reciprocating to rotary motion was a tremendous step forward. The advantage of rotary motion was the continuous application of force in one direction using wind, water, animals, or people as a source of power.

Water was used to turn millstones around 19 BC by the Roman architect Vitruvius. The millstones were 2-3 ft in diameter and  $\sim 6$  in thick. The application of water power through a gear mechanism laid the foundation for heavy industry. Gears had been described by Aristotle in 400 BC. Ctsesbus in 200 BC used gears in various clock movements where the forces were very light. The mill by Vitruvius was the first recorded use of gears in a massive mechanism designed to do productive work. Figure 1 shows the evolution of stone mills.

The millstone became a machine capable of working for extended periods of time, instead of being a hand tool producing large quantities of flour. This meant that the strength of the people or that of animals was not a limiting factor anymore. It is interesting to note that, except for subtle differences in design, the

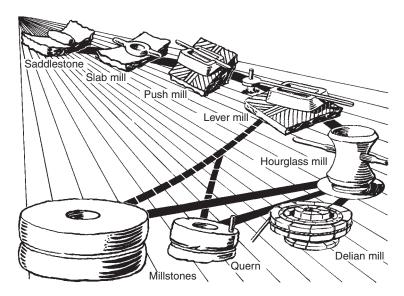
millstone has remained unchanged for over 2000 years.

Windmills were utilized in eastern Iraq by CE 644. A mill of this type is most efficient when built in an area that has winds blowing without changing direction. Western Europe had windmills by CE 1145. The use of windmills increased in number until the invention of the steam engine by James Watt in 1769. Steam engines were applied to stone mills in 1786; that allowed the construction of mills in areas that did not have reliable water or wind sources.

Milling with stones was a "sudden death" or "low grinding" process in which the wheat kernel was reduced to meal in one pass through the millstones. Some bran might be removed by sifting but a large amount would be reduced to meal or flour size and included with the final product. This was more of a problem with hard wheat than soft wheat.

### **High Grinding - Gradual Reduction**

Towards the close of the sixteenth century, the French miller Pigeaud developed a new system. Instead of passing the wheat once through the millstones, he ground it three or four times with sifting between each pass. On the first grinding, the upper millstone was raised slightly giving a larger gap. Sizings and middlings were produced with minimum flour. After sifting, the "overs" of the reel or sieve were ground again with a slightly smaller gap to remove the coarser endosperm particles. The endosperm collected was ground with a very close gap to produce as much flour as possible. This procedure gave higher quality flour than single pass "sudden death" milling.



**Figure 1** The evolution of the stone mill, from the hand operated saddlestone to powered millstones. (Reproduced with permission from Storck P and Teague WD (1952) *Flour for Man's Bread*, 76p. Minneapolis: University of Minnesota Press.)

Because of the better quality flour produced the method of "high grinding" spread rapidly in Europe and America. This was the start of the gradual reduction system.

The Hungarians improved the system by using a purifier developed by Ignaz Paur in 1807 to grade middlings produced on the first several passes through the millstones. This was especially desirable for hard wheat, which tended to produce coarser endosperm particles than soft wheat. With the Hungarian system, workers collected the product from each machine and transferred it by hand in buckets to the next operation. This required a lot of hand labor that was supervised by the miller who made the decision where each separation was sent. At that time the Hungarian system was the most complicated milling process but it produced the best flour.

The development of particle size classification ranged from handpicking, hand sieves, oscillating sieves, shaking sleeve bolters, cloth-covered reels with internal brushes, polygonal and cylindrical-sieving reels, and centrifugals, which are slowly revolving reels with rapidly revolving internal beaters. The Egyptians used hand sieves, but their capacity was limited. The brush sifter was a stationary inclined reel that had revolving brushes inside that forced the fine stock through the screen. John Milene patented this particular machine in England in 1765.

Reels varied in length from 12 to 28 ft (3.6–8.5 m) in length and 20–36 in (0.5–0.96 cm) in diameter rotating from 30 to 35 rpm. Capacity varied from 300 to 2800 lbs (136–1270 kg) per hour depending on mesh size and type of stock being sifted. Reel efficiency was low because only about one-third of the surface was used as the reel rotated. Rotating centrifugals had higher capacity than stationary centrifugals.

Plansifters made their appearance around 1880. Karl Haggenmaker of Switzerland built the first serious plansifter in 1888. The early plansifters were only three or four sieves high and were not free swinging. Free-swinging self-balancing sifters were developed in the US and Europe in the early part of the twentieth century. These sifters now come with 12, 17, 22, or 27 sieves per section.

A carpenter working in a French mill around 1775 invented what is regarded as the first purifier. This machine and others after it used air currents blown through falling stock to separate bran particles from heavier endosperm particles. These machines generated a lot of dust in the mill. In 1855 another Frenchman, Cabane, used air-aspirated sieves as a purifying device. The sieves separated sizings and middlings according to size and removed bran by air. The development of the purifier allowed the miller to produce

white flour from hard wheat that would compete with soft wheat flour in terms of color.

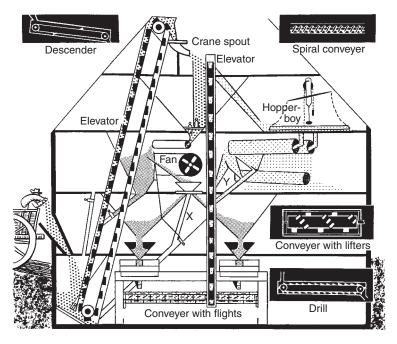
George Christenson had Edmund La Croix install a purifier in the Washburn mill in Minneapolis, Minnesota in 1870. This machine was different from the previous purifiers because it used a reciprocating sieve with air drawn through the cloth by negative pressure rather than positive pressure. This particular arrangement was based on a patent held by a Frenchman, Perrigault. The only problem with the Perrigault purifiers was the lack of a way to keep the sieve meshes clean. A mill employee, G. J. Smith, developed a traveling brush to clear the meshes and obtained a patent on the change in 1877. Since then, improvements on the basic purifier include air adjustments, drives, number of decks, cloth cleaners, sieve construction, clothing, feeding arrangements, and internal lighting.

About the same time that the purifier was being developed, Oliver Evans, an inventor unfamiliar with milling, contracted to build a mill near Wilmington, Delaware, USA. He wanted to build a mill that was better than any other previously constructed. He concentrated on using waterpower to drive conveyors moving stock in the mill. This would greatly reduce the manual labor requirements in milling. His mill, using bucket elevators, screw conveyors, and gravity spouts to move stock during processing, was the beginning of automatic milling. This allowed the miller to concentrate more on the milling process. The Evans mill started running in 1785 and eventually became the model for mechanized large-scale mills in the nineteenth century (Figure 2).

### **Roller Mills**

When the purifier came into general use, it started what was called "new process" milling because of the millers ability to produce flour superior to any previously produced. Combining the purifier and Evans automatic mill started a revolution in American milling that spread to Europe. About this time roller mills were starting to appear. The concept for the roller mill is credited to Giovanni Torriano in 1558 in Spain. It consisted of a hand-powered corrugated cone working inside a curved corrugated shell. Recorded history shows nothing else about roller mills until 1774 when a patent was granted in England for a roller mill to grind corn. However, nothing was mentioned historically until Helfenberger built his first roller mill in 1820. This attempt was not very successful.

Muller, a Swiss in Warsaw, Poland, made progress with improvements on the roller mill design in 1822. He constructed an all-roller flourmill in 1833 that failed almost immediately because of improper



**Figure 2** Oliver Evans 1785 "mechanized" flour mill, showing mechanical conveyors. (Reproduced with permission from Storck P and Teague WD (1952) *Flour for Man's Bread*, 71p. Minneapolis: University of Minnesota Press.)

placement of equipment in the flow. Another Swiss engineer Jakob Sulzberger was assigned to reconstruct the mill. His changes were so successful that numerous other flourmills were constructed to use roller mills. The mills used both roller mills and millstones in the flow. The break stages used rollers while the reductions used millstones. Apparently at that time, millstones were more efficient for grinding sizings and middlings than were rollers.

Roller mill development in relation to milling took place in Europe, particularly in Hungary, around 1877. The early roller mills were not very effective until high grinding or multi-pass grinding, which started in France, developed further. Later roller mills gave better control over grinding because of even distribution of stock to the nip of the rolls. Also, the gap between the rolls could be controlled more accurately to maintain even grinding. Early roller mills had steel rolls from 4.5 to 9 in (114–229 mm) in diameter. Porcelain rolls were tried but they wore out more quickly than steel.

Today's modern roller mills are set up to engage when stock is coming to the roll stand or disengage when the stock runs out. Feeder rolls automatically increase or decrease the stock to the rolls to maintain an even load to the length of the rolls. Newer roll stands can be programmed to automatically change roll gap when changing mill mixes. Double high roller mills are being used on selected break and reduction passages. This helps to eliminate sifting.

When roller mills were first introduced in mills using millstones, problems arose. First the capacity of the early roller mill was at least double that of a millstone. This required more handling and processing equipment. Reels could not handle the load, so the miller had to install more reels or go to plansifters both of which were more costly. Increasing capacity to cover the cost led to increased competition among the mills, driving many small operations out of business.

Milling was rapidly becoming a narrow-margin, high-volume, manufacturing process. That trend has continued unabated until today.

### **Grain Cleaning**

Grain cleaning was slower in developing, as compared to milling. Early man probably removed some impurities by hand before and after grinding grain. Sifting of the meal after stone grinding probably removed more impurities. A specific reference to grain cleaning was made by Oliver Evans in the mill he designed and built in 1783. Evans mill design scoured the grain between two stones then sent it through a revolving cylinder screen that had a fan for aspiration.

Frederick Kick, in 1888, discussed several graincleaning methods, one of which was throwing the grain into the air by shovel or hand. When the grain was thrown the heaviest kernels flew the farthest, while chaff and light kernels traveled the least distance. If a current of air blew across the airborne grain the separations were cleaner. This process took a lot of practice and was not suitable for large quantities of grain.

Kick classified grain-cleaning machines at that time into three categories:

- 1. dressing machines with sieves;
- 2. machine separating round seeds from wheat; and
- 3. machines for sorting grain.

Category 1 used sieves with air blowing through the grain as it passed through the sieve. Grain and impurities larger than wheat passed over the screen, while wheat and impurities the same size or smaller than wheat passed through the screen. Air blowing on the material through the sieve removed chaff and dust.

Category 2 used inclined surfaces to separate round seeds from wheat. Revolving cylinders with the first half perforated and the last half with indented areas are also included in this category. The perforations removed impurities smaller than wheat while the indents lifted round seeds that were the same diameter as wheat and dropped them into a trough in the cylinder above the grain flow.

Category 3 was an inclined reel made of perforated sheet metal or wire with the smallest openings at the head end and largest openings at the discharge end.

Wheat tempering (adding water to the mill grist to toughen the bran so it would not shatter) was also practiced but moisture control was difficult. Moisture determination of grain was time consuming and the control of moisture added was almost nonexistent.

Kick observed that corrugated rolls became dull because of the sand and small stones that were always in the wheat. This observation indicated that grain cleaning at that time had a lot of room for improvement.

### **Baking**

The history of baking dates back to well before written history, far back into antiquity. No one knows exactly when and where baking (and hence the oven) was discovered, but the Egyptians had well-designed ovens operated by skilled career bakers. The origin of milling may have preceded baking, probably coinciding with early nomadic tribes grabbing grass seeds, predecessors of present-day oats, barley, rye, and wheat, to chew on as they wandered across the savannas. Eventually they discovered that rubbing some of the husks off (the origin of milling) and then soaking the seeds in water, made them easier to chew and more palatable. Perhaps someone accidentally splattered some thin gruel on a hot stone to make a crisp, tasty snack. Or perhaps someone left a pot of porridge to set and some wild yeast, probably mixed with lactic acid bacteria, fell into it. The owner may have drunk it that way, or perhaps let it dry up in the pot while it was sitting in the coals, forming a flavorful hard cake. The dried cake didn't spoil. It could be carried on the hunt, softened in water, and eaten without taking time to gather grains. It could even be stored in the cave to eat when prey was difficult to find. At any rate, harvesting cereal grains, milling, baking, and brewing appear to have a common ancestor and are closely related even today.

# **Ovens**

Baking gradually evolved from bare coals and hot rocks into the more sophisticated practice we know today. Archaeologists have discovered the remains of ovens, grain bins, and grinding slabs dating from 7000 to 5600 BC in the Jordan valley of Jericho and of Hacilar in Turkey. Just as in nearly all other forms of technology with which we are familiar today, when civilization developed new baking equipment and techniques, it left some people behind who did not adopt it, keeping the ancient forms alive. Someplace in the world today, a woman is still baking her family's daily bread using a rock sitting in a bed of coals. Perhaps someone is still following the ancient nomadic practice by baking their bread on dried camel dung, providing the hearth and the fuel source all in one. By 3000 BC, the organized and agrarian Egyptians were using thick earthen jars sitting in coals to bake bread. And as before, the heated earthen jar evolved into the tandoor ovens still in common use in India and the Middle East. A piece of fermented dough is slapped onto the inside of the hot jar. When it falls off, it is baked. At  $\sim$ 1000 years BC, the same time that bakers learned to preserve their sour dough for the next day's production, they developed the ancestral bee-hive oven design (Figure 3). A stone or clay domed roof sat on top of a flat stone hearth. First, a fire was built inside until the oven was extremely hot throughout. Then the fire was scraped out, the inside swept free of ashes, and the fermented dough pieces placed on the hearth to bake.

Ovens changed slightly in form, but did not leave the basic "hearth inside a chamber" design until the 1800s, only two centuries ago. The Egyptians passed their technology to the Greeks who in turn passed it to the Romans. The Romans understood milling, sifting, fermentation, and baking, and distributed the knowledge throughout their empire, spreading it to Europe. The bakers were sometimes highly regarded and skilled people, and at other times they were slaves to the wealthy. Most bread was still baked at home or in communal ovens. Little change in baking technology occurred from the fall of the Roman Empire through the Middle Ages. Civilized society nearly

vanished from Europe until the eleventh century crusades brought the travelers into contact with other cultures, exposing travelers to their baking methods.

By the late eighteenth century, the beehive oven had evolved into the ancestral "deck" oven and for larger

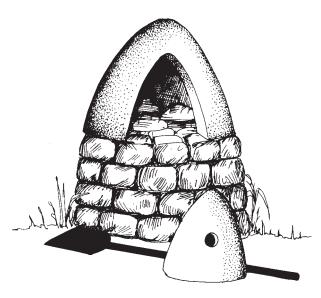


Figure 3 Beehive shaped ovens have been used to bake breads for thousands of years. (Reproduced with permission from Varilek PS and Walker CE (1983a) Baking and ovens: history of heat technology. Part I. Baker's Digest 57(5): 52-54, 56-57, 59.)

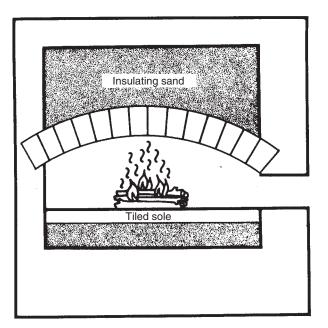


Figure 4 The faggot oven had the advantage of a large, smooth sole beneath a domed room containing a mass of stone or sand that held the heat more uniformly. (Reproduced with permission from Varilek PS and Walker CE (1983a) Baking and ovens: history of heat technology. Part I. Baker's Digest 57(5): 52-54, 56-57, 59.)

capacity, the deeper "peel" oven. Both designs live on today, baking pizzas and hearth breads. About this time, though, the heating methods begin to change. For thousands of years, the fire was placed directly in the oven to heat it, the fire was removed, and the bread baked as the oven gradually cooled (Figure 4). A huge advance occurred when the fire was located in a separate firebox, removing much of the soot, ashes, and dirt from the bakery. By 1900, large commercial bakeries were producing 25% of the bread in the USA, mostly in large peel ovens as much as 6 m (20 ft) long. A long-handled paddle (peel) was used to place the dough pieces onto the hearth, loading the oven from the back to the front. The same peel was then used to remove the baked bread, from front to back. The result, of course, was that the loaves received a different bake, depending upon where in the oven they were placed. By the late nineteenth century, bakeries began to mechanize and a wide variety of ovens evolved, finally breaking away from the hot masonry chamber into which loaves had been loaded and removed, one at a time, for many centuries past.

# **Baking Classifications**

Baking today may be classified by several methods: scale, food being baked, physical arrangement, and energy source.

### **Scale**

Today's small "corner hot bread shop" or "in-store bakeoff" may use small batch ovens that only operate a few hours per day, perhaps baking only a hundred loaves. The "baker" only needs to put the loaves (sometimes frozen doughs or even par-baked loaves) into the oven and remove them when the buzzer sounds. Larger-scale bakers are probably using rack or reel ovens, still manually loading them a batch at a time. They may bake a few hundred to a few thousand loaves per day. More judgment and skill is required on the part of the baker because the entire operation is probably a "scratch" bakery, preparing doughs and batters from individual ingredients, not from mixes or frozen items. Finally, modern largescale high-speed plant bakeries produce 100 loaves per minute on each of several lines.

### **Food Being Baked**

Except in very small bakeries, breads are baked in different ovens from sweet doughs, such as yeastleavened pastries. Cookies and crackers will be baked on a different design yet, and cakes and pies require still different designs. While smaller bakers may use a "one for all" oven, by far the best results

will be obtained using an oven designed for the specific food type.

### **Physical Arrangement**

Different physical arrangements for ovens began with the descendents of the primitive beehive ovens, such as hearth or deck ovens, rotary hearth, rotary rack, reel, traveling tray ovens with single or multiple laps, and finally today's long, continuous tunnel ovens used for crackers and cookies.

### **Heat Sources**

Except for small or primitive bakers and a few "boutique" bakers, wood is seldom used today. Refined petroleum, natural gas, or electricity are the most commonly used heat sources. Individuals living in very hot and sunny countries with a shortage of fossil fuels may use solar energy in a few isolated locations. Coal, once common, is now seldom used because it is so dirty. Modern ovens may combine several forms of heat and heat transfer, including high velocity air jets as in the present day pizzeria ovens, or adding microwaves to existing units to make them bake faster.

### Other Changes in Baking

As bakers progressed with technology, their way of life changed. First, one person, usually the wife/mother, gathered the grains, threshed and milled them by hand, mixed the dough, and baked it on her cooking fire. As harvesters, millers, and bakers began to specialize and fall into the male domain, their operations got larger and they began to develop machinery. Automation entered the bakery after it had already set milling onto the path that led it to today's large and efficient plants. Their paths were similarly taken.

Early "large-scale" bakers often crawled into their wooden troughs to knead their dough with their feet. Then, larger and more efficient mechanical mixers were developed, especially with the advent of electric motors. As each stage was mechanized, the increasing capacity put pressure onto each stage that followed, in a stepwise fashion.

Mixed fermented doughs were divided, rounded, and molded automatically. Pans were filled automatically. Ovens were made continuous instead of batch, so conveyors were developed to load and unload them. The loaves were de-panned by machinery and the loaves sent through large cooling conveyors.

Individual loaf-protective wraps began to appear and antimold preservatives were added as bakeries became larger and larger and their delivery routes began to stretch across state lines. The advent of pre-sliced bread put additional demands upon technology and formulation. Special fats were added to retard staling. In the 1940s, wartime labor shortages encouraged the development of even more highly automated continuous mix — continuous baking processes that produced bread with very fine cake-like crumb grain and a very soft texture. The shelf life of white pan bread extended from hours to days to a week. Recent advances have led to 2 weeks or longer shelf lives.

# **Future Challenges**

The grain producing, milling, and baking industries today face steadily increasing challenges. The expectations for more grain at a cheaper price to feed an expanding population conflicts with the farmer's dream for more profits. The millers and the bakers face the same conflicting expectations. The results have been steadily more sophisticated and automated systems, from the field to the table. This has resulted in a large, highly efficient, standardized process producing a similar product everywhere, the same product from all plants for all customers.

As any society becomes more efficient and more affluent however, it begins to demand and to be able and willing to pay for more specialized consumer goods, including breads. The proliferated demands in the 1990s for a wider variety of bread types, once available only from the small artisan bakers, has caused other competing demands on the large bakers. Perhaps they can now develop easily modified automated means to produce a wider variety of breads, cakes, cookies, and pies by computer control over the entire baking process, from initial ingredient selection and mixing through forming, baking, finishing, final packaging, and distribution.

See also: Bakeries. Breads. Cereals: Overview. Cultural Differences in Processing and Consumption. Oven Technologies. Wheat: Dry Milling

### **Further Reading**

Kick F (1888) *Flour Manufacture*. London, England: Crosby Lockwood and Son.

Koyim PA (1921) *Flour Milling*. London, England: George Routledge.

Scott JH (1951) Flour Milling Processes. London: Chapman and Hall.

Scott JH (1972) *Development of Grain Milling Machines*. Edinburgh, England: Audio Typing.

Simon ED (1930) *The Physical Science of Flour Milling*. Liverpool, England: The Northern Publishing Co.

Smith L (1962) Flour Milling Technology. Liverpool, England: The Northern Publishing Co.

Storck P and Teague WD (1952) Flour for Man's Bread. Minneapolis: University of Minnesota Press.

- Varilek PS and Walker CE (1983a) Baking and ovens: history of heat technology. Part I. Baker's Digest 57(5): 52-54, 56-57, 59.
- Varilek PS and Walker CE (1983b) Baking and ovens: history of heat technology. Part II. Baker's Digest 57(6):
- Varilek PS and Walker CE (1984a) Baking and ovens: history of heat technology. Part III. Baker's Digest 58(1): 24-27.
- Varilek PS and Walker CE (1984b) Baking and ovens: history of heat technology. Part IV. Baker's Digest 58(2): 12-15.
- Varilek PS and Walker CE (1984c) Baking and ovens: history of heat technology. Part V. Baker's Digest 58(3): 22-24, 26, 29.
- Zieher W (1987) Farmer, Miller, Baker: Bread through the Ages. Tielt, Belgium: Lannoo.

### **Relevant Websites**

http://www.aibonline.org. http://www.asbe.org. http://www.bri.com.au.



# NITROGEN IN GRAIN PRODUCTION SYSTEMS

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### Introduction

Nitrogen (N) is the largest component of the Earth's atmosphere, comprising  $\sim 78\%$  (by volume) of the air we breathe. It is usually present in the atmosphere as a dinitrogen gas (N<sub>2</sub>), which is colorless, odorless, tasteless, and relatively inert at room temperature.

Nitrogen was first identified by the chemist and physician Daniel Rutherford in 1772. It is now known to be present in a vast range of materials encountered in our day-to-day existence; foods, fertilizers, poisons, and explosives are a few general examples. Some specific examples are: nitrogen gas is used as a blanketing medium during the production of electronic components, as an agent for annealing stainless steel, in beverage processing, as a refrigerant for food protection and preservation, and as a major fertilizer in crop production. Atmospheric nitrogen is also responsible for the orange-red, blue-green, blueviolet, and deep violet colors of the aurora. More importantly, N is a component of a wide range of biological compounds, being fourth in abundance after carbon (C), hydrogen (H), and oxygen (O).

Nitrogen exists in organic and inorganic forms in the atmosphere, biosphere, hydrosphere, and geosphere. It can be present in gas, liquid (dissolved in water), or solid phases. The most common inorganic forms include dinitrogen  $(N_2)$  gas, nitric acid  $(HNO_3)$ , nitrate  $(NO_3^-)$ , nitrite  $(NO_2^-)$ , ammonium  $(NH_4^+)$ , gaseous nitrous oxide  $(N_2O)$ , gaseous nitric oxide (NO), gaseous nitrogen dioxide  $(NO_2)$ , and gaseous ammonia  $(NH_3)$ . The most common N-containing biomolecules belong to one of the following three chemical families: proteins (including enzymes) and related compounds (including peptides and amino acids), nucleotides

(largely RNA and DNA), and porphyins (e.g., cyto-chromes and chlorophyll).

Although N constitutes only 1-7% (w/w) of plant dry matter, it is the element most frequently limiting the plant growth. Sufficient nitrogen is fundamental for crop growth, development, and yield. Improvement in N use-efficiency is thus the key to produce safe and high-quality food and feed in an environmentally responsible way.

# The Nitrogen Cycle

Nitrogen is a dynamic element and is recycled continually by plants and animals. It can be transformed from one oxidation state to another through processes that comprise the N cycle (Figure 1). The term "cycle" is really a misnomer as the pathways of N in the biosphere are actually a simple web. Nitrogen moves among oxidation states, with ammonia (NH<sub>3</sub>) being the most reduced and nitrate (NO<sub>3</sub>) the most oxidized. The tremendous chemical stability of dinitrogen (N<sub>2</sub>) gas makes the conversion of this compound into ammonia (NH<sub>3</sub>) or oxides of N (NO<sub>3</sub><sup>-</sup>) energetically expensive and the rate-limiting step in this part of the "cycle." This causes N2 to accumulate in the atmosphere. Thus, cycling of nitrogen by various biological entities determines a major part of the composition of the Earth's atmosphere.

It is a paradox that while 78% of the Earth's atmosphere is N<sub>2</sub> gas, and plant stems, leaves, flowers, and fruits are immersed in this gas, biologically available N is generally the most limiting nutrient element for crop production. A solution to this problem evolved in the exquisitely adaptable prokaryotes. A limited number of microorganisms are able to react N<sub>2</sub> with electrons to form NH<sub>3</sub>, which they then incorporate into various biomolecules. When these microorganisms die, N is mineralized to simple forms (NH<sub>3</sub>, NO<sub>3</sub>) through decomposition by other microorganisms. In these forms, N is available to higher plants. Nitrogen is taken up by crop plants, which become food for animals, including human beings, and energy sources for bacteria or fungi; N is then

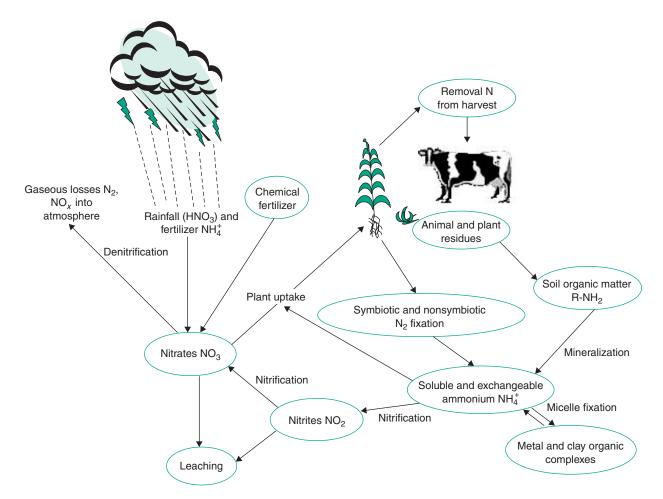


Figure 1 A diagram of the N "cycle" in a typical grain crop containing agri-ecosystem.

cycled back to the soil when dead cells and tissues of organisms (plant debris for example) or N-containing waste products (such as animal urine and feces) are broken down by soil microbes.

In natural ecosystems, N changes from one form to the other, but most of it remains within the nearly closed system in a dynamic balance. In agricultural systems, humans often add chemical N, fixed through industrial processes, a significant portion of which is eventually removed from the field in harvested material. Food, fiber, and livestock are now often produced primarily in one location and transported to other regions of the world for consumption, making crop production fields into nutrient flow-through systems. Nitrogen input into agriculture production systems is largely from chemical fertilizers, or N-fixing crops (including those grown as green manures). There are other minor sources, such as biosolid waste products, which are sometimes added to croplands. A small amount of N comes with precipitation, sometimes associated with lightning discharges, which reacts the N2 and O2 in the atmosphere. There is some N-cycling within agricultural systems, with livestock manures constituting the major component of this process.

Nitrogen outputs from crop production systems include removal of harvested grains and forage biomass from production systems and losses of various N forms through runoff (surface water), leaching (to ground water), denitrification (production of gaseous  $N_2O$ ), and volatilization (generally  $NH_3$ ). Because of leaching and gaseous losses, and aspects of plant N physiology, only  $\sim 50\%$  of the N applied to crop production systems is taken up by the plants, with the remainder being unusable or lost. Globally, various forms of N are in a dynamic balance.

Soil microbes play a wide range of roles in the N cycle. Because of this, these processes are dependent on temperature, moisture, and the quality and quantity of soil organic matter available to them. It is microbes that break down most of the organic material, leading to "mineralization" of organic N. Microbial populations in the soil can take up large amounts of N released from decomposing plant tissues, as long

as there is sufficient reduced carbon available, leading to "immobilization" of N. The conversion of ammonium ( $NH_4^+$ ) to nitrite ( $NO_2^-$ ) and to nitrate ( $NO_3^-$ ) is referred to as nitrification. All forms of N in the soil (except  $N_2$  gas) can be converted to  $NO_3^-$  by soil microorganisms, under aerobic conditions when soil temperatures are above freezing.

The amount of nitrification is controlled by the supply of NH<sub>4</sub><sup>+</sup> which is, in turn, controlled by decomposition rates, plant and microbial growth, and soil pH, or by N-fertilizer addition rates. Nitrate can be converted back into N<sub>2</sub>, or into nitrous oxide  $(N_2O)$ , through denitrification. This process often takes place under anaerobic (generally water saturated) soil conditions. In this process, soil NO<sub>3</sub> can be reduced to N<sub>2</sub> gas through a series of intermediate steps  $[NO_3^- \rightarrow NO_2^- \rightarrow NO \text{ (gaseous nitric oxide)} \rightarrow$  $N_2O$  (gaseous nitrous oxide)] and finally to  $N_2$  (gas). The intermediate and final products of denitrification, are released into the atmosphere. Denitrification may lead to the loss of 4-5% of soil NO<sub>3</sub> each day when soil remains water saturated. Microbes play a key role in most aspects of the N cycle, generally extracting energy by converting forms of N out of equilibrium with the oxidation state of their environment into forms that are in equilibrium, and extracting a reasonable portion of the energy liberated through this process. This causes N to move among forms that are biologically available or unavailable, mobile or immobile, oxidized or reduced, gaseous or solid.

### **Nitrogen Sources**

Nitrogen exists in inorganic or organic forms in our environment. Inorganic forms include NO<sub>3</sub>, NO<sub>2</sub>, NH<sub>3</sub>, and N<sub>2</sub> whereas organic N is found in a wide range of biomolecules. Protein is one of major classes of biomolecules and generally the largest repository of organic N in the biosphere. RuBisCo, the plant protein responsible for the uptake of  $CO_2$  by  $C_3$  plants, is the most abundant protein on the planet. The other two most abundant classes of biocompounds, carbohydrates, and lipids, generally have little N associated with them. Over 90-99% of N in the surface (or tillage layer; 0-25 cm) layer of most soils is in organic forms. Although there is an abundant potential supply of N in the Earth's atmosphere (N<sub>2</sub> gas), the unique geometry of the triple bond between the two N atoms results in a very stable compound that is very energetically expensive to break. Thus, this form of N cannot be used directly by most organisms. Whenever dinitrogen is converted into other forms, a large input of energy is involved. The major sources of this energy are: (1) lightning in the case of atmospheric fixation, which accounts for 5-8% of total N fixed and potentially available to the biosphere. This process results in the combination of oxygen from the atmosphere with dinitrogen, causing the formation of N oxides. These compounds dissolve in rain water and are carried to Earth; (2) reduced carbon and adenosine triphosphate (ATP) in the case of biological N fixation, conducted by certain prokaryotes alone or in symbiotic relationships with plants and sometimes animals; and (3) fossil fuels for industrial fixation.

Industrial fixation relies on the Haber–Bosh process, developed during the First World War for the production of explosives. This process uses a catalyst, high pressure, and high temperature (600°C), to combine N from the atmosphere with hydrogen (usually derived from natural gas or petroleum) to produce ammonia (NH<sub>3</sub>). Ammonia can be used directly as fertilizer, reacted with CO<sub>2</sub> to form urea ((NH<sub>2</sub>)<sub>2</sub>CO), and/or oxidized to form nitrate (NO<sub>3</sub><sup>-</sup>) which can be salted with other compounds (such as ammonium, NH<sub>4</sub><sup>+</sup>) to form other types of N fertilizers (e.g., ammonium nitrate). The large requirement for energy makes N fertilizer easily the largest component of fossil fuel consumption in crop production, being close to 50% of the total for crops such as maize.

The bulk of the soil N in noncropland systems comes from fixation by symbiotic bacteria (largely genera associated with legumes and collectively referred to as rhizobia and species of Frankia, usually symbiotic with woody plants), fixation by free-living bacteria and mineralization of organic matter (N released as decomposition of plant and animal residues). The potentially available N (90-99%) in the soil is in organic forms, the rest being in inorganic (or mineral) forms. The organic forms are not immediately available for plant use and have to be decomposed by soil microorganisms through mineralization. In addition, a very small amount of organic N may exist in relatively small soluble compounds, such as urea or amino-sugars, which may be slightly available to plants.

Symbiotic (largely legume) N-fixation and mineral fertilizer N are the major sources of N for grain production systems, with animal and green manures, crop residues, composts, sludges, soil micro- and macro-biota being the other possible sources. Again, organic N must be mineralized before it is available to plants. Sodium nitrate (NaNO<sub>3</sub>) and potassium nitrate (KNO<sub>3</sub>) are formed by the decomposition of organic matter. In certain dry areas of the world, these saltpeters are found in quantity and are used as fertilizers. Other inorganic N compounds are ammonia (NH<sub>3</sub>), nitric acid (HNO<sub>3</sub>), the N oxides (NO, NO<sub>2</sub>, NO<sub>3</sub>, N<sub>2</sub>O), cyanides (CN<sup>-</sup>), etc. However, inorganic N in soil generally comprises only a small portion of soil N. Hence, despite N being

one of the most abundant elements on the biosphere, N deficiency is probably the most common nutritional problem affecting crop plant production worldwide.

# Nitrogen Availability to Crop Plants

The availability of N sources is a fundamental requirement for metabolism as it allows N assimilation into amino acids (for which photosynthetically produced carbon compounds are also required) and their availability for protein synthesis. An adequate supply of N allows leaf growth and photosynthesis.

Inorganic (or mineral) forms of N, such as NH<sub>4</sub><sup>+</sup> and  $NO_3^-$ , comprise the majority of plant-available N. Other forms of N must be converted to one of these compounds by either natural or artificial means before they can be utilized by plants. When N is first released from organic matter, it is in the form of gaseous NH<sub>3</sub>, which is volatile and can be lost to the atmosphere. When NH<sub>3</sub> dissolves in water, it acquires a proton and becomes ammonium (NH<sub>4</sub><sup>+</sup>), which carries a positive charge and is attracted by the soil clay colloids and soil organic matter, which carry negative charges. Once attached to the soil matrix, NH<sub>4</sub><sup>+</sup> becomes part of the cation-exchange process whereby plants exchange a hydrogen ion (H<sup>+</sup>) for one of the positively charged molecules in the soil. Only a small portion of the soil NH<sub>4</sub><sup>+</sup> exists in the soil solution; this NH<sub>4</sub> is available to crop plants. On the other hand, NO<sub>3</sub> carries a negative charge, and so is not bound by clay particles. Thus, NO<sub>3</sub> molecules are free to move with the soil water and are more readily available to crop plants than NH<sub>4</sub><sup>+</sup>. However, in coarse textured or water-saturated soils, NO<sub>3</sub> is much more prone to leaching into groundwater than NH<sub>4</sub>; once in ground water  $NO_3^-$  becomes a potentially serious pollutant.

### **Nitrogen Fixation**

A symbiotic relationship between leguminous plants and N-fixing bacteria allows for plant-based biological N fixation. The major conversion of  $N_2$  into  $NH_3$  (a biologically available form) is the biological N-fixation process, carried out by single-celled prokaryotes. This process requires a great deal of energy, supplied as ATP, which is derived from the breakdown of organic matter, or through photosynthesis (e.g., cyanobacteria). It is estimated that biological N fixation accounts for the annual production of as much as  $250 \times 10^9 \, \mathrm{kg}$  of ammonia, which is twice the amount produced by the Haber–Bosch process.

Biological N fixation can be described as the following equation:

$$N_2 + 8H^+ + 8e^- + 16ATP = 2NH_3 + H_2 + 16ADP + 16Pi$$

This reaction is conducted by prokaryotes only (eubacteria and archea) through the enzyme dinitrogenase, and the closely associated dinitrogenase reductase. Dinitrogenase contains both iron and molybdenum in a cofactor referred to as FeMoco. The fixation of N is carried out while  $N_2$  is bound to dinitrogenase. Dinitrogenase reductase (the Fe protein) is reduced by electrons donated by a protein generally containing ferredoxin. Reduced dinitrogenase reductase (Fe protein) binds ATP and reduces dinitrogenase (molybdenum-iron protein), which provides electrons to  $N_2$  resulting in its reduction to  $2NH_3$ .

Due to its economic importance and agronomic value, the legume-rhizobia symbiosis is the most investigated process of biological N fixation. Many grain legumes are important crops due to this symbiotic relationship; these include soybean, bean, mung bean, faba bean, peanut, and pea as well as forage legumes such as alfalfa, clover, sainfoin, fenugreek, and vetch. During the establishment of this symbiotic process, rhizobia infect the roots of legume plants and cause the formation of nodules. It is in these nodules that the bacteria fix N and supply it to the plant. The plant provides reduced carbon (sugars) to the nodules, where it is converted to organic acids and supplied to the bacteria as an energy source. In most cases, the symbiosis can supply all N required for normal growth and development of the plant. The amount of N fixed by legume plants is estimated to range from 11 to  $250 \text{ kg N ha}^{-1} \text{ year}^{-1}$ . When fertilizer N is supplied, N fixation is inhibited through several long and short-term mechanisms, so that adding N fertilizer to N-fixing legumes does not increase the amount of N available to the plants. For grain legumes, however, a small amount of starter N fertilizer greatly helps crop growth prior to the time when nodules become functional and begin to supply N. The simplest approach for evaluating whether nodules in field-grown legumes are able to fix N is to observe their interior color: effective nodules contain leghemoglobin and are bright red, while nodules not yet functional are generally white and those that are no longer functioning are generally greenish. Leghemoglobin carries oxygen into N-fixing (rhizobia containing) nodule cells, ensuring the availability of large quantities of oxygen, but in a very controlled fashion, to the nodule-enclosed rhizobia.

On the other hand, all field crops in the grass family, such as the grain crops maize, sorghum, rice, and

Table 1 Annual N inputs and outputs of typical agricultural land

	N (kg ha <sup>-1</sup>
Inputs Plant residues Biological N fixation Natural fertilization through precipitation Chemical fertilizer	10-100 10-250 0-50 0-400
Outputs Volatilization of NH <sub>3</sub> Dinitrification Leaching Crop harvest	0-60 10-70 10-70 10-150

wheat, as well as forage grasses, and also nonleguminous broadleaf field crops (e.g., sunflowers, potatoes, sugar beets, cotton, etc.) are unable to form N-fixing root nodules. Therefore, these plants must obtain their required N from the soil and other exogenous sources.

At present, almost 90 genera of diazotrophic bacteria, which can reduce atmospheric dinitrogen to ammonia with varying efficiencies, are known. Biological N fixation can be carried out by a wide range of prokaryotes, alone or in symbiosis, including cyanobacteria free living and in symbiosis in lichens, cycades, the fern *Azolla* or the angiosperm *Gunnera*; and free-living soil bacteria. These types of N fixation contribute significant quantities of NH<sub>3</sub> to natural ecosystems, but not to most cropping systems, with the exception of paddy rice. Their contributions are generally less than 5 kg N ha<sup>-1</sup> year<sup>-1</sup>. Table 1 indicates the general annual input and output of N in agricultural ecosystems.

### **Symbiotic Signals**

Nitrogen fixing root nodules are the result of symbiotic interactions between leguminous plants and rhizobia. This begins with a recently elucidated signal exchange between the two symbiotic partners. Initially, the roots of leguminous plants secrete flavonoid compounds into the soil. These compounds activate a set of genes in the appropriate rhizobia. A structurally diverse mixture of lipo-chitooligosaccharides (LCOs) is produced by rhizobia after induction with flavonoids. LCOs consist of a chitooligosaccharide backbone of  $\beta$ -1,4-linked N-acetyl-D-gluconsamine (GlcNac) and a fatty acyl group attached to the nonreducing saccharide. Diversity of the fatty acyl substituents (length and degree of unsaturation) contributes to the diversity of LCOs. In addition, various groups can be added to the chitin backbone. The nodE gene determines the nature of the fatty acyl moiety, a major determinant of host range. LCOs are considered to be key factors in the specific recognition processes that underlie the formation of leguminous root nodules and their bacterial infection; in these associations only appropriate legume and rhizobium matches can come together and create a specific symbiosis.

### **Uptake**

Nitrate  $(NO_3^-)$  and ammonium  $(NH_4^+)$  are the two major mineral forms of N taken up and assimilated by plants. These compounds enter the plant roots by diffusion through the space between root cells until they cross the plasma lemma and enter the plant cell interior. Once inside the symplast (for a review of the "symplast," see www.worldreference.com) these compounds can be stored in vacuoles, assimilated into amino acids or proteins or moved into the stele (xylem) for transport elsewhere in the plant. Movement among the cells that make up the symplast is through the plasmodesmata that connect plant cells. All mineral N must enter the symplast before entering the stele. This can happen at any point in the root cortex, but must happen by the time mineral N molecules reach the casparian strip. During the course of the vegetative growth, both NO<sub>3</sub> and NH<sub>4</sub> enter the plant root and are transported via the xylem to the leaf (Figure 2). Once it reaches the leaf, nitrate reduction takes place, leading to the production of amino acids. Most of the newly formed organic acids are then translocated to the root where carboxyl groups are exchanged for an ammonium and the newly assimilated N is incorporated largely into leaf N compounds (protein). When a large quantity of N is required by developing fruits and filling seeds (reproductive growth stage), leaf proteolysis occurs and a great deal of the amino N in the leaf is ultimately exported to the filling fruits where it is accumulated as seed storage proteins (Figure 2). As a result, the phloem is enriched with amino compounds, which repress nitrate uptake and diminish the rate of nitrate reduction. Improved crop yield due to increased fertilizer N application suggested that the availability of N (usually nitrate in agricultural soils) is often the limiting factor in plant growth. The absorption of  $NO_3^-$  by roots is not only determined by the N demand of the plant, but also by its availability in the soil environment. Nitrate absorbed via roots is either reduced in situ into ammonium, imported into vacuoles (by high- and low-affinity transport systems) for storage, or transported via the xylem to the shoot where it can either be metabolized or stored as a reserve. A low-affinity root NO<sub>3</sub> uptake system may play a greater role in nitrate uptake. In some cases, a high-affinity system has been shown to be

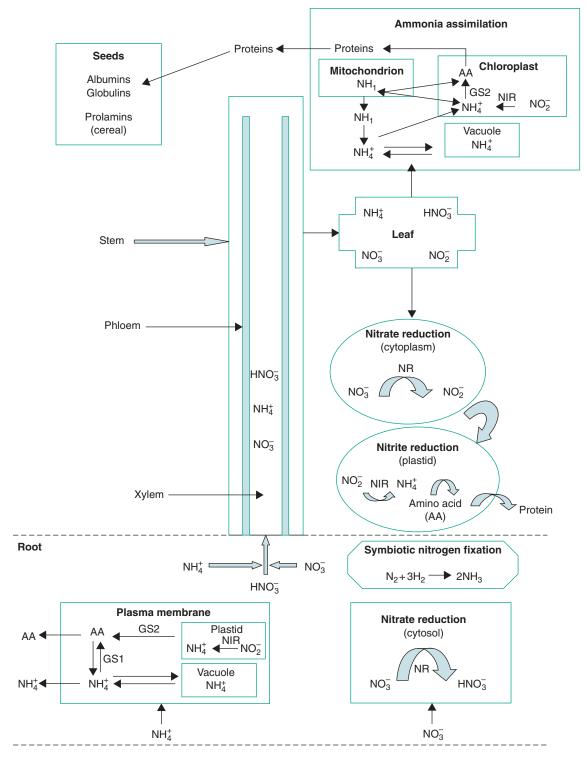


Figure 2 A diagrammatic summary of N metabolism in a grain crop.

the major mode of nitrate uptake through uptake competition studies and membrane voltage change studies. The low affinity transport system (LATS) (possibly a carrier system or an anion channel), and the inducible, high affinity transport system (HATS)

are regulated by cellular energy supply, intracellular nitrate levels, and a proton electrochemical gradient. The latter is regarded as an H<sup>+</sup>/anion cotransport carrier system that produces transient plasma membrane depolarization upon addition of nitrate.

The depolarization is counteracted by the plasma membrane H<sup>+</sup>-ATPase. The plasma membrane proton ATPase is induced by nitrate. In addition to the nitrate uptake system, plants have an inducible nitrate efflux system, requiring both RNA and protein synthesis. The efflux system, however, has a much slower rate than the uptake system.

In the agricultural soils of tropical and temperate regions (except paddy rice fields), nitrate accounts for 70–90% of soil mineral N. Nitrate in the soil solution is 10 times more mobile than NH<sub>4</sub>. Two reserves of soil NH<sub>4</sub><sup>+</sup> exist: a small portion of NH<sub>4</sub><sup>+</sup> is in the soil solution, and a large portion of NH<sub>4</sub><sup>+</sup> is adsorbed on soil colloids. Only water soluble NH<sub>4</sub><sup>+</sup> is available for plant uptake. Depending on the plant species, the bulk of the nitrate entering the plant is reduced to ammonia and assimilated into organic compounds in the roots or in the leaves. Plants can take up either  $NO_3^-$ ,  $NH_4^+$ , or mixtures of the two. In most cases, crop plants perform better when the mixture of NO<sub>3</sub> and NH<sub>4</sub><sup>+</sup> fertilizer is provided than either one alone. Uptake of NO<sub>3</sub> by roots is governed by the concentration of  $NO_3^-$  in the soil solution, the volume of soil exploited by roots and root density within that volume, and plant demand for N, the latter being largely related to plant growth, development, and production. The root uptake efficiency of N is affected by metabolic demand, N concentration around the roots, and conditions such as temperature.

Ammonium is mainly assimilated in roots. It or alanine, formed by organic acid ammonization, is the main form of N exported from symbiotic N<sub>2</sub>-fixing microorganisms to their host plants. In soil, NH<sub>4</sub><sup>+</sup> comes mainly from the mineralization of organic matter. When  $NO_3^-$  levels in the soil are low or completely exhausted, plants can rely on NH<sub>4</sub> released from cation-exchange sites or through mineralization of organic matter. During NH<sub>4</sub><sup>+</sup> assimilation in the root, excess  $H^+$  is generated  $(3NH_4^+ = 3R-NH_2 + 4H^+)$ and released directly to external solution. While NO<sub>3</sub> assimilation occurs excess OH<sup>-</sup> is produced  $(3NO_3^- = 3R-NH_2 + 2OH^-)$ . This excess OH<sup>-</sup> may be neutralized by carboxylation or by HCO<sub>3</sub><sup>-</sup> released from the roots. Thus an H<sup>+</sup> neutral synthesis of plant materials can occur with a ratio of assimilation of  $1NH_4^+$  per  $2NO_3^-$ . This is why many plant species grow optimally with a mixture of mineral N compounds (NO<sub>3</sub> and NH<sub>4</sub>). With a mixed N solution (NO $_3^-$  and NH $_4^+$ ), NH $_4^+$  is often the form of N preferentially taken up by the plant. Many forest species take up primarily NH<sub>4</sub><sup>+</sup>, since forest soils are relatively acidic, inhibiting the development of populations of bacteria that cause nitrification. The average NH<sub>4</sub><sup>+</sup> concentration in well-aerated soil is 10-100 times lower than NO<sub>3</sub>, rarely exceeding 0.1–2 ppm. However, absorption of NH<sub>4</sub><sup>+</sup> by plant roots can occur at very high rates, due to the presence of transport systems in the root plasma membrane with high substrate affinities. Like NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> uptake by roots is diurnally regulated, with maximum uptake during the light period. Uptake is stimulated by sugar supply, implying that the greater uptake during the day is related to the availability of photosynthate.

# **Assimilation of Nitrogen**

Both nitrate and ammonium are taken up and assimilated by plants. In general,  $NO_3^-$  is the predominant form of inorganic N taken up by grain crop plant roots (except paddy rice). Under most circumstances, absorbed nitrate is reduced to ammonium in roots and/or leaves, and is then utilized in the synthesis of amino acids and proteins. Whatever the source of ammonium in plants, this ion is first converted into amino acids, the building blocks of protein.

Nitrate is assimilated into organic compounds by GS/GOGAT (GS – glutamine synthetase/glutamate synthase; GOGAT – glutamate oxidase, glutamine amino tranferase). The GS/GOGAT pathway is considered the major pathway for incorporating reduced N into organic molecules. This pathway recycles glutamate; amino groups are eventually transferred to other amino acids and utilized for protein synthesis and also for synthesis of other key biomolecules such as RNA, DNA, and porphyrins.

GS and GOGAT are located in the cytosol (for a review of the "cytosol," see www.worldreference. com) and the chloroplast (for a review of "chloroplast," see www.worldreference.com). Their activity in particular plant tissues is closely linked to specific primary N assimilation, NH<sub>3</sub> recycling during photorespiration, or general N remobilization. There are two pathways for incorporation of N into amino acids: (1) Direct incorporation where glutamate dehydrogenase is involved:  $\alpha$ -ketoglutarate + NH<sub>3</sub> + NADH  $\rightarrow$  glutamate + NAD<sup>+</sup>; (2) GS-GOGAT cycle: GS catalyses the ATP-dependent conversion of glutamine utilizing ammonia as substrate and is represented by two protein groups, plastid (GS1) and cytosolic (GS2) isoenzymes; GS1 is located exclusively in chlorophyllous tissues while GS2 is located predominantly in roots, nodules, and floral organs. GOGAT catalyzes the conversion of and α-ketoglutarate to glutamate: glutamine glutamine +  $\alpha$ -ketoglutarate + NADP + H + H<sup>+</sup>  $\rightarrow$ 2 glutamate + NADP<sup>+</sup>. The assimilation of NH<sub>3</sub> may be by either glutamate dehydrogenase or GS/ GOGAT; the first of these requires quite high concentrations and the bulk of uptake is thought to be through GS/GOGAT.

Following the incorporation of NH<sub>3</sub> into glutamine, N can be transferred directly to the same position in asparagine by asparagine synthetase. Amino transferase can transfer the amino group to aspartate, which is converted to asparagine. It has been suggested that the reaction catalyzed by Fd-GOGAT is the key regulatory element controlling N partitioning and redistribution during plant growth and development. To date, research has focused on modifying ammonia assimilation in transgenic plants and this has demonstrated that the amplification or shifting of ammonium assimilation in a particular organ or tissue has strong effects on plant growth and development. Thus, there appears to be great potential for improving N-use efficiency in crop plants.

### **Reduction of Nitrate and Nitrite**

Once NO<sub>3</sub> enters the root symplast, it may follow five pathways:

- 1. efflux back to the apoplast [for a review of "apoplast" see www.worldreference.com];
- reduction of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup>, then NH<sub>4</sub><sup>+</sup> by nitrate reductase (NR) using the reduced form of nicotinamide adenine dinucleotide (NADH) and by nitrite reductase (NIR) using ferredoxin (Fd) in root cells, leading to the production of amino acids;
- accumulation into vacuoles of root cells, involving transport across the tonoplast [for a review of "tonoplast" see www.worldreference.com];
- 4. secretion into xylem vessels for long distance transport to the shoots; and
- 5. reduction of NO<sub>3</sub> in the leaves, often using excess reductant in upper, CO<sub>2</sub> limited, leaves.

High concentrations of nitrate can be found in vacuoles, indicating that nitrate not only acts as a nutrient but also participates in osmotic maintenance. The first step in the nitrate assimilation pathway is the reduction of nitrate to nitrite, catalyzed by assimilatory NR. When reduction occurs in leaves, the nitrite formed by NR activity is then translocated to the chloroplast, where it is further reduced to NH<sub>4</sub><sup>+</sup> by NIR. During the nitrate reduction process, NR activity appears to be the rate-limiting step in the conversion of  $NO_3^-$  to  $NH_3/NH_4^+$ , because it is (1) the first enzyme in the pathway  $NO_3^-$  of assimilation, (2) substrate inducible, (3) relatively unstable, and (4) it's activity - relative to other enzymes in the NO<sub>3</sub> assimilation pathway – is low and its  $K_{\rm m}$  (related to binding affinity) for NO<sub>3</sub> is high. Further, there is evidence that when nitrate is the dominant N source, nitrate reductase activity (NRA) is the limiting factor to the growth of many plants. NR has therefore been intensively studied in order to understand it's catalytic efficiency and regulation. Nitrite is reduced to NH<sub>4</sub><sup>+</sup> by NIR using ferredoxin (Fd). Nitrite reductase is located in plastids and possesses Fe–S and heme cofactors. Subsequently, the NH<sub>4</sub><sup>+</sup> is incorporated into carbon compounds: amides (amino acids) and ureides.

Nitrate reduction occurs mainly within the chloroplasts of green leaves, but can also occur within plastids of roots. Induction and the rate of nitrate uptake depend on the external nitrate concentration, light, pH, temperature, the concentration of other ions, anaerobic conditions, metabolic inhibitors, and biological genotypes. Since nitrate reductase contains Mo, Mo deficiency can cause N deficiency.

# **Nitrogen Storage**

Seed storage proteins comprise 70% (up to 100% in some developing countries) of total intake of dietary protein by humans. It also provides the major protein source in the diet for nonruminant livestock. The amino acids in the leaves, produced from assimilated NO<sub>3</sub> with energy and carbohydrates produced by photosynthesis, are transported to the embryo and the cotyledons via mass flux in phloem vessels of the leaf. The main transport forms of amino acids are: glutamine, asparagine, serine, alanine, glutamate, and aspartate. As a result, storage proteins are the products of the secretary pathway, which resides within the endomembrane system of the cell. In general, N assimilates available to developing grains are mainly used for the synthesis of proteins. These proteins then are accumulated and stored in specialized tissues such as the endosperm of cereals, other endospermic monocotyledons, or the cotyledons of the embryo for the pulse legumes and other nonendospermic dicotyledons.

Grains of cereal crops usually contain 7–19% protein while the seeds of legume crops contain up to 40% protein, in both cases on a dry matter basis. Tuber crops are rich in soluble carbohydrates and low in protein. For example, the crude protein concentration in sweet potato is twice that of potato (12.1% versus 5.4%). Thus, when tuber crops are used as human food or animal feed, they are a good source of energy, but require protein supplementation.

Almost all storage proteins in the seeds of major crops can be grouped into three categories according to their functions: (1) globulins (soluble in dilute saline solutions), the most common storage proteins, are present in all angiosperm seeds; (2) albumins (soluble in water); (3) prolamins (soluble in dilute alcohol) and restricted to the seeds of Gramineae (grass) family. This protein represents the main storage protein in cereals such as wheat (gliadins),

barley (hordeins), oat (globulins), rice (glutelins), rye (secalins), and maize (zeins). Both albumins and globulins have biological functions. This is particularly true of enzymes during seed germination. In most cases, storage proteins have no biological activity and only act as a source of N, sulfur, and carbon skeletons for the developing seed. Thus, storage proteins are not as evolutionarily constrained as those of other proteins, such as enzymes, although there is a requirement that proteins should be efficiently synthesized, packaged, and stored, and then remobilized during the process of seed germination.

# Interactions between Nitrogen and Carbon Metabolism

The products of photosynthesis are the building blocks of agricultural production. From a production point of view, the efficiency of photosynthesis is mainly determined by the availability of light, CO<sub>2</sub>, water, heat (temperature), and key elements in the soil, with N being the most likely to be limiting. Precipitation is outside management control in crop production systems, although irrigation may provide reliable water where water resources are available. Nitrogen application, including aspects such as quantity, timing, and method is part of most crop management regimes. Grain yield is largely a function of accumulation of photosynthetic assimilates. The interactions between carbon dioxide (CO<sub>2</sub>) and nitrate assimilation and their dynamics are key elements of crop production. As a result, an adequate supply of N, its assimilation into amino acids (for which photosynthetically produced carbon compounds are required), and their availability for protein synthesis, are essential for metabolism.

Nitrate is reduced to NH<sub>3</sub> by NR and NIR using electrons from photosynthetic electron transport. The NH<sub>3</sub> is then converted into amino acids by the GS/GOGAT reaction (the main pathway by which plants convert ammonia into amino acids), and the "carbon-skeletons" are provided as organic acids derived from the tricarboxylic acid cycle. Carbohydrates for organic acid synthesis, ultimately from photosynthetic CO<sub>2</sub> assimilation, and ATP for the GS/GOGAT reaction are produced by photosynthesis and respiration. Thus, there is close interaction in the very earliest phases of N and carbon metabolism, both using light energy, with some 10% of the electron flux in photosynthesizing leaves used for N reduction.

During the last 4-6 decades grain yields of major field crops have increased  $\sim 2.5$  fold, largely due to an increase in the production and storage of carbon in grains by efficient crop varieties and increased use of

N fertilizer. Although large variations in grain protein concentrations exist among varieties within a species, an increase in yield has generally resulted in a slight to severe decrease in the protein/starch or oil ratio. For example, in the case of maize, the increase in the genetic-based yield potential after 1967 was, on average, accompanied by a decrease in the concentration of protein ( $\sim 1.5\%$ ) and an increase in that of starch ( $\sim$ 2%). In the case of soybean, genetic selection leading to a seed yield increase of 100 kg ha<sup>-1</sup>, increased seed oil yield by 1 kg ha<sup>-1</sup>, and decreased protein yield by  $2-3 \text{ kg ha}^{-1}$ . For wheat, an increase in yield of  $\sim 2 \text{ kg ha}^{-1}$  led to a decrease in the protein concentration of more than 2% of the dry weight. Plant geneticists and breeders continually make efforts to break this relationship, hoping for increases in yield without decreases in quality.

# **Nitrogen and Environmental Health**

Nitrate accumulation in plants occurs when the speed of nitrate uptake exceeds the rate of reduction and subsequent assimilation into amino acid and proteins for growth. A high nitrate accumulation in edible parts of plants consumed as vegetables is a potential health hazard. Although nitrate itself is not toxic, it can easily be reduced, internally, to the toxic compound nitrite. Reduction to nitrite in vegetables can occur postharvest as well as after ingestion, in saliva and in the gastrointestinal tract. One of the symptoms of nitrite toxicity is methemoglobinaemia, in which NO<sub>2</sub> binds tightly to hemoglobin reducing the ability of the blood to carry oxygen and leading to respiratory dysfunction. Chronic nitrite toxicity may lead to the formation of carcinogenic nitrosamines. The frequency of gastric cancer could be reduced by avoiding high intake of nitrate.

According to the International Fertilizer Association, annual world consumption of N fertilizer is equivalent to  $\sim$ 83 million tons (Mt). North America accounts for 15% of this total world consumption. Nitrogen that is not taken up by plants may lead to gaseous loss to the atmosphere, or an increased level of nitrate in the soil. This nitrate is vulnerable to run off in surface water or leaching into groundwater, causing significant environmental pollution. High concentrations of  $NO_3^-$  in drinking water due to surface and ground water contamination can also lead to methemoglobinaemia.

Nitrous oxide ( $N_2O$ ) is a greenhouse gas with a heat-trapping capacity that is  $\sim 310$  times greater than that of  $CO_2$ . Since the last century  $N_2O$  emission has been increasing 0.3% year<sup>-1</sup>. Emissions of  $N_2O$  from cultivated lands are estimated at 3.0–3.5 Mt  $N_2O$ -N year<sup>-1</sup>. The bulk of current  $N_2O$  emissions

are caused mainly by the application of fertilizer N and biomass burning. When soils become anaerobic, or sometimes during the conversion of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup>, N<sub>2</sub>O is produced. N<sub>2</sub>O escapes the soil as a gas and accumulates in the atmosphere where it is both an agent of stratospheric ozone destruction and a very potent greenhouse gas. High levels of soil organic matter and nitrate combined with low oxygen levels promote rapid denitrification. Increasing emissions of greenhouse gases are likely to accelerate global climate change. Average global surface temperature is expected to rise 0.6–2.5°C (33.1–36.5°F) in the next fifty years, and 1.4–5.8°C (34.5–42.4°F) in the next century.

Reducing the contamination of drinking water associated with excess application of inorganic fertilizer and manure in agriculture production is a high priority. There is an urgent need to develop new crop production systems that maximize N use efficiency and are less hazardous to the environment. However, the reduced application of fertilizers conflicts with the need for greater crop yields to feed rising world populations. Crop yields would drop to a very low level if N-fertilizer applications were to cease. Many countries that use substantial amounts of fertilizers and export agricultural produce would barely be able to feed their own population in the absence of Nfertilizer applications. To overcome the potentially dangerous social problems of food shortage, environmental degradation and pollution, agricultural yields per unit land area must be increased at the same time as dependence on applied fertilizers is decreased. Agriculture uses N fertilizer very inefficiently (even in developed countries), in part because other environmental conditions are often limiting. Thus, understanding the process of N uptake and assimilation, and mechanisms associated with increased N-use efficiency by crops would be a benefit not only in the context of environmental concerns but also crop quality. Consequently, selection of superior genotypes with efficient N-use and development of sustainable production systems for specific regions will solve or at least reduce the negative impact of fertilizer N use on the environment.

### **Conclusions and Prospects**

Nitrogen is essential to the growth and reproduction of all plants, and animals. Improved understanding of N dynamics in the biosphere is of both economic and environmental importance in developing sustainable N management strategies for crop production. Absorption of nitrate and ammonium by plant roots is the primary pathway for the entry of N into our food

chain. Nitrate and ammonium enter plants through the cell walls and root membranes. This mineral N has to be incorporated into carbon-nitrogen compounds in order to build cellular metabolites, especially protein. Some leguminous plants can convert atmospheric N to bio-available forms through a symbiotic relationship with specific soil bacteria. This rhizobia-legume symbiosis plays a major ecological and economical role on a global scale. A major recent achievement in understanding the rhizobia-legume symbiosis has been the characterization of signal molecules produced during initial communication between leguminous plants and N-fixing bacteria. The specific lipo-oligosaccharide (LCO) signal compounds, also called Nod factors, secreted by rhizobia, are considered to be a new class of growth regulator, affecting plant growth and development and ensuring the formation of N-fixing nodules. Consequently, a better understanding of the whole plant system, from genes, to plant-microbe relationships, to biomass production, partitioning and grain yield formation might achieve the long-term objective of improved crop N-use efficiency, leading to increased grain crop yield with reduced impact on the environment. For cereals and other nonleguminous grain crops, development of superior genotypes and sustainable production systems with efficient N use continues to be one of the major goals for plant geneticists, crop physiologists, agronomists, grain crop producers, environmental scientists, and policy makers.

See also: Cereals: Protein Chemistry. Nitrogen Metabolism. Organic Growing of Grains. Protein Chemistry of Dicotyledonous Grains. Protein Synthesis and Deposition. Starch: Synthesis.

# **Further Reading**

Follett RF and Hatfield JL (eds.) (2001) Nitrogen in the Environment: Sources, Problems, and Management. New York: Elsevier.

Lea PJ and Morot-Gaudry JF (eds.) (2001) *Plant Nitrogen*. Berlin: Springer.

Morot-Gaudry JF (ed.) (2001) Nitrogen Assimilation by Plants: Physiological, Biochemical and Molecular Aspects. Enfield, NH: Science Publishers.

### **Relevant Websites**

http://www.sws.uiuc.edu – Illinois State Water Survey.

http://www.fertilizer.org – International Fertilizer Industrial Association.

http://www.hort.purdue.edu — University of Purdue.

# NITROGEN METABOLISM

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### Introduction

The success that flowering plants enjoy is in no small way a consequence of the effectiveness of their seed as a perennating organ. The embryo is in a metabolically dormant state and is accompanied by a reserve of nutrients that can be mobilized on germination to support the activated meristems of the new plant. The diversity of composition exhibited by mature seeds reflects variation in the size and nature of this stored reserve. Some are high in carbohydrate, others in protein, and others in oil. Yet all are viable and are able to convert the reserves into translocatable solutes with a balance of C and N that supports the active root and shoot meristems before uptake from soil provides an alternative source of nutrients and before the seedling is autotrophic for C through photosynthesis. The reserves are fashioned from organic assimilates that supply C, N, and S through the long distance translocation channels of the vegetative structure of the plant.

Metabolism of N has been studied in a restricted range of seed types, mostly those of cereals, legumes, and oilseeds of agricultural importance and more recently in the model species Arabidopsis thaliana. Because of the high proportion of protein stored in legume seeds these have received most attention in this regard. However it is well to realize that plants produce an almost bewildering array of N-containing compounds, many of which are amino compounds that accumulate in seeds and are likely to impart unique nutritional, toxicological and pharmacological properties. Our understanding of this whole area in angiosperms is thus limited and fragmentary, and at this stage we must infer the commonality of processes that are so far only adequately documented for a few species.

# **Nitrogen Nutrition of Developing Seeds**

Before considering seed nutrition it is useful to review the basic structural features of seeds and the consequences of this structure to solute import into and transfer within the component tissues of a seed as it develops. The outer seedcoat tissues are maternal while the endosperm and embryo within this coat are filial. There is no vascular connection between the two. Thus, whether stored reserves of the seed accumulate in the endosperm, the embryo, or associated tissues, in all cases solutes that supply C and N (assimilates) pass across an apoplastic compartment. It follows that in receiving translocated solutes before they pass to the embryo the seedcoat may regulate both the rate and nature of solute transfer.

Although developing seeds and their surrounding supporting structures may have the capacity for photosynthesis during early stages of development, this is limited. Consequently their major source of C is assimilates translocated in phloem and xylem. These are principally in phloem as sucrose, longer raffinose-based oligosaccharides, or in some species cyclitols, like mannitol and sorbitol. The same translocation streams supply N but typically phloem contains 10-20 times more N than xylem. However, even though both streams may carry a wide array of amino acids, as well as other N-solutes (e.g., proteins, nucleic acids, plant growth regulators), usually one or two low molecular weight organic compounds predominate. For many dicotyledonous species, including temperate legumes, these are the amides, glutamine and asparagine or their parent acids, glutamate, and aspartate, especially in cereals. In some species arginine, proline, or non-protein amino compounds like homoserine, ornithine, citrulline, canavanine, and putrescine predominate. In others, particularly nodulated legumes of tropical origin, the ureides, allantoin, and allantoic acid, account for almost all the N in xylem and a significant component in phloem-N. There are some very unusual N-solutes that translocate the bulk of N, for example djenkolic acid (two cysteines bridged at their S groups by a methylene) occurs at very significant levels in the xylem of some Acacia spp., and no doubt the plant kingdom employs many other forms of translocated N that have yet to be discovered. Thus protein synthesis in sink organs does not receive a mixture of amino acids that can be simply used to form protein, but rather a mixture in which just one or two solutes provide the bulk of N that has to be metabolized to generate the appropriate proportions of the 20 protein amino acids. In their passage from sites of phloem unloading to the embryo, N-solutes may be transiently stored, for example in the seedcoat, and are metabolized in highly specific ways within the tissue compartments they traverse.

There seems little doubt that almost all the N entering seeds is in an organic form. Although the literature records quite high levels of ammonium ion in both xylem and phloem, it is not likely that this is the norm and may, in many cases, be a consequence of hydrolysis of the amide groups of glutamine and asparagine during or subsequent to collection of vascular exudates. Xylem transports considerable quantities of nitrate from the root system to the major transpiring surfaces of the shoot but negligible quantities of this inorganic ion are transferred to phloem. As a result, fruit tissues and especially developing seeds are unlikely to receive significant levels of nitrate and consequently exhibit low or negligible capacity for its assimilation.

Early in legume fruit/seed development when a significant proportion of translocated N is delivered in xylem, the C:N ratio of incoming solutes is more narrow than that required by the developing tissues, and "excess" N is transiently stored. The endosperm in many dicotyledonous seeds accumulates a significant proportion of this N as ammonium ion and levels approaching 0.1 M have been recorded for periods of 3-4 weeks in white lupin (Lupinus albus) seeds. Although the ammonium is promptly reassimilated when the developing embryo expands to fill the endospermic apoplast, such high levels are normally regarded as extremely toxic and mechanisms that prevent this ammonium entering the embryo to any great extent must be functioning at this time. In pea (Pisum sativum), the major forms of accumulated N in the endosperm are homoserine, glutamine, and alanine, in cowpea (Vigna unguiculata) endosperm there is negligible ammonium ion but 30–55% of the N is histidine, while in chickpea (Cicer arietinum) ammonium and citrulline predominate. The significance of ammonium or other N solutes that accumulate to such high levels in endosperm when embryo development is characterized by cell division is unknown, but it is interesting to note that coconut endosperm ("milk"), used in pioneering studies of plant cell and tissue culture, contains high concentrations of N, including ammonium ion, along with plant growth regulators.

A positive correlation has been established between rates of ammonia emission (i.e., Nloss) and ammonium concentration in leaves. There are no emission data available for developing seeds/fruits, but given the high levels of ammonium ion that may accumulate in liquid endosperm it seems possible that transient periods of N loss occur due to its volatilization. It is perhaps not too surprising then that the highest rates of ammonia emission in barley occur during grain filling.

The mix of solutes in phloem is not constant during reproductive growth, the C: N ratio changing to more

closely match the C and N requirements of the developing seed. In white lupin, sucrose levels in phloem decline progressively after anthesis while the concentration of amino compounds increases, almost doubling over the period of grain filling. This pattern of change is consistent with a declining rate of photosynthesis and increasing senescence of leaves, with release of protein-N as amino compounds, during reproductive development. However, further studies with lupin have shown that in fact the phloem N level also increases to narrow the C:N ratio as a consequence of solute specific mechanisms that achieve an enrichment of phloem with asparagine as the translocation stream passes through stem segments immediately before entering the inflorescence. Similar specific exchanges of amino acids have also been found for developing cowpea fruits/seeds. The precise tissue or cellular site where these transfer mechanisms act and the molecular basis for their regulation is unknown but a role for transfer cells has been inferred.

The developing seed is supported on or within fruiting structures that not only bear the vasculature which brings the translocation streams to the seed but which serve also as intermediate sites for metabolism and transient accumulation of nutrients, including N. For example, in the period from anthesis to 11 days after anthesis, in cowpea fruits 21 mg N enters in phloem and 17 mg N in xylem. Of this N less than half (15 mg) reaches the developing embryo and endosperm, the rest is sequestered into the pod wall (16 mg) and the testa (7 mg). Importantly, however, the incoming N-solutes are metabolized extensively before being transferred to the embryo. At this early stage of development xylem-N is ~70% ureide and phloem-N  $\sim$ 20% ureide, but ureides account for less than 20% in the podwall and testa and no more than 2% of soluble-N in the liquid endosperm or embryo. Later in development (12–22 days after anthesis) phloem accounts for 80% of N entering the fruit and, although the ureide level is low, ureide-N accumulates preferentially in the seedcoat. Despite this enrichment literally no ureide is transferred to the apoplast surrounding the embryo. Rather, ureide-N is converted to glutamine and this is the major source of N for protein synthesis. Similar observations have been made for soybean, another plant that translocates significant levels of ureide-N in xylem and to a lesser extent in phloem.

In pea a major N-solute entering the seedcoat in phloem is homoserine and, although this may accumulate transiently in endosperm, most is converted to threonine and glutamine in the seedcoat during embryo expansion. Even in species that have an asparagine-rich phloem stream reaching the seedcoats,

a significant proportion of asparagine-N is converted to glutamine-N during passage to the apoplast and embryo. Whether this apparent preference for N as glutamine in the embryo's nutrition reflects some metabolic requirement or the specificity of transporters involved in transferring solutes across tissue compartments of the seed has yet to be clearly resolved. In maize the endosperm of the developing kernel supplied assimilates through the pedicel and even though the major phloem N-solute is aspartate this is largely metabolized with its N being transferred to glutamine. The maize pedicel expresses a unique cytosolic form of glutamine synthetase, GS<sub>p1</sub>, which is developmentally regulated, increasing as storage protein synthesis increases. Glutamine is the major amino acid released from the pedicel to the apoplast surrounding the endosperm and a transporter expressed in the basal endosperm transfer cells apparently enhances its uptake. Thus specificity of both metabolism and transport combine to ensure that a preferred N-solute reaches the embryo tissues.

# **Transport of N-Solutes within Seeds**

Rates at which solutes that supply C and N to the embryo are transported are likely to contribute to determining the potential accumulation of assimilates or "sink strength" of seeds and consequently grain yield and harvest index. Studies with four cultivars of bean (Phaseolus vulgaris) that differed in their rates of seed growth found that although sink size, as assessed by surface area and volume of cotyledons, were major determinates of growth rate, the rate of transfer of dry matter was also a contributing factor. Not surprisingly, maximal sucrose fluxes into cotyledons were positively correlated with levels of expression of the sucrose/H<sup>+</sup> symporter and accompanying H<sup>+</sup>-ATPase in the dermal cells facing the apoplast between the cotyledons and the seedcoat. Specifically in relation to N nutrition, activity of amino acid transporters has been suggested to regulate the rate of seed protein synthesis. Although most research into solute unloading from phloem and transport within developing seeds has involved sucrose, some of the amino acid transporters expressed in seed tissues have been identified and their location studied in relation to likely function.

In pea, two cDNA clones (PsAAP1 and 2) belonging to the AAP (amino acid permease) family of H<sup>+</sup>/amino acid co-transporters have been isolated from cotyledons. Like the sucrose transporter, PsAAP1 is highly expressed in specialized epidermal transfer cells abutting the seedcoat and shows broad specificity, mediating transport of acidic, neutral, and basic amino acids. Similar broad-spectrum members

of the AAP family have been described for developing *Arabidopsis* and castor bean seeds. In *Arabidopsis* AtAAP1 is localized to endosperm and cotyledons while AtAAP2 is restricted to the vasculature of siliques and the funiculus. Thus, AtAAP2 is thought to mediate exchange of amino acids from xylem to phloem. Such an exchange has been identified as a specific asparagine transfer in the upper stem segments and raceme of lupin to enrich phloem with N, narrowing the C:N ratio to match more closely seed demands for these commodities.

The broad substrate specificity found for most plant amino acid transporters does not conflict with the idea that translocated N is characterized by just one or two amino acids. Most of the amino acid transporters from plants have been characterized by yeast mutant complementation or functional analysis in *Xenopus* oocyte assays and specificity *in vivo* may be quite different. Indeed, differences in affinity for amino acid species have been described. Furthermore, specific transporters are likely to be found when more extensive genomic data is available for a wider range of plants.

As noted above seed tissues produce and may transiently accumulate significant amounts of ammonium ion. It appears that this is a feature of their metabolism throughout development, raising the question of transport mechanisms for the ion within seed tissues. Preliminary data indicates that genes homologous to the high affinity ammonium transporter family (AMT), AtAMT1 from Arabidopsis, are not expressed in the seedcoat and cotyledons of developing lupin seeds. However, the recently described high affinity AMT2 transporter gene is expressed in Arabidopsis siliques and this may be the transporter that functions in seeds. In view of the widely diverse levels of ammonium that form in seed tissues at different stages of development it seems likely that other transporters/channels with quite different kinetic features are also involved.

The *Arabidopsis* genome has revealed transporters for other types of N-solute that are expressed and could have significant roles in seed metabolism. These include oligopeptide transporters, such as AtPTR2-B, that might be responsible for the movement of peptides released from storage proteins during germination to new meristems or in the transport of signal peptides from sites of synthesis to sites of action. The possible importance of peptide transport in seed development was highlighted in a study where antisense lines for *AtPTR2-B* were generated in *Arabidopsis*. The transgenic plants showed normal silique development but formed fewer, larger seeds than controls. Specifically about half of the embryos were arrested at an early stage of their development,

suggesting peptide transport was essential for N nutrition at this time or that transfer of some bioactive peptide, essential for embryogenesis, was limiting.

Plants also express high affinity transporters specific for purines and purine derivatives (PUPs) including bases, nucleosides, purine alkaloids, and cytokinins. Northern analysis for *AtPUP1* in *Arabidopsis* has however shown only a low level of expression in developing siliques compared to other tissues and their functional significance in seed development or in the mobilization of N during germination remains to be established.

A novel superfamily of transporters has recently been described for allantoin and other oxidized derivatives of heterocyclic N solutes in Arabidopsis. In view of the predominance of ureide-N in translocation streams serving developing fruits in certain tropical legume species, but with the metabolic requirement that ureide-N is not transferred from the maternal tissue to the embryo, it will be interesting to see whether or not fruit and seed tissues express these transporters. Enzymes of purine oxidation (urate oxidase and allantoinase) have been demonstrated at elevated levels during germination of a number of seeds, and it has been inferred that purines are released due to intense nucleic acid hydrolysis. If indeed allantoin provides a supplementary source of translocated N to the newly formed root and shoot apices then the novel allantoin transporters may be important in phloem loading at this time.

# **Primary N Metabolism**

The primary pathways of N metabolism in seeds are the reactions that utilize incoming N-solutes to form the 20 protein amino acids. As noted above N-solutes translocated to tissues surrounding a developing seed or to the embryo itself are characterized by one or two predominant compounds. Among these asparagine and glutamine appear to be the most common. Although some incoming asparagine is incorporated directly into seed protein the majority is metabolized (in white lupin more than 80% over 13 weeks of development). Supplying <sup>15</sup>N (amide)-labeled asparagine to developing lupin fruits in phloem resulted in label being recovered in 15 amino acids following hydrolysis of seed protein. In addition to asparagine, glutamine, aspartate, and glutamate, those labeled included arginine and histidine, those derived from pyruvate (alanine, leucine and valine) and 3-phosphoglycerate (glycine and serine), those of the aspartate family (lysine and isoleucine) as well as aromatic amino acids (tyrosine and phenylalanine). Clearly seeds express all the major pathways for synthesis of protein amino acids.

There are two potential pathways releasing asparagine-N to amino acid synthesis. Hydrolysis of the amide-group by asparaginase produces ammonia and aspartate, the latter providing an amino donor to the many aminotransferases (AT) that have been detected in seeds. The second pathway utilizes an asparagine-AT that transfers the amino group to glyoxylate forming glycine and 2-oxosuccinamic acid. The acid is reduced to 2-hydroxysuccinamate and deamidated to ammonia and malate. Although both potential pathways are expressed in developing pea fruits, asparagine-AT is largely confined to pod tissue and is very low in developing seeds. Asparaginase on the other hand is expressed at high levels in seedcoats early in development, transiently in endosperm and in filling cotyledons of legume seeds. The same could be said for predominant non-amino acid solutes like the ureides. Although there are a number of enzymic routes for the degradation of allantoin that are expressed in different plants, differing even between cultivars, the N is released as ammonia. Thus reassimilation of ammonia is a major activity of seed tissues whether in the seedcoat, endosperm, or the components of the embryo. Not surprisingly, both glutamine synthetase (GS) and glutamate synthase (GOGAT) activities are expressed in seed tissues, providing a high affinity utilization mechanism for ammonia that is likely to function in reassimilation throughout seed development. Seeds express glutamate dehydrogenase (GDH) activity and this may also participate in reassimilation. While the role of GDH has been the subject of considerable debate and conflicting opinion the consensus view that has emerged for most tissues appears to be one of oxidation of glutamate rather than synthesis. However, the very high concentrations of ammonium ion that form transiently in the endosperm of some seeds would be kinetically favorable for GDH to function, for a short while at least, in its assimilation.

Among the protein amino acids that are synthesized largely *in situ* to meet the needs of seed storage protein accumulation are those of the aspartate family; namely lysine, threonine, isoleucine, and methionine. Not surprisingly expression of the first committed step in the aspartate pathway, catalysed by a bifunctional aspartate kinase (AK)-homoserine dehydrogenase (HSDH) in *Arabidopsis*, is coincident with storage protein synthesis in the embryo. Cereals are deficient in lysine and in some, threonine and tryptophan, while legume seeds are poor sources of S-amino acids, particularly methionine. As a consequence there has been considerable research into regulation of the pathway for these "essential" amino acids. There have also been numerous attempts to

exploit this knowledge to enhance the nutritional and/or processing qualities of both cereal and pulse grains. AK activity is subject to feedback inhibition by pathway products, specifically lysine or threonine. However, in the high lysine opaque-2 mutants of maize very significant levels of the aspartate family accumulate as a pool of free amino acids in the mature endosperm. Accumulation results in part from lysine degradation being compromized, because lysine-ketoglutarate reductase activity is suppressed, but other studies have identified changes in sensitivity of the monofunctional AK (Ask2) to feedback regulation by lysine as a major genetic determinant of free amino acid content. In a mapping population with the mutant Ask2 was a good candidate gene for a quantitative trait locus (QTL) affecting free amino acid content in the harvested grain. Dihydropicolinate synthase (DHDPS) converts the product of AK (aspartate 4 semialdehyde) to an intermediate in lysine synthesis and is also subject to feedback inhibition by lysine during seed development.

Specific features of C metabolism within the developing seed are required to accommodate some aspects of amino acid synthesis. Phosphoenolpyruvate carboxykinase (PEPCK) is present in a wide range of developing seeds. In grape seeds, PEPCK is expressed in the inner layer of the seedcoat, in the chalaza and in cells at the boundary of the storage tissue; expression coinciding with maximum deposition of storage reserves. Feeding asparagine to developing grape seeds caused a strong induction of PEPCK indicating that the enzyme functions in the metabolism of N-solutes after unloading from phloem. These data are consistent with an anaplerotic role for PEPCK in supplying acetyl-CoA when the TCA cycle is involved in the interconversion of C skeletons for 4C and 5C amino acids.

Because seeds are formed within enclosing structures that limit gas exchange the concentrations of respired CO<sub>2</sub> within the structures may build up to quite high levels and refixation of some of this C is achieved through RubisCO (Ribulose bis P carboxylase/oxygenase) and especially PEP carboxylase. In lupin embryos some of this refixed C is incorporated in sugars and TCA cycle intermediates (malate, isocitrate, and citrate), but as much as one-third provides C for a range of amino acids, principally alanine, aspartate, and serine, but also glutamate, glutamine, asparagine, glycine, valine, leucine, and isoleucine. In Vicia faba PEP carboxylase is up-regulated in cotyledons during grain filling. Again this suggests an anaplerotic role for the provision of 5C skeletons to the TCA cycle functioning in amino acid synthesis in an intensely respiring tissue.

# Synthesis of Stored N Reserves in Seeds

The major dietary sources of plant protein are seeds of cereals and legumes. Legumes and some oilseeds typically contain 30-50% dry weight as protein while cereals are lower,  $\sim 10\%$ . The "storage" proteins constitute up to 90% of the seed's protein at maturity and as a consequence it is this group that largely determines the nutritional and processing qualities of grain. Features of the synthesis, localization, and properties of the major storage proteins have been reviewed extensively and, more recently, details of the regulatory mechanisms that determine their synthesis in response to changes in plant nutrition and environmental conditions are being revealed.

While most of the incoming N is used for protein synthesis, seeds may also accumulate quite a significant pool of free protein amino acids that persists to maturation and constitutes a "store" at maturity. The amides glutamine and asparagine are common constituents of this soluble-N pool but other amino acids may predominate in some species.

One of these soluble reserves is arginine. In soybean arginine is synthesized in developing cotyledons as well as being supplied from the seedcoat to the embryo through the apoplast. Its accumulation to more than 60% of the free amino acid pool apparently occurs because arginase is not expressed in the embryo. The accumulation of lysine and other amino acids of the aspartate family in maize endosperm was noted above and doubtless there are numerous other examples that occur in nature.

Seeds accumulate significant quantities of nonprotein amino acids. Some of these, such as homoserine, are intermediates in the synthesis of the 20 protein amino acids while others are accumulated as end products of metabolism. Some, such as the arginine analogue canavanine, are highly toxic to insects and other potential herbivores apparently serving to protect the developing or mature grain. Canavanine can reach levels that are 6% of the dry weight in the seeds of Canavalia ensiformis and so represent a significant stored reserve of N. Mature groundnut (Arachis hypogea) seeds contain high levels of  $\gamma$ -methylene-glutamine that is mobilized to the developing seedling following germination but is not metabolized to any great extent, apparently accumulating again in the next generation of seed.

There are hundreds of nonprotein amino and imino acids formed by plants with the overwhelming majority of these accumulated in seeds. Many of these occur in sufficient amounts that they could be construed as a stored reserve but the physiological and biochemical studies to confirm this role have yet to be undertaken.

# Mobilization of N Reserves in Germinating Seeds

While the N reserves in seeds are principally in the form of storage proteins, as germination proceeds N from nucleic acids or metabolic proteins that might have been formed before dehydration or subsequently following imbibition is also available. The possibility that allantoin is formed from nucleic acid catabolism has been indicated earlier. Additionally seeds mature with what can be a significant soluble N pool and this appears to have a specialized role in the first few hours/days of germination when the newly activated meristems of the embryo begin to grow rapidly.

Arginine has been recognized as a common constituent that fulfils this role. Its accumulation as the seed matures results from suppression of an enzyme, arginase, which would normally catabolize the amino acid. However, within a day after imbibition arginase transcript level and activity increase sharply in the cotyledons to release urea and, as a result of urease activity, ammonium ion for reassimilation and translocation to the new meristems. It is likely that the translocated N-solute at this time is glutamine. Thus arginine is a "compact" form of stored N (C:N ratio of 1.5) that provides an almost instant N source, and is no doubt used in this role in a wide range of species (e.g., pumpkin and pea seeds). Whether other soluble N-compounds that accumulate in maturing seeds function as initial sources of N for germination is not known.

Ammonia released from oxidation of free and mobilized amino acids can account for 50% of the N made available during germination of *Arabidopsis* seeds indicating, as during seed development, a central role for GS/GOGAT in reassimilation. QTLs for enhanced germination efficiency in maize have been identified that are co-localized with two of the GS1 (cytosolic forms of glutamine synthetase) structural genes, *gln3* and *gln4*, emphasizing the importance of ammonia reassimilation in regulating the germination process. Such markers offer the potential to breed for complex traits related to seed N metabolism in grain improvement program.

### **Future Prospects**

Despite the wealth of knowledge about the composition of seeds and the nature of their stored reserves, factors that determine which reserves are formed and stored and the roles of environmental cues in these processes have yet to be established. Clearly there is tissue-specific and temporal expression of genes that encode biosynthetic enzymes but the nature of the mechanisms that integrate metabolism so that stored

N-reserves are generated to maximize the available resources of C, N, and S need to be resolved. Although functional genomic or proteomic analyses have been applied to developing seed tissue they have yet to reveal new information about more detailed aspects of the pathways and regulation of N metabolism and transport. As genomic analysis is extended to a wider range of plants this information will become available providing new tools for both understanding and manipulating grain development. Significant changes to grain composition through selection from germplasm collections, conventional breeding and the exploitation of direct genetic engineering will be an ongoing area for research as novel, more nutritious and health promoting food ingredients are sought. Because plants engage in a bewildering diversity of N metabolic end products, many of which accumulate in seeds, it is important that critical studies of N metabolism are extended beyond the handful of species for which information is currently available.

See also: Carbohydrate Metabolism. Cereals: Protein Chemistry. Enzyme Activities. Grain, Morphology of Internal Structure. Protein Chemistry of Dicotyledonous Grains. Protein Synthesis and Deposition. Starch: Chemistry. Wheat: Grain Proteins and Flour Quality.

# **Further Reading**

Editorial (1995) The plant cell – special review issue on plant biochemistry. *Plant Cell* 7: 795–1111.

Atkins CA (2000) Biochemical aspects of assimilate transfers along the phloem path: N-solutes in lupins. *Australian Journal of Plant Physiology* 27: 531–537.

Azevedo RA, Arruda P, Turner WL, and Lea PJ (1997) Review Article Number 121: the biosynthesis and metabolism of the aspartate derived amino acids in higher plants. *Phytochemistry* 46: 395–419.

Bewley JD and Black M (1985) Seeds. Physiology of Development and Germination. NY: Plenum Press.

Kozlowski TT (ed.) (1972) Seed Biology, vol I. Importance Development and Germination. NY: Academic Press.

Lea PJ and Ireland RJ (1999) Nitrogen metabolism in higher plants. In: Singh BK (ed.) *Plant Amino Acids*, pp. 1–47. NY: Marcel Dekker.

Murray DR (1987) Nutritive role of seedcoats in developing legume seeds. *American Journal of Botany* 74: 1122–1137.

Rosenthal GA (1982) Plant Nonprotein and Imino Acids. NY: Academic Press.

# **Relevant Websites**

http://www.mpiz-koeln.mpg.de. http://www.dijon.inra.fr.

# **NOODLES**

Contents

Starch

**Asian Wheat Flour Noodles** 

### Starch

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### Introduction

Noodles form a diverse category of foods that utilize starchy staple crops throughout Asia. Most are in the form of long strips so that traditionally noodles have often been served during special celebrations to symbolize "long life." Today, noodles are consumed as part of ordinary daily meals, snacks, or salads and may be eaten hot or cold. They can be cooked in a variety of ways such as boiling, steaming, stir frying, and deep frying. Noodles may be broadly classified into two general types, i.e., wheat and nonwheat noodles. "Nonwheat noodles" comprise starch noodles (e.g., those from sweet potato) and those from flour (meal) of crops such as maize or rice. Some grains can be used for either starch or flour noodles (e.g., rice). Wheat flour is a unique material, because a simple addition of water coupled with energy input through mixing enables the formation of dough that can be kneaded and stretched to make noodle sheets and strips. This is due to the properties of its unique protein (gluten) that is mainly responsible for wheat noodle structure, although recent studies show that wheat starch properties also contribute to noodle texture. As nonwheat raw materials do not contain gluten, another mechanism of structure formation is utilized to produce noodle sheets and strips, based on the possession of appropriate starch functionality and its manipulation during processing. These products are called nonwheat noodles or often more appropriately starch noodles. Examples include noodle sheets, strips, and threads from flour and starch of cereals, legume starches, and root crop starches. These products are either sold fresh or dried for greater storage

stability. The method by which different botanical source substrates are processed into noodles is discussed in this article. The variety of these Asian products is presented in Table 1, listing the regional names, English equivalents, the countries where they are produced, and their outstanding characteristics and uses. These products are generally bland tasting but readily absorb flavors from other ingredients with which they are cooked. Starch noodles are commercially produced from cereals such as rice and maize and from root crops such as potato (Solanum tuberosum), canna (Canna edulis), arrowroot (Maranta arundinacea), cassava (Manihot esculenta), and sweet potato (Ipomea batatas) in many Asian countries.

# **Types of Asian Starch Noodles**

Rice noodles come in a variety of forms such as thin threads that may be bundled or packed in a compact block (Figure 1a). They are also produced as well-separated rice sticks that vary in diameter and are generally straight cylindrical rods (Figure 1b). There are also flat noodles or ribbons that are well separated and packed as folded bundles. This noodle is commonly used in Vietnamese soups with meat and vegetables (Figure 2a). Distinct characteristics of dried rice noodles are their off-white color, slight opacity, and brittleness of the raw noodles. If enough caution is not taken during packaging and transport, the noodles will easily be broken.

In The Philippines, common starch noodles are almost entirely produced from maize starch, which has been found to be a good substitute for rice, the traditional substrate. The consistency of maize starch in terms of quality, as well as supply and price, has driven this gradual shift to reduced use of rice. It is used as the base for the Philippine "pancit palabok," which is boiled noodles served with a traditional sauce and toppings (Figure 2b). Instant rice noodles in clear soups are found in many Asian supermarkets and come in thread and ribbon forms (Figure 1c). They are either boiled in water or soaked in boiling

Table 1 Different types of starch noodles in Asia

Substrate	English name or equivalent	Country	Regional name	Special characteristics
Rice	Rice vermicelli	China, Malaysia, Philippines Vietnam, Thailand	mei fun, kuey teow, Bijon, bihoon, banh hoi, sen lek, sen mee	Soak the dried noodles in hot water to soften; thin noodles used for soups, stir fries, salad
	Rice sticks	Malaysia Vietnam	Laksa, to banh pho	Soften them in hot water; for soups, salad and spring rolls
	Flat rice noodles; ribbon noodles	Thailand	Banh pho; junta boon; chanta boon	
		China	Ho fun, sha he fen	
	Rice flake noodles	Malaysia	kuay chap, banh uot mien	Shaped like tortilla chips; soften in hot water
Mung bean	Bean threads	China	Baifun, Sai	Soups, stir-fries, salads, desserts, and drinks
	Translucent, silver, shining	Vietnam, Indonesia,	fun, soo hoon, su, un,	
		Thailand Philippines	sotanghon	
	Transparent	China	Fansi, fun see	Flavorless, but readily absorb flavors
	Glass, crystal noodles	Japan, Thailand, Indonesia	Harusame, woo sen, boon tau, pekjysan, tanghoon	Soak until soft
	Mung bean, rice		Tientsin fen pi	
Cassava	Tapioca stick	Thailand	hu tieu bot loc	
Sweet potato	Sweet potato vermicelli	Korea	Dangmyun, tangmyun	Soak in hot water before use
Maize starch	Maize starch noodles	Philippines	Pancit bihon, luglug	Wash with water before use, stir fry
Buckwheat (mixed with noodles wheat flour)	Buckwheat noodles	Japan	Soba, naeng myun	Buckwheat flour and potato starch; may be served hot or cold
	Buckwheat noodles	Japan	cha soba	With green tea
	Buckwheat noodles	Japan	nama, hashiwari	With greater amount of buckwheat flour
	Buckwheat noodles	Japan	yaki, chuka	With high fat content

water for 3 min before being served and are offered with assorted flavors of soup base such as chicken, seafood, and beef. They may also be conveniently packed in cups to which boiling water is poured and can be directly consumed after a few minutes (Figure 1e).

Another type of noodles is produced from the legume, mung bean (*Vigna radiata*) (**Figure 1d**). These noodles are much whiter, and usually thinner in diameter than rice noodles, very glossy, clear and with high tensile strength both in the raw and cooked

form. The strands are well separated, bundled neatly, and do not break easily. Another difference between rice noodles and bean thread noodles is the cooking instructions that come in the package. Rice noodles are usually provided with detailed instructions on how the product is to be prepared for cooking, such as soaking, boiling, and washing in cold/hot water before it is used in soup or stir-fried dishes. Emphasis is given to the time required so that a desired textural quality in the cooked product is attained. In contrast, mung bean noodles are normally



Figure 1 Different types of Asian noodles: (a) thin rice vermicelli; (b) rice sticks; (c) instant vermicelli; (d) bean noodles; (e) instant starch noodles-in-a-cup; and (f) buckwheat noodles.

not provided with these instructions, because they can withstand long soaking and boiling times without adverse effects on the characteristic texture.

Another variant of starch noodles are the wrappers used in many food preparations. Steamed Chinese

"dimsum" are normally wrapped in wheat flour dough sheets but may also be wrapped in starch sheets made from wheat and cassava. These provide a nearly transparent skin that shows the colorful mixture of the vegetable and meat filling of the product. These



Figure 2 Prepared dishes and the starch noodle/wrapper used: (a) rice flat noodles used in beef soup; (b) maize starch noodles in pancit palabok; and (c) rice paper used for steamed spring rolls.

wrappers may also be mixed with vegetable juices from spinach or carrots to give a dimsum dishes diverse natural colors. Wraps or sheets may also be sold in the dried form and used as spring roll wrappers. A variant is shown in Figure 2c, a Vietnamese steamed spring roll with mixed vegetable and meat filling wrapped in round rice paper.

### **Noodle Processing**

The general procedure by which starch noodles are made is presented in Figure 3. A portion of the flour or starch is gelatinized to serve as binder which allows formation of dough or batter which may be extruded or sheeted and further molded into the desired shape. The variations possible in each step in different products are presented in the following sections.

### **Substrates Used for Starch Noodles**

The generally considered best substrate for starch noodles is from the legume, mung bean (Vigna radiata), which normally has high amylose content (greater than 30%). The eating and cooking qualities of mung bean noodles are usually the benchmark for high standards when working on experimental starch substrates and process parameters for starch noodles. Aside from its tensile strength and chewiness, bean noodles are also known for clarity and gloss not observed in noodles from other substrates. This characteristic is the reason why they are referred to as "spring rain," "invisible," "transparent," or "glass" noodles. It also has high tensile strength both in raw and cooked form. Legume starches generally exhibit a characteristic type C Brabender pasting profile characterized by the absence of peak and breakdown, and high hot paste stability and setback, similar to

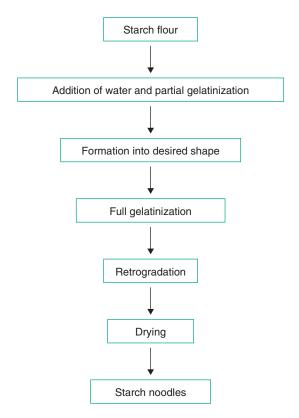


Figure 3 Schematic flow diagram for starch noodle production.

cross-linked starches. Studies on noodles from different cereal and root crops have shown that textural quality of noodles produced is significantly correlated to the pasting profile determined using a visco-amylograph. The pasting profile characterized by high hot paste stability and high setback has been used by several workers as screening criteria for evaluation of different substrates and starch modification processes for suitability for noodles.

In The Philippines, as maize starch has become a common replacement for rice in starch noodles, initial process steps involving soaking, washing, and wet milling of rice grains have been eliminated. It has simplified the process, reduced costs, and also produced noodles with consistent quality in terms of color, surface smoothness, and texture. Although noodles from vellow maize make distinctly vellowish noodles as compared to white maize, this difference does not seem to adversely affect most purchase decisions of consumers. The use of starch (instead of rice flour) offers additional stability from rancidity. It is also common practice to add cassava starch to improve texture of rice and maize starch noodles. Fresh starch noodles or wrappers are likewise prepared from a composite of wheat starch and cassava starches to attain a characteristic translucency and texture not possible when the starches are used individually in the formulation. Generally, blending of starches serves to improve texture which may be reflected as increased strength and elasticity imparted to starch sheets during processing. This makes the sheet less vulnerable to breakage and consequently reduces production losses. Another common recommended noodle improver is "konjac" flour, composed mainly of glucomannan and extracted from elephant's foot yam (*Amorphophallus* sp.). It may be added at the rate of 3-10% of starch, and its addition not only improves texture but also increases the soluble fiber content of the product.

A traditional product from composite flour with wheat is buckwheat noodles (Figure 1f). Its structure is partially dependent on the wheat flour component. It is brownish in color and can be found in both the dry and fresh form and can be served hot or cold. Buckwheat noodles are known in Japanese as "soba." This refers to long and thin brownish noodles made from a composite of buckwheat flour and wheat flour, the ratio of which varies according to the type of the product. The most common buckwheat noodles combine 60–70 parts buckwheat to 30–40 parts wheat flour, to which 45–48 parts water is added. Korean buckwheat noodles may include potato starch. Other formulations may include the addition of green tea that imparts a characteristic color.

### **Partial Gelatinization of Moist Starch**

In the preparation of rice noodle, the grains are soaked overnight, washed, and wet-milled to a fine consistency, after which excess water is drained. When purified starch is used as substrate such as in the case of maize, potato, and sweet potato, water is added to the dry starch to make ~20% moisture content (Figures 5a and 6a). This initial moisture enables mixing with minimal lumpiness that facilitates easy addition of more water. For rice and maize noodles, the dough is formed into fist-sized balls and cooked in boiling water for  $\sim$ 30 min. The outer layer is cooked in the process but the inner portion is left uncooked. A kneading process is employed to fully disperse the gelatinized starch in the uncooked portion to produce noodle strands that do not readily break during extrusion.

In the preparation of buckwheat noodles, the most common process involves mixing buckwheat flour into wheat flour, to which water is added, the amount depending on the protein content of the mixture. The flour is mixed in a circular motion with gradual addition of water. The binding capacity of the flour is greatly improved by using boiling water during the initial mixing stages which gelatinizes the buckwheat flour starch.

For jelly noodles from sweet potato and potato, a batter with appropriate flow characteristics is required to enable smooth application on to a metal plate or cloth canvas to form a sheet. The metal sheet conveyor is always kept clean so that the smooth sheet is peeled off easily. The addition of hot water of  $\sim 70-80^{\circ}$ C with simultaneous mixing enables partial gelatinization and development of right viscosity and smoothness (Figure 5b). In a similar manner, Chinese dimsum wrappers from starches are prepared by adding boiling water to wheat starch and cassava starch to enable the formation of dough which can be formed into a ball and rolled thin to a circular sheet used to enclose the filling, otherwise the sheet breaks easily when folded.

### **Shape Formation**

In rice and maize starch noodles, smooth balls are made from the kneaded dough and loaded into a cylindrical tube holder ready for extrusion by a hydraulic press that forces the dough through a die with specified opening corresponding to the noodle diameter (Figures 4b-4d). For flat noodles, the cutting step is done after sheet formation that is set through gelatinization. In fresh-noodle processing, the cutting edge is heavily coated with oil to prevent starch sheet and noodles from sticking to metal-cutting surfaces. When dried flat noodles are prepared, the sheet undergoes a short drying process before a cutting step is employed. The process may involve a drying cabinet with air drying/tempering as shown in Figures 5d and 5e, in the production of potato jelly sheet noodles.

Dimsum wrappers are formed by carefully picking up the flattened soft pliable circular dough, folding 3—5 times on one side of the circle, pinching to keep the folds in place, putting a tablespoon of filling into the dough circle and sealing tightly with another pinch. For dried starch wrappers, the shape is attained when batter is poured on to a shaped mold normally a round shallow container. Rice spring roll wrapper usually has a delicate weave design that it picks up from the bamboo tray on which it was molded and dried. A Vietnamese dish uses this rice paper as wrapper for steamed or fried spring roll (Figure 2c).

The different preparation methods for starch noodles are determined to a great extent by the flow characteristics of the starch dough and are reflected in the finished product. It was observed in some studies that the starch dough becomes more cohesive when phosphorylated cassava starch is added to potato starch resulting in thicker noodle strands even when using the same die opening, compared to use of pure mung bean, sweet potato, or potato starches. Even at similar

moisture content ( $\sim$ 50%), for starch dough in which gelatinized starch is used as binder, different starches exhibit different flow characteristics so that a different extrusion or sheeting condition may be needed. In dried rice noodle production, a hydraulic press extruder is used while for sweet potato noodle production, an open mechanically driven screw-type extruder is used (Figure 5c). Reports on different substrates vary in the proportion of 1:1 to 1:3 (w/w) uncooked and gelatinized starch and moisture content which range from 45% to 60%. Lower and higher ratios of gelatinized starch produced slurry that was either too dry or soft to extrude. However, a much lower amount of gelatinized starch binder ( $\sim 5-7\%$ ) has been used successfully in the preparation of mung bean noodles. This may be attributed to the difference in the degree of gelatinization of binder, extrusion conditions employed, the unique starch properties of substrate being used, and/or the additive or improver applied. Indeed, the process of making starch noodles remains an art based on experience in the handling of a particular substrate.

### **Full Gelatinization of Shaped Dough**

The shaped noodles or sheet is fully gelatinized by either boiling or steaming. In rice noodles, the noodle strands may be extruded directly into boiling water in a cooking vat for 2-5 min or steamed on trays in steaming chambers for 15-20 min. Once the extruded noodles are dropped into boiling water, they are removed when sufficiently cooked or when strands begin to float on the surface (Figure 4d). This is due to the change in density of the noodle strand as it is cooked or gelatinized. Uncooked starch granules have a relatively high density,  $\sim 1.5 \,\mathrm{g\,cm}^{-3}$ , so that uncooked noodles settle directly to the bottom of the cooking container; but as they gelatinize, starch granules swell as they absorb more water and consequently float on the surface of boiling water. The extruded noodles can also be steamed in a conveyor for 15 min at 120°C. Full gelatinization of the extruded noodles serves to set noodle structure and improve texture and flavor on cooking. Degree of gelatinization of steamed extruded rice noodles is reported to be from 65% to 70%, with the surface ~98% gelatinized and the core only 55% gelatinized.

For flat noodles, the starch sheet is steamed through the application of heat under the metal or canvas conveyor which moves over the heating/steaming components. In effect, the speed by which the sheet moves sets the cooking time. In the processing of canna starch noodles in Vietnam, the steam sheeting of canna starch dough is followed by a stretching step

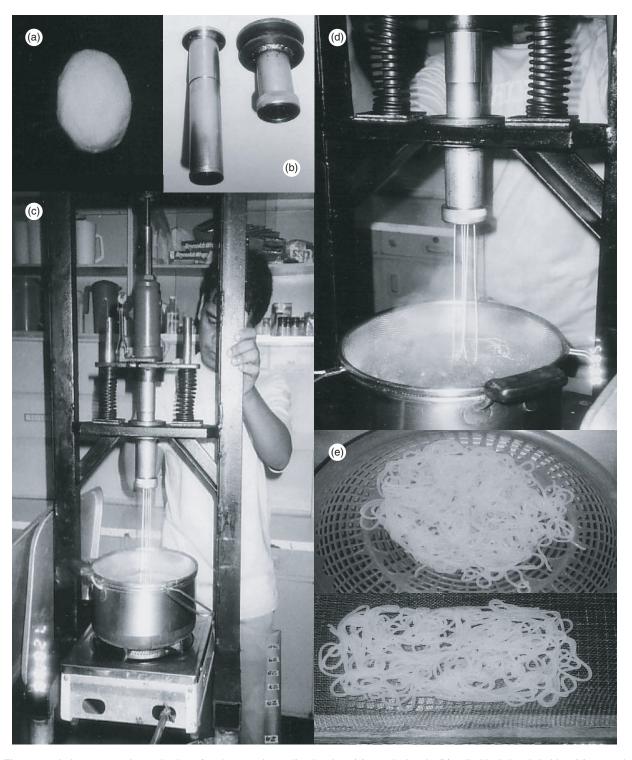


Figure 4 Laboratory scale production of maize starch noodle showing: (a) starch dough; (b) cylindrical dough holder; (c) manual extruder assembly; (d) extrusion of dough into boiling water; (e) drained noodles; and (f) noodles on a drying tray (Institute of Food Science and Technology, University of the Philippines, Los Baños College, Laguna, The Philippines).

not found in rice or mung bean/sweetpotato jelly sheet noodles. For Chinese dimsum, cooking enables not only setting the wrapper shape but it also cooks the vegetable and meat fillings.

# Retrogradation

In the production of starch noodles, processes are employed to enhance retrogradation, such as the



Figure 5 Potato starch noodles production showing: (a) mixing starch batter; (b) application of batter on a metal sheet; (c) cooked sheet peeled off from metal sheet; (d) drying starch sheet; (e) tempering of starch sheet; (f) sheets in rollers; (g) cutting; (h) drying; and (i) potato noodles (Changchun, People's Republic of China).

low-temperature conditioning step applied after the gelatinization of the noodle strands or sheets. Retrogradation is the process that finally sets the noodle structure that can withstand normal cooking temperatures in soups and stir-fried meat and vegetable dishes. This may involve freezing and thawing as in

mung bean noodles, or a simple washing in cold water and tempering as in rice, potato, and sweet potato noodles at room temperature (Figures 5e and 6g). It is interesting to note that in village level production of sweet potato noodles, the conditioning stage is attained naturally since processing is an annual



Figure 6 Sweet potato starch noodles production showing: (a) mixing starch; (b) starch dough; (c) extrusion of dough; (d) cooking of noodles; (e) cooling of noodles; (f) hanging of noodles; (g) tempering of noodles; (h) drying; and (i) sweet potato noodles. (Pinyin County, Jinan, Shandong, People's Republic of China).

activity during the cold months in October or November when night temperature in Sichuan and Shandong, China, drops to  $0-5^{\circ}$ C. After an overnight conditioning, the noodles are separated and allowed

to dry under the sun and drying is further enhanced by the low relative humidity which normally drops to  $\sim 50\%$  during this time of the year. In many production systems in Asia, favorable conditions

Starch is made up of two molecular forms, the unbranched glucose polymer, amylose, and the branched glucose component, amylopectin. Earlier findings revealed that amylose crystallization in the retrograded B-form kept the structure intact in rice noodles and mung bean starch noodles. The following discussion deals with the retrogradation process as it relates to starch noodle processing and the formation of a thermally stable structure that can withstand normal cooking conditions and temperatures. Starting from the gelatinization process in excess water, the starch granules swell and gradually lose their molecular order; the amylose solubilizes and a starch gel or paste is formed. Amylose and amylopectin aggregate to form crystalline double helices stabilized by hydrogen bonds in the process known as retrogradation, forming three-dimensional crystalline structures of the B-type. An A-type crystalline structure can be obtained if retrograded starch is formed in gelatinized starch stored at high temperature (e.g., 100°C) for several hours. It is now generally accepted that both A- and B-type starch structures consist of double helices. These crystallites are highly stable, showing a melting endotherm at  $\sim 120-150^{\circ}$  C and are resistant to enzyme digestion. It is thus evident that these structures are responsible for the integrity of the noodle structure. Amylopectin molecules can also crystallize by association of the short lateral chains. Amylose retrogradation is a rather fast process, taking place in a few hours; however, amylopectin requires longer times (days or week). Amylopectin crystallites are less stable than amylose ones, with a melting point close to 55-70°C. Even though amylopectin is almost always found in greater amounts, its role in starch noodle structure is not as significant as compared to that of amylose, but its presence may have implications on noodle texture.

Retrogradation of starch leads to the formation of enzyme resistant starch (RS). In studies on gelatinized starch containing both amylose (25%) and amylopectin (75%), as in normal maize and rice starches used in starch noodles, resistant starch yield depends strongly on storage time and temperature. Resistant starch formation in gelatinized starch can also be described as the crystallization of amylose in a partially crystalline polymer system. In such a polymer system, nucleation is favored at glass transition temperatures ( $-5^{\circ}$ C), while propagation is favored under conditions above the glass transition but below the melting temperature. At a high storage temperature ( $100^{\circ}$ C), resistant A-type crystalline structures were formed while at lower temperatures ( $0-68^{\circ}$ C),

formation of B-type crystals was observed. Crystallinity of the resistant fractions increases with storage of the starch gel. This also appears to be consistent with the promotion of retrogradation in starch noodle production, in which gelatinized noodles are exposed to freezing temperatures  $(-5-0^{\circ}\text{C})$  in mung bean thread noodles or plain washing in water for conditioning and tempering in rice and maize starch noodles at room temperature (25–35 $^{\circ}$ C). In studies that compared starches and flours from wheat, maize, rice, and potato for optimum RS production, the recommended process involves gelatinization in excess water, cooling to room temperature followed by overnight freezing and a low-temperature drying at 60°C. These processes are parallel to that required in most starch noodle production systems.

Formation of RS is evidently affected by the water content and temperature. A minimum of water is necessary for plasticization of the environment and for incorporation into the crystal structure. The dependence of retrogradation on starch concentration has been reported by several workers. It was reported that a bell-shaped distribution of retrogradation,  $\Delta H$ , as a function of starch concentration occurred with maximum values in the range of 50-60% starch. These DSC data support X-ray diffraction studies which reported that 50% starch gels produced the most intense X-ray pattern. It is interesting to note that this is the approximate moisture and starch content range that is required in starch dough for extrusion or sheeting in starch noodle processing. Although these findings support the apparent thermal stability of starch noodles and sheets when exposed to normal cooking conditions, actual studies on the nature of starch noodle structure and properties from different substrates are very limited.

# **Drying**

Most starch noodles are traditionally air-dried under the sun (Figure 6h) but much commercial production is now dried in mechanized driers which are part of a continuous process (Figure 5d). In China, in the production of jelly sheet noodles from potato and sweet potato, the gelatinized sheet is scraped and moved on to a fine wire screen conveyor that leads to a heating cabinet (Figure 5d), after which the sheet comes out ready for tempering followed by cutting, drying, and packing (Figures 5g and 5h).

### **Nutritional Benefits**

There is renewed interest in starch noodles due to the nutritional benefits associated with their consumption. The definition of dietary fiber has broadened in recent years to include resistant starch. Starch noodles provide

not only an alternative to wheat-based pasta for celiac patients, but are also now considered a source of dietary fiber from retrograded starch recognized as a form of resistant starch. Other forms of enzyme-resistant starch include physically inaccessible starch, locked in the plant cell; the native granular starch found in food containing uncooked starch; chemically and thermally modified starches; and the indigestible starch fraction formed after heat-moisture treatment of starch (i.e., the retrograded starch formed when starch noodles are produced).

Retrograded starch is left undigested until fermented in the large intestines. It can be considered invisible fiber, because its consumption is not coupled with coarseness and discoloration normally associated with traditional sources of fiber such as brans and wholegrain cereals. In earlier work, retrograded amylose was considered to be non-nutritive; however, it was demonstrated that amylases gradually degrade the structure. Glucose and oligosaccharides are released from retrograded starch over an extended period through the normal digestive process. Studies have shown that extruded noodles from high amylose rice varieties significantly reduced starch digestibility (by 15%) and the glycemic index in normal individuals (by 36%). This has been attributed to starch gelatinization and retrogradation during processing. The low glycemic response from noodles implies that these foods may have health benefits to both normal and diabetic individuals through sustained energy level and prolonged satiety.

Dietary fiber (DF) is a significant food component that can minimize the risk of colon cancer, cardiovascular disease, and diabetes. Studies on test animals demonstrated that resistant starch offers advantages comparable to traditional sources of dietary fiber in providing fecal bulk and encouragement of the growth of beneficial microflora. The physiological effects of RS are comparable to those of fermentable dietary fibers. A lower acidity in the colon is attained through the fermentation of carbohydrates by gut bacteria to short-chain fatty acids (SCFAs) such as acetic, propionic, and butyric acids. SCFA production offers some defense against colon cancer by epithelial proliferation, increased absorption of minerals, and bacterial metabolism of bile salts and balance of bacterial microflora.

Food consumption patterns are affected by factors of convenience in current fast-paced lifestyles and consumer demand for healthy natural food products. This development had triggered a lot of research on resistant starch, its health implications, and methods of analysis as a dietary fiber for appropriate food labeling purposes. Starch noodles can now be presented with a new image as a nutritious energy

food that can be recommended as a vehicle for the modulation of glucose release in the blood for diabetic patients and sustained energy level for athletes as well as rich source of dietary fiber. Optimization studies on resistant starch formation in starch noodles with the use of different substrates and process variables may present alternative ways by which resistant starch can be offered as a functional food ingredient in both traditional and nontraditional food products.

See also: Gluten and Modified Gluten. Maize: Wet Milling. Noodles: Asian Wheat Flour Noodles. Nutraceuticals from Grains. Starch: Chemistry.

# **Further Reading**

Alden L (2000) The Cook's Thesaurus Noodles (http://www.foodsubs.com./noodles/html).

Bhattacharya M, Zee SY, and Corke H (1999) Physicochemical properties related to quality of rice noodles. *Cereal Chemistry* 76: 861–867.

Chang SM and Lii CY (1987) Characterization of some tuber starches and their noodle quality. *Academia Sinica International Bulletin of Chemistry* 34: 9–15.

Collado LS, Mabesa LB, Oates CG, and Corke H (2001) Bihon-type noodles from heat moisture treated sweet potato starch. *Journal of Food Science* 66: 604–609.

Eerlengen RC, Deceuninck M, and Delcour JA (1993) Enzyme resistant starch: II. Influence of amylose chain length on resistant starch formation. *Cereal Chemistry* 70: 345–350.

Galvez FC, Resurrection AVA, and Ware GO (1994) Process variable, gelatinized starch and moisture effects on physical properties of mungbean noodles. *Journal of Food Science* 59: 378–381, 386.

Jang JK and Pyun YR (1997) Effect of moisture level on the crystallinity of wheat starch aged at different temperature. Starch/Starke 49: 272–277.

Kim YS, Wiesenborn DP, Lorenzen JH, and Berglund P (1996) Suitability of edible bean and potato starches for starch noodles. *Cereal Chemistry* 73: 302–308.

Lii CY and Chang SM (1981) Characterization of red bean (*Phaseolus radiatus* var. *aurea*) starch and its noodle quality. *Journal of Food Science* 46: 78–81.

Mestres C, Colonna P, and Buleon A (1988) Characteristics of starch networks within rice flour noodles and mung bean starch vermicelli. *Journal of Food Science* 53: 1809–1812.

Panlasigui LN, Thompson LU, Juliano BO, *et al.* (1992) Extruded rice noodles: starch digestibility and glycemic response of healthy and diabetic subjects with different habitual diets. *Nutrition Research* 12: 1195–1204.

Schoch TJ and Maywald EC (1968) Preparation and properties of various legume starches. *Cereal Chemistry* 45: 564–573.

Sing JY, Voraputhaporn UW, Rao PV, and Jambunathan R (1989) Physicochemical characteristics of pigeon pea

and mungbean starches and their noodle quality. Journal of Food Science 54: 1293-1297.

Udesky J (1988) The Book of Soba, pp. 21-42. Tokyo: Kondansha International.

Wolever TMS and Jenkins DJA (1985) The effect of fiber and foods on carbohydrate metabolism. In: Spiller G (ed.) Handbook of Dietary Fiber, pp. 87-119. Bocca Raton, FL: CRC Press.

### **Relevant Websites**

http://www.grains.org - US Grains Council website with information on the various types of value enhanced corn, their growing location and the market channel contacts.

http://www.namamillers.org - North American Millers' Association site with information on the products produced by corn dry milling.

# Asian Wheat Flour Noodles

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### Introduction

Asian noodles, which have relatively simple origins in China, have evolved into a vast array of varieties. These types vary with respect to formulation, process, and quality attributes of the finished products. The aim of this article is to describe the various noodle types and manufacturing processes, and the effects of processing variables and raw materials on product quality.

#### Classification of Noodles

Noodles are often classified on the basis of raw materials, dimensions of the strands, and by the method of manufacture. A similar approach is used here.

# **Classification Based on Ingredients**

The main ingredients in Asian noodles are flour, salt (sodium chloride), and water, and in the case of alkaline noodles, solutions of sodium carbonate, potassium carbonate, sodium bicarbonate, or sodium hydroxide. The alkaline solutions are known as lye water or "kansui." On this basis, noodles can be classified as nonalkaline or alkaline depending on the presence or absence of alkaline salts. There has been a tendency for nonalkaline noodles to be referred to as "white salted," but

this category is not exclusively white and containing sodium chloride. In Japan a creamy colored noodle is preferred, and in China this noodle type is manufactured with and without salt. Also, sodium chloride may be used in addition to alkaline salts in the manufacture of alkaline noodles. While there is also a tendency for Japanese-style white salted noodles to be referred to generally as "udon," in Japan there remains a preference for such noodles to be referred to by specific names that relate to noodle strand size – of which udon is one (see below and Table 3).

In addition, ingredients such as starches, gums, phosphate salts, and colorants are commonly used for specific effects in both nonalkaline and alkaline noodle types.

"Soba" is a popular noodle consumed in Japan, and is usually made from a mixture of buckwheat flour and high-protein wheaten flour, water, and salt. The high-protein content of the wheaten flour compensates for the lack of gluten-forming protein in buckwheat flour. Basic formulations for various noodle types are given in Table 1.

# **Classification Based on Manufacturing Process**

A small percentage of noodles are hand-made and valued for their fine texture, including types made by stretching, cutting, or shaving of a hand-mixed dough. Most noodles are made by machine, with the basic steps of dough mixing, sheeting, combining of sheets, resting, rolling, and cutting being used in most noodle plants. Subsequent treatment varies and noodles may be manufactured in various final forms for sale, including fresh (raw), semidried, dried, boiled, steamed, steamed and dried, steamed and fried, and frozen types. These types are described in more detail later in this article.

# **Classification Based on Noodle Size**

Noodles commonly have a rectangular cross-section but may be square or round depending upon the thickness of the noodle sheet and the shape and size of the cutting rolls used. In Japan, there is a wide range of nonalkaline types which differ markedly in their width (Table 2). Throughout Asia, an extensive range of noodle types and sizes is manufactured among both alkaline and nonalkaline noodles (Table 3).

# The Noodle-Making Process and Its Effects on Noodle Quality

#### **Basic Processing Steps**

Mixing Vertical or horizontal dough mixers and a variety of mixing processes are used to blend

**Table 1** Typical noodle formulations

Noodle type	Country or region	Preferred color of product				Basic ing	redients	Other key ingredients	Reference
or region	or region	for market <sup>a</sup>	Flour Water		NaCl		Alkali	Ingrodiente	
			(parts)	(parts)	(parts)	(parts)	(type)		
Nonalkaline									
Udon	Japan	Creamy – creamy yellow	100	34	2 <sup>c</sup>				1
Gua mian (dried noodles)	China	White – creamy yellow	100	d					2
Gua mian (dried noodles)	China	White	100	25-32	2-3				3
Mee sua (steamed and dried)	SE Asia	White	e	e	e				4
Mee teow (steamed and dried)	SE Asia	White	е	e	e				4
Alkaline									
Ramen (raw)	Japan	Light yellow	100	32	1	1.0	Na <sub>2</sub> CO <sub>3</sub> : K <sub>2</sub> CO <sub>3</sub> (40:60)		1
Steamed Chinese noodles	Japan	Light yellow	100	34	1	1.0	Na <sub>2</sub> CO <sub>3</sub> : K <sub>2</sub> CO <sub>3</sub> (40:60)		1
Cantonese (raw)	Asia	Light-medium yellow	100	36	1	1.2	Na <sub>2</sub> CO <sub>3</sub>		5
Hokkien mee (parboiled)	SE Asia	Intense yellow	100	36	2.5	0.8	NaOH		5
Hokkien mee (parboiled)	SE Asia	Intense yellow	е	е	е	е	NaOH, KOH		4
Mee pok (raw)	SE Asia	Light yellow	e	е	e	е	Na <sub>2</sub> CO <sub>3</sub> , K <sub>2</sub> CO <sub>3</sub>		4
Mee kia (raw)	SE Asia	Light yellow	e	е	e	е	Na <sub>2</sub> CO <sub>3</sub> , K <sub>2</sub> CO <sub>3</sub>		4
Wanton mee – Singapore style (raw)	SE Asia	Intense yellow	е	е	е	е	Na <sub>2</sub> CO <sub>3</sub> , K <sub>2</sub> CO <sub>3</sub> , NaHCO <sub>3</sub> , KHCO <sub>3</sub>	Eggs or egg white, approved colorant	4
Wanton mee – Hong Kong style (raw)	SE Asia	Intense yellow	е	е	е	е	Na <sub>2</sub> CO <sub>3</sub> , K <sub>2</sub> CO <sub>3</sub> , NaHCO <sub>3</sub> , KHCO <sub>3</sub>	Eggs or egg white, approved colorant	4
Bamee	Thailand	Intense yellow	100	28	3	1.5	Na <sub>2</sub> CO <sub>3</sub>	Egg	6
Instant steamed and fried	SE Asia	Light yellow	100	34-37	1.6	0.2	Na <sub>2</sub> CO <sub>3</sub> : K <sub>2</sub> CO <sub>3</sub> (50:50)	Guar gum, polyphosphates <sup>f</sup>	6

<sup>&</sup>lt;sup>a</sup>Ideally the noodles should also be bright.

References: (1) National Foods Research Institute, Ministry of Agriculture, Forestry and Fisheries (1985) Quality Assessment of Wheat - Sensory Test for Noodles. Japan: MAFF; (2) Professional Standard of the People's Republic of China (SB/T 10137-93); (3) Huang S (1996) China - the world's largest consumer of paste products. In: Kruger JE, Matsuo RB, and Dick JW (eds.) Pasta and Noodle Technology, pp. 301-329. St. Paul, MN: American Association of Cereal Chemists; (4) Singapore Standard CP64:1996; (5) Commercial source; (6) Hou G and Kruk M (1998) Asian Food Technology. Technical Bulletin, Vol. XX, Issue 12. Manhattan, KS: American Institute of Baking.

<sup>&</sup>lt;sup>b</sup> Higher water levels are added with hand-made noodles; also with vacuum mixing, e.g., udon -40-43%.

<sup>&</sup>lt;sup>c</sup> Higher levels of salt in hand-made noodles.

<sup>&</sup>lt;sup>d</sup>Water addition based on Farinograph water absorption.

<sup>&</sup>lt;sup>e</sup>Ingredient listed in main reference but amount not indicated.

<sup>&</sup>lt;sup>f</sup>A wide range of ingredients and additives is permitted.

ingredients and, depending upon the amount of water added, to develop a crumb or dough. Saline or alkaline solutions are usually incorporated with the dry ingredients at levels of  $\sim 28-35\%$  (based on flour weight) for nonalkaline noodles and  $\sim 28-34\%$  for alkaline noodles. Moisture additions may increase from 40% to 47% if vacuum is applied during mixing. In forming the initial dough crumb, the main focus is even hydration of the dry ingredients and there is little gluten development. Estimation of optimal water addition is generally based on an assessment of crumb size (for low water additions) and satisfactory sheeting and handling properties. The amount of water added affects almost all processing operations and final product quality. Low water addition and crumb formation is favored in the preparation of noodles that will be dried prior to distribution. Use of higher amounts of water under vacuum mixing

**Table 2** Classification of Japanese-style white salted noodles in Japan by width

Noodle type	Width (mm)	Cutting roll size
Somen	1.0-1.2	30-26
Hiyamugi	1.3-1.7	24-18
Udon	2.0-3.8	16-8
Hiramen (Kishimen)	5.0-7.5	6-4

Data from Japanese standard JIS, B9201.

Table 3 Dimensions of various types of Asian noodles

has advantages in minimizing gluten damage during subsequent sheeting, combining, and rolling operations, increasing product yield of raw noodles, and reducing cooking time for processes involving boiling or steaming.

The maintenance of close control of dough temperature is important in the mixing stage of the noodle process, and too low or too high a temperature can have adverse effects on product quality. In experimental studies, the Japanese National Foods Research Institute specifies that room temperature should be  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and the crumb or dough temperature  $26^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . These specifications could also be used as a guide for commercial noodle manufacture.

Initial sheeting and combining The crumb or dough is split into two and each is passed through a set of sheeting rolls. The two sheets are then combined through a single set of rolls to facilitate gluten development. At this stage, the sheet is usually rested for periods of up to 1 h, although longer rest periods are used in some plants. The rest period allows for relaxation of the gluten structure and minimizes damage to the gluten during subsequent reductions in sheet thickness. Studies at the Bread Research Institute of Australia that were reported in 1987 showed that rested Cantonese noodle doughs had a more continuous protein matrix, fewer airspaces and less

Noodle type	Country or region	Final roll gap <sup>a</sup> (mm)	Raw noodle thickness (mm)	Raw noodle width (mm)	Reference
Nonalkaline					
Udon	Japan	$\sim$ 1.7	2.5	3.0	1
Gua mian (dried noodles)	China	1.0	Not specified	2.0	2
Gua mian (dried noodles)	China		0.6-1.4	0.8 - 6.0	3
Mee sua (steamed and dried)	SE Asia		~0.8	Very fine	4
Mee teow (steamed and dried)	SE Asia		~0.8	Fine	4
Alkaline					
Ramen (raw)	Japan	∼1.1	1.4	1.5	1
Steamed Chinese noodles	Japan	~1.1	1.4	1.4 <sup>b</sup>	1
Cantonese (raw)	Asia	$\sim$ 0.6	1.0	1.5	5
Hokkien mee (parboiled)	SE Asia	$\sim$ 0.9	1.2	2.0	5
Hokkien mee (parboiled)	SE Asia		c	С	4
Mee pok (raw)	SE Asia		0.8	5.0	4
Mee kia (raw)	SE Asia		1.2	1.2	4
Wanton mee - Singapore style (raw)	SE Asia		1.0	1.0	4
Wanton mee - Hong Kong style (raw)	SE Asia		0.9	0.9	4
Bamee	Thailand		1.5	1.5	6
Instant steamed and fried	SE Asia		0.9	1.4	6

<sup>&</sup>lt;sup>a</sup> Final roll gap is set to give the required noodle sheet thickness.

<sup>&</sup>lt;sup>b</sup>Noodles for the market are made with round-grooved cutting rolls but a square cutting roll is preferred for experimental purposes.

<sup>&</sup>lt;sup>c</sup>Hokkien mee is manufactured over a range of width and thickness.

References: (1) National Foods Research Institute, Ministry of Agriculture, Forestry and Fisheries (1985) Quality Assessment of Wheat – Sensory Test for Noodles. Japan: MAFF; (2) Professional Standard of the People's Republic of China (SB/T 10137-93); (3) Huang S (1996) China – the world's largest consumer of paste products. In: Kruger JE, Matsuo RB, and Dick JW (eds.) Pasta and Noodle Technology, pp. 301–329. St.Paul, MN: American Association of Cereal Chemists; (4) Singapore Standard CP64:1996; (5) Commercial source; (6) Hou G and Kruk M (1998) Asian Food Technology. Technical Bulletin, Vol. XX, Issue 12. Manhattan, KS: American Institute of Baking.

protein contraction on the sheet surfaces. The betterdeveloped protein matrix was considered to result in improved noodle-eating quality.

**Rolling of the sheet** The sheet is then passed through a series of rolls to progressively reduce the sheet thickness. This stage of the process can impact substantially upon noodle-eating quality. Important factors include rate of reduction in sheet thickness, moisture content of the sheet, direction of sheeting, and the temperature of the rolls. The reduction in sheet thickness should ideally not be greater than 30% at any pass through a pair of rolls, otherwise the gluten structure may be damaged and the eating quality of the noodle impaired. The moisture content of the sheet is important with higher moisture levels protecting the gluten structure from damage. The superior texture of hand-made noodles has been associated with improved gluten development through a combination of higher dough moisture content and kneading of the dough after mixing. The kneading leads to randomized development of the gluten rather than the unidirectional development achieved through conventional sheeting. New techniques have attempted to achieve the characteristics of hand-made noodles in mechanized production. Strategies employed have included the use of corrugated or waved rolls that are reported to simulate the effects of multidirectional sheeting.

The temperature of the sheeting rolls is important, and water-jacketing of the rolls assists in controlling sheet temperature. Changes in temperature also affect sheet elasticity and viscosity. These factors, in turn, influence the roll gap settings required to achieve the desired final sheet thickness. Excessive roll temperatures can lead to surface drying of dough sheets, and the Japanese National Foods Research Institute has reported that low roll temperatures cause roughness of the noodle surface.

Cutting After reduction of the noodle sheet to its final thickness, the noodles are cut lengthways through cutting rolls. Cutting rolls are available to produce noodles that vary in width from  $\sim 1.0$  mm to 7.5 mm. The final dimensions of the noodle strands are determined by the cutting rolls and the thickness by the sheeting process. These dimensions influence time taken for drying (for dried noodles) and cooking (for steamed and boiled noodles).

#### **Processing for Specific Noodle Types**

**Dried noodles** Drying preserves noodles and prolongs their shelf life by reducing water activity to

a level that greatly restricts or stops quality deterioration and microbial growth. Various temperature, humidity, and time profiles are used commercially. A common method for drying "udon" involves temperature and humidity ranges of 35-50°C and 70-75% RH. Temperature is lowered in steps over a period of 6 h, and final moisture content is generally less than 14%. Other methods involve drying at 20-35°C for 10-20 h and at temperatures under 20°C with ambient low humidity air in the Japanese winter. The latter is traditionally used for the drying of hand-made "somen," and has the advantage of giving slightly whiter color due to reduced browning. Very high-temperature drying ( $>70^{\circ}$ C), as used for pasta, is not commonly applied. As a result of high ambient temperatures in Southeast Asia, noodles may be simply dried in the sun. Although this use of natural resources is economical, the lack of control of both temperature and humidity could lead to inconsistent quality.

Boiled noodles Several types of noodles are boiled prior to sale. These include boiled udon and the alkaline "Hokkien" noodles manufactured in Southeast Asia. After cooking, boiled udon noodles are rapidly cooled in chilled water, drained, automatically packed, and refrigerated at  $\sim 5^{\circ}$ C. In contrast to boiled udon, Hokkien noodles are only parboiled by the manufacturer and the final cooking is done immediately before eating. Hokkien noodles are cooled either by air or chilled water, drained, sprayed with oil to minimize sticking, and are usually packed in plastic bags.

Long life noodles are boiled noodles, which can be stored at ambient temperature for long periods. In this case the noodles are formed, partly boiled, cooled, soaked in a dilute organic acid solution such as lactic acid, surface treated with oil or  $\alpha$ -amylase to stop sticking and then packed and pasteurized at temperatures exceeding 90°C.

Boiling time required varies according to noodle size, but a typical time period for complete boiling would be 20 min for raw udon, with a moisture content of  $\sim$ 35% and with cross-sectional dimensions of 3.0 mm  $\times$  2.5 mm. Boiling time decreases with increased moisture content and increases with the size of the noodle strand.

The Japanese National Foods Research Institute indicates that pH of water used in boiling udon should be maintained at 5.5–6.0 to minimize boiling losses. The pH of the water is not so critical for alkaline noodles, which leach alkaline salts into the boiling water. Where pH control is required, organic acids such as lactic, malic, and citric acids are suitable.

Frozen noodles In the manufacture of frozen noodles, raw noodles are boiled, washed in chilled water, drained and rapidly frozen at  $-30^{\circ}$ C or below. Highquality frozen noodles are usually made by mixing under vacuum, allowing increased water addition. The additional water improves gluten hydration and development, reduces gluten damage during sheeting, and reduces boiling time by allowing more rapid heat transfer into the noodle. Rapid freezing is also beneficial as it ensures small water crystals which are less likely to disrupt the gluten network than the larger water crystals formed at slower freezing rates. Frozen noodles can have very high quality, approaching that of fresh (raw) noodles. The process is critical and poor results can occur if any of the key steps are not finely tuned.

Instant steamed and fried noodles Steamed and fried instant noodles were introduced in the late 1950s and early 1960s in Japan and Korea. They have fast rehydration rates on final cooking and this convenience makes them very popular. Their basic processing, up to cutting, is common with other noodle types. Dough moisture should be low enough to promote fast dehydration during frying. However, dough moisture must also be sufficiently high to promote full gelatinization of starch during steaming and adequate development of the gluten structure. Adequate gluten development is needed for its contribution to noodle texture and to minimize excessive softening of noodles in hot soup.

The process deviates after cutting. First the noodles are waved and formed into the appropriate shape for sale. The wave conformation assists in even penetration of steam and frying oil. Various devices ranging from hinged baffles to rubber flaps, both placed immediately after the cutting rolls are used to form the waved noodles. The devices allow the buildup and release of the noodles that cause the wave pattern to form. The noodles are then steamed, and steaming should be extensive enough to cook the noodles right through, that is, to the disappearance of the doughy core at the center of the noodle strand. This allows full swelling of the starch on rehydration, which is associated with good texture.

The frying step simultaneously cooks and dehydrates the product. In addition, frying creates voids through violent steam release, which increases the surface area of noodles and assists in speeding rehydration rates. Frying further gelatinizes the starch, the block of noodles is set into shape to facilitate easy packaging, and a desirable golden color is developed. Instant steamed and fried noodles have a relatively high fat content ( $\sim 15-22\%$ ) compared with steamed and dried instant noodles (1-3%).

Some manufacturers, to decrease oil uptake, take measures such as partially drying the noodles prior to frying or using edible coatings, sometimes polysaccharide-derived. Much of this work is the subject of patent coverage.

Instant steamed and dried noodles Instant steamed and dried noodles are manufactured in several forms, with the most popular being a waved form in blocks, similar to instant steamed and fried noodles.

These noodles may become increasingly popular with consumers because of their low fat content.

# Raw Materials and Their Effects on Noodle Quality

#### Wheat

The principal consideration in selecting wheat for the milling of noodle flours is that it be well-filled, of good appearance, and not damaged by weather or grain drying. Wheat used for the manufacture of noodle flour should have an appropriate balance of protein content, protein quality (as indicated by dough properties) and starch quality for the targeted noodle type. The presence of excessive levels of  $\alpha$ -amylase, either through preharvest sprouting or late-maturity α-amylase, can have deleterious effects during processing and on the quality of the final product these include increased boiling losses and reduced eating quality. High levels of protease, associated with rain damage, may lead to increased breakage of noodles during drying and poor color in both alkaline and nonalkaline noodles.

In addition to having access to sound wheat of high milling quality, the milling process is also important. The highest-quality noodle flours are associated with low extraction milling and low ash levels in the flour. This can be achieved either by milling to a low extraction rate, or by milling conventionally and utilizing flour streams least contaminated by bran particles.

# Flour

Typical flour specifications for the different types of noodles are given in Table 4.

Flour attributes and noodle appearance Flour extraction levels and ash contents have a profound influence on noodle appearance. Higher flour extraction levels generally lead to duller noodles with a higher propensity to darken during dough processing. Low flour extraction and ash levels are preferred for the manufacture of noodles that retain a clean, bright appearance after cooking.

Table 4 Typical specifications for noodle flours

Noodle type	Country/region	Moisture (%)	Protein (%)	Wet gluten (%)	Ash (%)	Falling number (sec)	Reference
Nonalkaline							
Udon	Japan		8.0-10.0		0.36 - 0.40		1
Dried noodles	China						2
Superfine grade		≤ <b>14.5</b>		≥28	$\leq$ 0.55	$\geq$ 200	
Common grade		≤ <b>14.5</b>		≥26	$\leq$ 0.70	$\geq$ 200	
Dried noodles	China		9.5 - 11.0				3
Alkaline							
Ramen	Japan		10.5-12.0		0.33 - 0.38		1
Cantonese	SE Asia		∼12.0				4
Cantonese (high quality)	SE Asia		12.0-13.0				5
Cantonese	SE Asia		10.0-12.0				5
Wonton	SE Asia		>13.0				5
Hokkien	SE Asia		∼10.5				4
Hokkien	SE Asia		10.0-11.5				6
Bamee	Thailand		11.5-13.0		$\leq$ 0.46		7
Instant noodles (ramyon)	Korea						8
Common		13.5 - 14.0	10.3-10.6		0.50 - 0.54		
High quality		13.5 - 14.0	9.4 - 10.6		0.40 - 0.44		
Cup noodles		13.5 - 14.0	11.0-11.5		0.40 - 0.45		
Instant	Taiwan		11.5-13.0		0.45 - 0.55		9
Instant	Philippines		$\sim$ 10.0				9
Instant	Indonesia		11.0-12.0				9

References: (1) Nagao S (1996) Processing technology of noodle products in Japan. In: Kruger JE, Matsuo RB, and Dick JW (eds.) *Pasta and Noodle Technology*, pp. 169–194. St. Paul, MN: American Association of Cereal Chemists; (2) Chinese National Flour Standard (SB/T 10137-93); (3) Huang S (1996) China – the world's largest consumer of paste products. In: Kruger JE, Matsuo RB, and Dick JW (eds.) *Pasta and Noodle Technology*, pp. 301–329. St. Paul, MN: American Association of Cereal Chemists; (4) Moss HJ (1984) *Proceedings of Singapore Institute of Food Science and Technology Conference*, pp. 71–75. Singapore: Singapore Institute of Food Science and Technology; (5) Miskelly DM (1996) The use of alkali for noodle processing. In: Kruger JE, Matsuo RB, and Dick JW (eds.) *Pasta and Noodle Technology*, pp. 227–274. St. Paul, MN: American Association of Cereal Chemists; (6) Shelke K, Dick JW, Holm YF, and Loo KS (1990) *Cereal Chemistry* 6: 338–342; (7) Hou G and Kruk M (1998) *Asian Food Technology*. Technical Bulletin, Vol. XX, Issue 12. Manhattan, KS: American Institute of Baking; (8) Kim SK (1996) Instant noodles. In: Kruger JE, Matsuo RB, and Dick JW (eds.) *Pasta and Noodle Technology*, pp. 195–225. St. Paul, MN: American Association of Cereal Chemists; (9) Nagao S (1995) Wheat usage in East Asia. In: Faridi H and Faubion JM (eds.) *Wheat End Uses Around the World*, pp. 167–189. St. Paul, MN: American Association of Cereal Chemists.

Raw noodles discolor with time. This is associated with polyphenol oxidase (PPO), activity, flour extraction rate, weather damage, and variety. Wheat breeders in the exporting nations have been working diligently to decrease PPO levels, and hence raw noodle darkening, in all potential noodle wheat but noodle darkening is still an issue. In addition to PPO in the flour itself, PPO is preferentially localized in the bran. Higher flour extraction levels lead to higher numbers of bran particles in flour and increased visible speck counts in raw and boiled noodles. If raw noodles are held for too long before cooking, the specks become darker and more visible due to the effects of PPO. Increased flour protein content also decreases noodle brightness in a range of noodle types but has little effect on noodle darkening over time.

The yellowness of the flour, mainly due to the presence of xanthophyll pigments, has a significant effect on the color of raw and cooked noodles. In Japan, the main preference is for creamy-colored udon, associated with a moderate level of yellow pigment in the flour. In addition to the effect of yellow pigment in the flour, the yellowness of ramen and other types of alkaline noodles is also influenced by the effect of the

alkali on flavonoid compounds in the flour. Flour particle size also influences noodle brightness, with decreased particle size, at equivalent starch damage levels associated with brighter noodles.

Flour and noodle texture The principal characteristics of flour that affect noodle texture are: protein content, reflecting the relative proportions of the two main components of the flour, protein and starch, and the compositions of these two components. Flour lipids may also play an important role.

Flour protein, in its hydrated form as gluten, provides the basic structure of a noodle strand. Increased protein content in the flour, and also increased gluten strength, reflecting the composition of the protein, have significant effects on various aspects of noodle processing and texture. Increased protein content has been related in the literature with increased noodle hardness. However, there is conflicting evidence in the literature regarding the relative importance of protein content and gluten composition. In studies where sample sets with small ranges of protein content have been selected, a clear effect of protein composition on noodle texture can be observed. Where the

range of protein content is greater, protein content seems to dominate the effects of gluten composition on noodle texture. In the last few years study of the effects of gluten composition on noodle texture has become a dynamic area of research and readers are advised to closely monitor the developments regarding this issue.

Starch also has a significant effect on noodle texture. Initial studies on the effect of starch on noodle texture focused on the pasting characteristics of isolated starch, and showed that high starch paste peak viscosity and correspondingly high levels of paste breakdown during shear were preferred for Japanese udon. Later studies indicated that the starch component of flour for udon should have high-swelling properties and these could be predicted from flour swelling volume (FSV) tests. The high-swelling starch type was found to be closely linked to the desired soft and elastic texture in udon.

Early studies also indicated a possible requirement for low paste breakdown in starch isolated from flour for Japanese ramen. Recent studies have indicated that flour with low-swelling starch is required to give the firmness, lack of stickiness, and other desired characteristics in ramen. However, this may not be a common requirement of all noodles made with the inclusion of alkali.

As research has progressed, it has become apparent that the swelling characteristics of flour appear to have a primary effect on boiled noodle yield and texture. Associations between paste viscosity measurements and noodle texture appear likely to reflect the common dependence of these characteristics on flour swelling.

More recently the genetic underpinnings of the main differences in starch characteristics for wheats preferred for udon have been elucidated. Wheat amylose is synthesized by three populations of the enzyme Granule Bound Starch Synthase (GBSS), which are coded for by genes on chromosomes 4A, 7A, and 7D of wheat. Each of the genes has at least two alleles, one of which is "null." Presence of one or more null alleles leads to a decreased synthesis of GBSS, decreased synthesis of amylose, and a corresponding increase in amylopectin. The increased relative proportion of the highly branched amylopectin leads to greater swelling of starch granules during gelatinization, which can be detected as increased FSV, and as the unique soft and elastic texture desired in udon and some other specialized noodle types.

#### **Effects of Salt**

High levels of salt, up to 8%, are used in some dried noodles, particularly hand-made. Salt influences the ease or rate of drying of noodles, modifies enzymatic activity, and prolongs shelf life. It also reduces boiling time, causes a softer mouth-feel and makes dough handling easier.

#### Effects of Alkali

Common alkalis used in the manufacture of alkaline noodles include: sodium carbonate, potassium carbonate, and sodium bicarbonate. Mixtures of the alkalis are commonly used, with 1% (flour basis) of a 40:60 mixture of sodium carbonate and potassium carbonate being incorporated with 1% (flour basis) of salt in the Japanese National Foods Research Institute method for ramen.

In Malaysia and Singapore, sodium hydroxide is sometimes used in the manufacture of Hokkien noodles. Here the use of this very strong alkali is favored for its positive effects on noodle brightness and yellowness, but this may be offset to some degree by a more rapid deterioration in cooked noodle texture with time.

The use of alkali has a strong influence on noodle texture due to its effects on gluten and starch properties and these differ according to the type of alkali used. Sodium hydroxide has a more substantial effect on starch gelatinization than sodium carbonate, even to the extent of inducing gelatinization of starch at room temperature. Noodles made from sodium hydroxide are reported to have a softer texture than those prepared from sodium carbonate and this is likely to be associated with increased starch swelling. A consequence of this is that the optimum starch type required for flour used in the manufacture of alkaline noodles may vary according to the type of alkali used.

#### **Effects of Other Ingredients**

Ingredients that have been used in noodle formulations include starches, gums, emulsifiers, enzymes, and colorings. For frozen noodles, nonwheat starches are frequently included in formulations. Modified starches designed to enhance freeze-thaw stability or slow retrogradation rates can improve the texture of the final cooked product through a softer but more elastic texture. In instant noodles, potato starch or other modified starches can be used to alter noodle texture and to increase the rate of rehydration on final preparation.

There are many types of gums available that currently or potentially have applications in noodles. These include; guar and locust bean gums, alginates, including propylene glycol alginate (PGA), carrageenans, xanthan, gellan, and cellulosic gums. Gums are added in low amounts (often between 0.5% and 1.5% of flour weight) and are commonly used to make noodles firmer, increase water-holding capacity in pre-boiled noodles, increase freeze—thaw stability and reduce ice crystal formation in frozen noodles, and to reduce fat uptake in fried noodles. In choosing a gum, one needs to consider the type of gum, the viscosity of the gum, its ease of hydration, and its mesh, or particle size. Hydration and particle size are crucial factors in some noodle formulations because of the low water additions used. As a rule, gums should be fully hydrated prior to addition to the flour.

Monoglycerides and other emulsifiers can be used to modify dough sheeting and handling characteristics or to restrict starch swelling on cooking. The benefit of the latter is to reduce surface erosion and cooking losses during boiling, but this needs to be balanced against some reduction in cooked noodle yield.

More recently a range of enzymes has been developed for use in noodles. Transglutaminase, which forms glutamyl-lysine cross-links in gluten, can be used to increase break strength in uncooked dried noodles as well as produce firmer boiled noodles. Lipases that produce monoglycerides and other lipid species *in situ* have been developed commercially and are claimed to reduce surface stickiness and increase firmness and cooking tolerance of noodles in a similar fashion to the use of monoglycerides. Oxidoreductases, such as glucose oxidase, are claimed to increase noodle firmness, and to reduce cooking losses and stickiness. Surface application of amylases is also suggested for reduction of surface stickiness in packaged and precooked noodles. The patent literature and technical literature from enzyme suppliers provide rich sources of further suggestions for the use of enzyme technology in noodle applications.

Proteins, such as gluten or whey protein isolates can also be added to improve noodle firmness. Addition of egg white tends to make noodles both firmer and less elastic.

Final noodle color may also be adjusted by the addition of food colorants.  $\beta$ -carotene is often used as a means of adjusting udon to a creamy yellow color. In Japan, riboflavin (vitamin B<sub>2</sub>) may be used in alkaline noodles to accentuate the yellow color of ramen. In Southeast Asia, tartrazine is a common colorant used in the manufacture of intensely yellow Hokkien noodles.

See also: Noodles: Starch. Wheat Proteins and Flour Quality. Wheat: Ultrastructure of the Grain, of the Flour and of the Dough.

# **Further Reading**

Crosbie GB (1991) The relationship between starch swelling properties, paste viscosity and boiled noodle

- quality in wheat flours. *Journal of Cereal Science* 13: 145–150.
- Crosbie G, Miskelly D, and Dewan T (1990) Wheat quality for the Japanese flour milling and noodle industries. *Journal of Agriculture, Western Australia* 31: 83–88.
- Crosbie GB, Ross AS, Moro T, and Chiu PC (1999) Starch and protein quality requirements of Japanese alkaline noodles (ramen). *Cereal Chemistry* 76: 328–334.
- Hatcher DW and Kruger JE (1993) Distribution of polyphenoloxidase in flour mill streams of Canadian common wheat classes milled to three extraction rates. *Cereal Chemistry* 70: 51–55.
- Hou G and Kruk M (1998) *Asian Food Technology*. Technical Bulletin, Vol. XX, Issue 12. Manhattan, KS: American Institute of Baking.
- Huang S (1996) China the world's largest consumer of paste products. In: Kruger JE, Matsuo RB, and Dick JW (eds.) Pasta and Noodle Technology,, pp. 301–329.
  St. Paul, MN: American Association of Cereal Chemists.
- Konik CM, Mikkelson LM, Moss R, and Gore PJ (1994) Relationships between physical starch properties and yellow alkaline noodle quality. *Starch/Staerke* 46: 292–299.
- Kruger JE, Anderson MH, and Dexter JE (1994) Effect of flour refinement on raw cantonese noodle color and texture. *Cereal Chemistry* 71: 177–182.
- Miskelly DM (1984) Flour components affecting paste and noodle colour. *Journal of the Science of Food and Agriculture* 35: 463–471.
- Miskelly DM (1998) Modern noodle based foods raw material need. In: Blakeney AB and O'Brien L (eds.) *Pacific People and Their Food*,, pp. 123–142. St. Paul, MN: American Association of Cereal Chemists.
- Miskelly DM and Moss HJ (1985) Flour quality requirements for Chinese noodle manufacture. *Journal of Cereal Science* 3: 379–387.
- Moss HJ, Miskelly DM, and Moss R (1986) The effect of alkaline conditions on the properties of wheat flour dough and Cantonese-style noodles. *Journal of Cereal Science* 4: 261–268.
- Nagao S (1996) Processing technology of noodle products in Japan. In: Kruger JE, Matsuo RB, and Dick JW (eds.) *Pasta and Noodle Technology*,, pp. 169–194. St. Paul, MN: American Association of Cereal Chemists.
- National Foods Research Institute, Ministry of Agriculture, Forestry and Fisheries (1985) Quality Assessment of Wheat Sensory Tests for Noodles. Japan: MAFF.
- Oda M, Yasuda Y, Okazaki S, Yamauchi Y, and Yokoyama Y (1980) A method of flour quality assessment for Japanese noodles. *Cereal Chemistry* 57: 253–254.
- Oh NH, Seib PA, Deyoe CW, and Ward AB (1983) Noodles: I. Measuring the textural characteristics of cooked noodles. *Cereal Chemistry* 60: 433–438.
- Ross AS, Quail KJ, and Crosbie GB (1997) Physicochemical properties of Australian flours influencing the texture of yellow alkaline noodles. *Cereal Chemistry* 74: 814–820.

Shelke K, Dick JW, Holm YF, and Loo KS (1990) Cereal Chemistry 67: 338–342.

Toyokawa H, Rubenthaler GL, Powers JR, and Schanus EG (1989) Japanese noodle qualities: II. Starch components. *Cereal Chemistry* 66: 387–391.

#### **Relevant Websites**

http://www.instantramen.or.jp - The home page of instant ramen and the official website of the Japan

Convenience Foods Industry Association. Information in English and Japanese on the history of instant noodles, their manufacture, the ingredients used, classification of types, and other instant ramen facts. http://www.wsu.edu — The home page of the Western Wheat Quality Laboratory of the United States Department of Agriculture — Agriculture Research Service. Information on evaluation of the biochemical and genetic bases of the milling and baking characteristics of wheat for commercial food production.

# **NUTRACEUTICALS FROM GRAINS**

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#### Introduction

This article deals with a class of grain components selected, not on the basis of their chemical structure, but rather by their physiological functionality. Nutraceuticals are a diverse group of chemical compounds, but they all demonstrate a beneficial impact on our bodies. Whilst it is clear that food is eaten to provide energy and the building blocks for growth, it has become increasingly popular in the "West" to consume foods for their ability to improve and protect our health. After a brief introduction to the concept of functional foods and nutraceuticals, this article presents the major classes of grain nutraceuticals and describes their potential health benefits. Although most nutraceuticals mentioned here are found in all grains, their concentrations vary widely. Where a particular grain has high levels of the nutraceutical under discussion, it is specifically mentioned.

#### **Background**

In the late 1980s, a trend in food products was emerging in which foods were recognized as contributing more than simply sustenance and nutrition. Not only was food seen as a quantitative source of protein, carbohydrates, and fats to maintain (human) viability, but it was recognized that some food components were capable of improving health and well-being and therefore capable of effecting qualitative changes in

health. The resulting foods are known as functional foods, indicating that their consumption serves a biological function, such as reducing cholesterol level, improving heart health, or improving bowel health, to name but a few. The chemical components responsible for this functionality are known as nutraceuticals. The definition of nutraceuticals has evolved but generally they are food components, which provide medical or health benefits to the consumer. Where these compounds are consumed in purified form (as tablets or as potions) in addition to a normal meal, they are better described as food supplements.

The design of functional foods and the focus on nutraceuticals to improve health represents quite a paradigm shift in health treatment in the West. Western medicines have traditionally been administered to patients to treat disease, but in the East, medicine is also given to maintain health and prevent disease. The move to functional foods can be seen as a move towards the traditional Chinese medicine paradigm, where the emphasis is on prevention rather than cure. Many cultures have medicinal plants, but the Chinese and Indian (Ayurvedic) systems are particularly comprehensive. The UK materia medica, for example, lists some 300 plants. The Chinese materia *medica*, on the other hand, lists some 7000 plants. It is, therefore, understandable that the first country to regulate the use of such bioactives in foods was Japan, where East meets West.

The Government of Japan, in collaboration with industry, introduced Foods for Specified Health Uses (FOSHU) laws to regulate functional foods and nutraceutical claims. The system began to evolve in the late 1980s and was implemented in the early

1990s. At the time of writing, there are several hundred functional foods on the FOSHU list. The existence of this accreditation agency in Japan has resulted in a growing distinction in the marketplace, between functional and "normal" food products, based on verified functionality. It has also generated growing customer awareness. For the food manufacturer, this distinction has meant an ability to achieve the higher prices common for FOSHU-accredited products in the market place.

In Australia and the USA, nutraceuticals have traditionally encompassed primarily the vitamins, fiber, and minerals. More recently, phytostanols and phytoestrogens have been touted; so too have omega 3 fatty acids and polyphenols, but there is a strong movement away from describing functional foods in terms of their nutraceutical components and towards descriptions in terms of physiological effect.

Margarines containing hypocholesterolemic nutraceuticals are, for example, advertised not for their phytostanol content, but for their ability to lower cholesterol levels. Other functionalities of interest, at the time of writing, include cancer prevention, cardiovascular health, obesity reduction, arteriosclerosis prevention, stamina enhancement, immune function improvement, bone health, eye health, mental health, and prevention of neuro-degeneration. Nutraceuticals are also targeted by food manufacturers at particular age groups and lifestyle groups. For 14–34 year olds, for example, these include functional soft drinks, "booster"/energy drinks, confectionary, quick-fix tonics, hangover cures, and performance enhancement (sports) drinks; and for 35–60 year olds, the major driving force in the nutraceuticals market, agerelated disease prevention predominates for conditions such as osteoporosis, mental health, joint health, eye health, heart health, and bowel health.

There have been a large number of individual market studies on the functional food phenomenon, but for a good review the reader is recommended to a "meta review" by Sloan. Of the ten key trends identified in the review, the major ones representing potential for grain-derived nutraceuticals are:

- condition-specific marketing (Foods targeting specific medical conditions such as diabetes, high cholesterol etc.);
- gender, age, and ethnic positioning (Foods to minimize menopausal symptoms, hormone related osteoporosis etc.);
- weight, satiety, and appetite suppression (Food with low glycemic index);
- functional snacks and natural functional ingredients (both well suited to grain products); and

• nontraditional food markets (eye health, oral health, cosmetic application of food components).

Grains have traditionally been consumed for their part in staple diets, providing quantitative sources of protein, carbohydrates and, to some extent, vitamins. The differences between the grains in these respects are not large, although the amino acid composition, carbohydrate composition, and digestibility do vary from one grain type to another. There are, however, significant differences in the minor components and their bioactivities (nutraceutical properties). The nutraceuticals from a particular grain can be delivered to the consumer by including the whole grain in the target food, by including nutraceutical-rich fractions, or by adding purified nutraceuticals.

Whereas the focus of the consumer is, thus, increasingly on the physiological functionality of a food product, the focus of the food manufacturer needs to be on the optimal combination of ingredients to deliver functionality and flavor. The focus of the ingredient supplier or grower needs to be on the nutraceutical levels of their ingredients. The following sections discuss some of the major nutraceuticals found in grains.

# **Storage Compounds**

Grains, by their nature, are rich in storage compounds such as amylose, amylopectin, storage proteins, and vitamins (e.g., tocopherol, tocotrienols). Traditional milling and processing results, primarily, in liberation of the components of the starchy endosperm containing these storage compounds and, in wheat, this is also the major quantitative source of beta-glucans. It has been suggested, however, that the nonstarch (bran and germ) fraction, which may account for up to 25% of the grain in wheat, for example, may contain up to 95% of the important nutraceuticals and phytochemicals. Bran contains, for example, phenolic acids, lignans, flavonoids, vitamins, and phytosterols and the potential exists to render these more bioavailable by further processing.

#### Starch

Starch is a polysaccharide comprised primarily of amylose and amylopectin — two polymers distinguished by their degree of branching. Presentation of carbohydrates to the digestive system as amylose induces a slower breakdown in the stomach in the first instance, consequently, resulting in a slower release of sugar, and thus, a lower glycemic index than the same amount as pure sugars. The slow release of sugar reduces peak blood glucose levels and the resultant diabetic stress that this generates. The starch

polysaccharides continue to be metabolized, however, throughout the digestive system with some forms being more resistant to digestion than others. These resistant starches fall into four categories, termed RS1, 2, 3, and 4.

RS1 refers to resistance conferred due to physical entrapment of starch, as found in partly milled grains, seeds, or legumes. RS2 includes starch granules that are highly resistant to digestion by  $\alpha$ -amylase until gelatinized. This type is found in high amylose maize starch. RS3 relates to the retrograded starch polymers from food processing of grains such as chickpea, rice, etc. RS4 includes chemically modified, commercially produced resistant starches. These are likely to be degraded by amylases to alcohol-soluble fractions and are used in many baby-foods applications.

#### **Protein**

Although, the exact composition of seed storage proteins may vary considerably from one plant variety to another, it appears that the subunit structure and physical properties are quite similar across many dicotyledonous and monocotyledonous plants. The surface properties of the 11S subunit globulins, for example, appear similar and their hydro-phobicity has been suggested to underlie their hypocholesterolemic (cholesterol-lowering) activity. Certain protein isolates have, as a result, been used as nutraceuticals based on the hypolipidemic effect associated with certain 11S globulins.

Although seed storage proteins are usually not bioactive, it should be remembered that during digestion peptides and amino acids produced may have bioactivity. Hydrophobic peptides, for example, have been found to bind to bile resulting in a reduction of blood serum cholesterol. Also, a correlation between plasma cholesterol level and the hydrophobicity of peptic pancreatic digests exists. The peptic hydrolysates of wheat gluten and rice prolamin and glutelin proteins have been shown to possess an opioid-like activity. Such peptides have been termed exorphins because of their exogenous origin and their morphine-like nature.

Recent studies have focused also on the potential of various protein concentrates for the treatment and prevention of hypertension. This has been attributed to angiotensin-converting enzyme (ACE) inhibitory peptides in the storage proteins of corn, rice, and soybean.

#### **Fiber**

Crude fiber is contained mainly in the seedcoat and relatively little is carried through processing into the

starch, protein, or oil fractions, which are the major food-processing streams. The major crude fiber components are cellulose and hemicellulose and they appear in the bran fraction after milling. The term "soluble fiber" has been applied to nonstarch carbohydrate fractions which are water soluble but not digested in the stomach or small intestine. If metabolized by gut flora in the large intestine, the soluble fiber can be considered a pre-biotic. This is because it supports the growth of the organisms and the resulting production of short chain fatty acids such as butyric acid, which has been shown to be an effective inhibitor of bowel cancers. Such an effect has been demonstrated for brewers spent grain, which is derived from germinated barley. Patients with mild ulcerative colitis are reported to have responded well to this grain, fed as a prebiotic, resulting in increased stool butyrate concentrations. Crude fiber can also function simply as a fecal-bulking agent, reducing the residence time of food (and toxins) in the bowel, and increasing stool weight. Bran consumption has been demonstrated to elicit a reduction in LDL-cholesterol (bad cholesterol), but the effect is dependent on the source of the bran. Oat and rice bran are reported to be more effective than wheat bran in reducing plasma lipoprotein risk factors for cardiovascular disease.

#### Oil

Legumes generally contain higher levels of lipids than do cereals. Neutral lipids are comprised primarily of triglycerides, whereas polar lipids are comprised largely of phospholipid. The fatty acid compositions of the lipids vary widely between the various grains as do their absolute levels.

It is the fatty acid molecule which appears to confer a particular lipid functionality and any discussion of oils in a nutraceutical context currently revolves around fatty acid composition. Most of the legume lipids are a good source of essential fatty acids such as linoleic and linolenic acids. Oleic and linoleic acids are the major fatty acids in chickpeas, peanuts, soybeans, lentils, garden peas, and broad beans. Unsaturated fatty acids are essential for proper function of brain and eyes and are implicated in joint health and cardiovascular health.

There are two types of essential fatty acid, omega 3 and omega 6. They are both polyunsaturated, meaning that they have at least two double bonds somewhere in the fatty acid backbone. In omega 3 fatty acids, the first double bond is after the third carbon atom from the methyl end. In omega 6 fatty acids, the first double bond is after the sixth carbon atom. Unsaturated fatty acids such as omega 3 and omega

6 fatty acids tend to have lower melting points (are more fluid at room temperature) than saturated fatty acids of the same number of carbon atoms. Melting point is significant because these fatty acids become incorporated in cell membranes and the correct fluidity is essential for optimal cell performance. Fatty acid chain length and number of unsaturated bonds further affect the melting point. When ingested, the typical C<sub>18</sub> omega 3 and omega 6 fatty acids found in grains are built up to form  $C_{22}$  molecules, e.g.,  $\alpha$ -linolenic acid, an omega 3, 18:3, fatty acid is converted in the body to eicosapentaenoic acid (EPA) a C<sub>20</sub> molecule with 5 double bonds and then to docosahexaenoic acid (DHA), a C<sub>22</sub> molecule with 6 double bonds. These so-called omega 3 long chain polyunsaturated fatty acids have been found to be essential for proper brain and eye development. Similarly the omega 6, 18:2 C<sub>18</sub> molecule linoleic acid is converted to docosapentaenoic acid, containing 5 double bonds.

As excessive amounts of omega 6 fatty acids may be detrimental to health, and omega 3 fatty acids diminish this effect, the ratio of omega 3: omega 6 fatty acids is important for optimal health. There is some disagreement as to the best ratio, with suggestions ranging from 1: 1 to 1:6. Most vegetable oils such as corn and safflower oil contain some omega 6, but very little omega 3 fatty acids. Flax seed oil is an exception with up to 58% of its fatty acids being omega 3.

Dietary deficiency of omega 3 fatty acids is reported to correlate with depression, schizophrenia, and Alzheimer's disease. Although it is not clear whether this is cause or effect, dietary supplementation of DHA has been reported to give positive results. It is also reported to reduce the inflammatory responses of arthritis and to improve the function of insulin receptors. Flaxseed oil, with its high omega 3 fatty acid content is reported to reduce high blood pressure, cholesterol level, and the risk of heart disease. It is also claimed to help treat eczema, psoriasis, arthritis, and menstrual pain. Unfortunately, the very structure which results in this bioactivity also renders the oil prone to oxidation and rancidity.

Vegetable seed oils can be rich in fat-soluble vitamins such as vitamin E (alpha tocopherol) and tocotrienols. Tocotrienols (high in rice bran oil) are claimed to reduce fat deposits in artery walls, blood cholesterol level, and growth of breast cancer cells. They are also said to provide protection from UV radiation and ozone-induced oxidative skin damage and to reduce blood clotting.

# **Phytosterols and Phytostanols**

Phytosterols are desmethyl sterols, which share a common ring structure with cholesterol. Just as

cholesterol plays a critical role in membrane structure and performance in humans, the phytosterols are essential to the membrane structure and function of plant cells. This similarity in structure is reflected in the ability of phytosterols to significantly alter the rate of uptake of both dietary and endogenously produced cholesterol from the gut, resulting in a reduction of blood serum cholesterol levels. The precise mechanism has not been determined. One theory is that phytosterols reduce cholesterol solubility and hence availability by formation of phytosterol/cholesterol complexes. It is also argued that phytosterols and cholesterol compete with each other for micellar uptake by endothelial cells.

Phytostanols are almost identical to phytosterols in structure, but are fully saturated, whereas phytosterols have one double bond in their ring structure. This chemical difference has a significant impact on their functionality. Phytostanol esters are reported to result in lower blood levels of both phytostanols and phytosterols than is the case when phytosterol esters are consumed.

Most of the phytosterols and phytostanols in corn fiber are reported to be contributed by the aleurone layer, which is reported to contain 8 times more phytosterols than the pericarp. The native phytosterols and phytostanols are not very soluble and are processed by the manufacturer to produce fatty acyl ester derivatives, which improve both solubility and ease of formulation into foods. The esters are believed to be hydrolyzed in the small intestine. There is some conflicting data, but the phytosterol esters and phytostanol esters appear to be equally efficacious in reducing blood serum LDL cholesterol levels by up to 14%. Neither ester has any significant effect on HDL levels. It has been estimated that daily consumption of phytosterols or phytostanols can reduce the risk of heart disease by up to 40%, depending on age and other factors. Both sterol and stanol esters have, however, been found to reduce the absorption of alpha and beta carotene and of vitamin E and it has been suggested that a maximum daily dietary intake be set to avoid adversely affecting carotenoid concentrations in the blood.

The major source of phytosterols is vegetable oil. Phytostanol esters are often derived from hydrogenation and subsequent esterification of a phytosterolrich by-product fraction from pine tree pulping. Phytostanols do, however, also occur naturally in plants, and corn fiber oil has been shown to be one of the richest sources of stanols and stanol esters. The esters are of either fatty acids or phenolic acids, such as ferulic acid. In addition to the enhanced bioavailability of phytostanols and phytosterols engendered by esterification, further improvement in

bioavailability can be effected by coupling with proteins and lecithin, possibly due to an emulsification or solubilization mechanism.

### **Flavonoids**

Flavonoids are a range of  $C_{15}$  aromatic compounds. The term flavonoids was first used for the family of vellow colored compounds with a flavone moiety, but was later extended to include various polyphenols including less intensely colored flavanones, flavon-3-ols (catechins without a C<sub>4</sub> carbonyl group), and red and blue anthocyanidins. Some flavonoids appear to function as plant defence systems. Catechins, for example, are astringent and have parasite deterrent properties, while isoflavones are important phytoalexins (natural plant defence chemicals) and other polyphenols function in the plant to protect it from harmful UV solar radiation. In the plant, flavonoids are generally glycosylated, while the free (unglycosylated flavonoid) aglycones are less common. The glycosides may further be acylated and both of these modifications can adversely impact on the bioavailability of the bioactive aglycone. The antioxidant potential of flavonoids is dependent on the number and arrangement of the hydroxyl groups across the structure and the presence of electron-donating and electron-withdrawing substituents. In a systematic study conducted by Miller et al. of the net antioxidant activity of various foods, it was shown that whole grains have almost equivalent antioxidant activity to fruits and vegetables when compared on a "per serving" basis.

#### **Isoflavones**

Isoflavonoids are found primarily in leguminous plants. They are derived from flavanones, which are found in all plants. The major dietary sources of isoflavones for humans are soybean, chickpea, and lupin seed products. A number of the isoflavones have estrogen-mimetic effects and are thus known as phytoestrogens. Epidemiological studies have demonstrated a link between consumption of soy isoflavones and reduced risk of breast cancer and prostate cancer. Isoflavones have also been shown to be active in the chemo-prevention of osteoporosis and cardiovascular disease.

Recent studies on the pharmacokinetics of isoflavone glycosides again demonstrate the important effect of digestion and gut microbial metabolism on their efficacy. Two of the commonly studied phytoestrogenic isoflavones are genistein (found in high levels in lupin and soybean) and daidzein. In their glycosylated forms, they are known as genistin and daidzin.

The glycosides are more actively absorbed in the small intestine than are the aglycones, but it is only the aglycone that has estrogenic activity. The absorbed glycosides are broken down continuously by the liver without yielding active phytogestrogen. Gut flora can cleave the glycoside by means of a glucosidase enzyme, increasing the concentration of the bioactive aglycone. The net yield of the bioactive moiety from a given meal depends on a kinetic balance between these processes. In some traditional fermented soy products, such as "miso" and "tempeh" the deglycosylation has already occurred and the bioactive phytoestrogen aglycones are present at higher levels than in unprocessed soybeans.

# Lignans

Lignans are another class of phytoestrogens. They possess a 2,3-dibenzylbutane structure and exist as minor constituents of many plants where they form the building blocks for the formation of lignin, which is found in the plant cell wall. Lignans from plants are believed to be precursors of the primary animal lignans enterolactone and enterodiol, being converted into these forms by intestinal bacteria. Flaxseed is one of the most abundant plant sources of lignans, with the lignan Secoisolariciresinol diglycoside (SDG) comprising the major fraction. Enterolactone and enterodiol appear to promote homeostasis, or balance, of female estrogen levels. In postmenopausal women, the effect is estrogenic whereas in premenopausal women the effect is antiestrogenic. A Finnish study found that women with high enterolactone levels in their blood had a lower risk of breast cancer and animal studies have shown reduced breast cancer and colon cancer with lignan in the feed. In males, lignans appear to interfere with the conversion of testosterone to dihydrotestosterone (DHT). DHT is required for normal growth of healthy prostate cells, but an excess amount is believed to lead to benign prostatic hyperplasia (swollen prostate). Lignan intake in the diet is reflected in the lignan concentration in the urine and higher levels are found in the prostate fluid of men with a lower risk of prostate cancer than those at increased risk.

# **Anthocyanins**

Anthocyanins are generally highly colored pigments associated with flowers and attraction of pollinating insects. They are, however, flavonoids and can be found throughout a plant. They have been reported to be in relatively high concentrations in sunflower hulls and although the pigment quality is not generally sufficient for food-coloring applications, they would retain some bioactivity. In other plants, such as

specially bred purple sweet potatoes in Japan, certain anthocyanins have been shown to have antimutagenic activity, hepatoprotective activity, and antioxidant activity. The color of purple maize and red wheat is derived from the anthocyanin content.

#### **Catechins**

Catechins are a class of flavonols. They are C<sub>15</sub> compounds and their derivatives are composed of two phenolic nuclei connected by 3 carbon units. The phenolic rings are generally di- or tri-hydroxylated and the molecule as a whole is highly reactive, with properties of metal chelation, oxidative radical scavenging, and inhibition of nitrosation. Tea catechins have also been shown to have anticariogenic activity, by means of their antibacterial activity, which inhibits plaque formation.

# **Nonstarch Polysaccharides**

#### Glucans

Beta-glucan is a water-soluble, viscous, linear, highmolecular-weight polysaccharide. A Canadian study reported in 1994 found that a purified oat gum containing 80% beta-glucan, when fed to hypercholesterolemic subjects over a 4 week period, reduced LDL and total cholesterol levels by 10% and 9.2% respectively. A reduction of the LDL cholesterol level, whilst leaving the HDL cholesterol level constant, significantly reduces the risk of heart disease. Many studies on rolled oats have shown similar results.

Barley is also a source of  $\beta$ -glucans and besides its effect on serum cholesterol has been studied with respect to prebiotic activities.  $\beta$ -Glucan supports butyrate production in the colon, which has been demonstrated to inhibit various stages of growth of cancer cells.

#### Saponins

Saponins are glycosides with both hydrophilic and hydrophobic regions. In solution, they spontaneously form micelles. In the presence of bile, the hydrophobic portions intertwine with the bile and large micelles are formed which are too large to be absorbed across the gut wall. Saponins also interact directly with cholesterol, producing an insoluble complex that prevents cholesterol absorption from the intestine. However, saponins can also have negative bioactivity of their own, depending on their source and structure. They are a source of many of the bioactives in traditional herbal medicine and some have been shown to bind iron and reduce iron uptake. As is the case with any bioactive (and therefore potentially nutraceutical) compound, caution is necessary in incorporating saponin-rich fractions in foods.

#### Conclusions

Grains are a staple food in the Western diet and their contribution to nutrition is well established. The growing consumer demand for functional foods provides the opportunity to "mine" the grain components, many of which, such as bran, are discarded in low-value waste streams, for use as nutraceutical ingredients. Technologies need to be developed for cost-effective separation and purification of these potential nutraceutical compounds and systematic studies of the bio-functionalities of such purified fractions are necessary to underpin health claims. Clinical trials are essential to demonstrate purported nutraceutical activity, but are notoriously variable and often generate conflicting results. Caution must be applied to the interpretation and extrapolation of the findings of individual studies. Given the massive production of grains and the growing number of success stories, the prospects for a grain-based nutraceutical industry are encouraging.

See also: Carbohydrate Metabolism. Cereals: Chemistry of Nonstarch Polysaccharides. Consumer Trends in Consumption. Cultural Differences in Processing and Consumption. Food Safety through the Production Chain. Fortification of Grain-Based Foods. Grain Crops, Overview. Labeling of Grain-Based Foods. Nutrition: Beriberi, A Deficiency Related to Grains; Guidelines for Grain-Based Foods; Effects of Food Processing; Mineral Composition; Vitamin Composition. Soybean: Sov-Based Fermented Foods. Starch: Analysis of Quality. Whole-Grain versus Refined Products. Appendix: Grain Composition Tables; Foods for Celiac Diets.

# **Further Reading**

Dixon RA and Sumner LW (2003) Legume natural products: understanding and manipulating complex pathways for human and animal health. Plant Physiology 131: 878-885.

Hicks KB and Moreau RA (2001) Phytosterols and phytostanols: functional food cholesterol busters. Food *Technology* 55(1): 63–67.

Marcone MF (1999) Biochemical and biophysical properties of plant storage proteins: a current understanding with emphasis on 11S seed globulins. Food Research International 32: 79-92.

Miller HE, Rigelhof F, Marquart L, Aruna Prakash RD, and Kanter M (2000) Antioxidant content of whole grain breakfast cereals, fruits and vegetables. Journal of the American College of Nutrition 19: 312–319.

Setchell KDR and Cassidy A (1999) Dietary isoflavones: biological effects and relevance to human health. *Journal of Nutrition* 129: 758–767.

Sloan AE (2002) The top 10 functional food trends: the next generation. *Food Technology* 56(4): 32–57.

Tham DM, Gardner CD, and Haskell WL (1998) Potential health benefits of dietary phytoestrogens — A review of the clinical, epidemiological and mechanistic evidence. *Journal of Clinical Endocrinology and Metabolism* 83(7): 2223–2235.

Tungland BC and Meyer D (2002) Nondigestible oligoand polysaccharides (dietary fibre): their physiology and role in human health and food. *Comprehensive Reviews* in Food Science and Food Safety 1: 73–92.

### **Relevant Websites**

http://www.nutrition.org. http://www.apps.fao.org. http://www.who.int.

# **NUTRITION**

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Beriberi, A Deficiency Related to Grains Guidelines for Grain-Based Foods Effects of Food Processing Mineral Composition Soy-Based Foods Vitamin Composition

# Beriberi, A Deficiency Related to Grains

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#### Introduction

Grains have been the major source of both energy and protein for the world's population for millenia. However, it is now realized that, when the diet lacks a variety of other foods, the grains are also needed to supply a considerable number of other essential micronutrients if those eating them are to remain healthy. But the processing of grains to remove the bran and germ, in addition to its favorable effects of increasing shelf life by removing much of the oils that can become rancid, and providing foods with a smoother feel by removing most of the fiber, does

also cause the loss of a large proportion of their other nutrients.

It now appears that the emergence of the disease pellagra amongst communities in the southern states of the USA from around 1910 is explained by the introduction of machinery that allowed the replacement of stone-ground "whole corn meal" with "de-germed meal," and that this, combined with white wheaten flour and little in the way of lean meat, eggs, and milk, resulted in a diet deficient in the vitamin niacin. Until this came to be understood in the 1930s, several thousand people died and even more suffered a miserable and humiliating disease.

The following article summarizes the way in which another disease, beriberi, that was causing huge suffering in the Far East, particularly in the earlier period 1870–1910, came to be understood as the consequence in most cases of people having polished white rice as their staple food with little in the way of supplementary foods. It was the work of the pioneers struggling to understand this disease that

led to the discovery of the class of micronutrients that now is known as vitamins.

It is a complicated story with many false leads, but it provides a valuable lesson in how science does eventually advance, and the dangers of over-simplification of the problems of the real world. It also shows how it finally proved possible, as a result of collaboration between industry and academia, to "enrich" highly milled grain products so that they still provided a good contribution of vitamins and minerals.

# **Occurrence in Asia**

The first Western physicians allowed to work in Japan in the 1870s were surprised to discover the existence of a serious disease previously unknown to them and "second only to smallpox in its ravages." In Japan, it was known as "kakké," but was soon recognized as being identical to the disease known in South-east Asia as "beriberi," a native name now universally adopted, which may originally have meant "great weakness." Characteristically, it began with a feeling of weakness in the legs and a loss of feeling in the feet. Then, in many but not all cases, the legs and then the trunk would swell with retained water. Finally, the heart would be affected so that the subject gasped for breath, and would die from heart failure.

Older records from both Japan and China showed that it had been known for some centuries, although it had been the opinion of two eighteenth century Japanese physicians that the disease had become worse after  $\sim$ 1750. The early records also indicated that it was largely a disease of the wet summer months and could attack even the well-off.

#### Infection or Malnutrition?

Those most at risk were men in the newly modernized Japanese army and navy, and also prisoners. As these were all people living together in large groups, and with the excitement in this period at other diseases being traced to the transmission of pathogenic bacteria, this seemed a likely cause for beriberi also. Yet, it was difficult, on this basis, to explain the frequent observation that a naval ship would leave its base with all its crew in good health, yet, after a month or more in isolation at sea, the disease would sweep through the crew.

Kanehiro Takaki, a surgeon on the naval staff, was directed in 1878 to work on the problem. He knew that the ships had been built in Britain and that they followed the general practices of the British navy where there was no beriberi. The only difference that caught his attention was in the rations issued to the men: the Japanese issues contained less protein and did not meet the high standard in force at that time in Europe. He therefore persuaded his superiors to permit a trial of modified rations with a proportion of the rice being replaced by meat, condensed milk, vegetables, and barley. The change was a complete success, and it was found that even just the use of barley in place of one half of the rice staple was enough to prevent the disease, which Takaki now believed to have resulted from a deficiency of protein in the earlier rations.

The Japanese army, perhaps as the result of interservice rivalry, did not follow the navy and in the short Russo-Japanese war of 1904-05, some 100 000 of their soldiers had to be invalided home from Manchuria suffering from beriberi.

# A Disease in Chickens

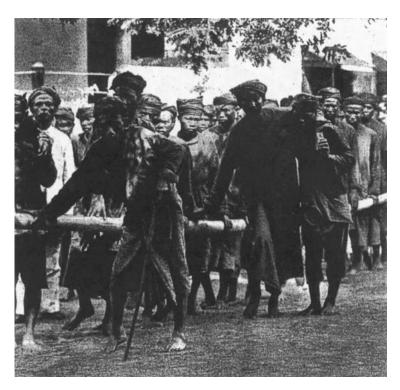
Meanwhile, the disease had become an equally serious problem in the Dutch East Indies (now Indonesia) (Figure 1). After a punitive military expedition had to be withdrawn because of a beriberi epidemic, the Dutch government dispatched a small commission to try to identify the bacteria responsible for the disease. After a few months, it was thought that the microorganism had been found, and Christiaan Eijkman, a young Army physician, remained behind to confirm its activity in animal models.

Some of the chickens that Eijkman had injected with blood from beriberi patients developed signs of leg weakness, but so did some of his uninjected controls, suggesting that the condition was so infectious that it could "jump" from cage to cage. Autopsies of the affected birds showed degenerated peripheral nerves. But in the following months, none of the next batch of birds developed the condition. Eijkman discovered that when the leg weakness had appeared, the man in charge of the birds had been feeding them on cooked white rice left over from feeding the beriberi victims in the adjoining hospital, instead of buying rough, feed-grade rice.

A long series of feeding trials confirmed that birds fed on white rice would become sick with leg weakness, whereas those given supplements of rice polishings (still present in feed-grade rice) remained healthy. This was only an animal disease, but a survey by the medical inspector of prisons in Java showed that prisoners who had been receiving white rice as their staple issue were susceptible to beriberi, whereas those receiving brown rice were not.

# The Concept of a Vitamin

Eijkman, who believed that the disease was a kind of starch poisoning, now had to be invalided home with malaria. His successor, Gerrit Grijns, found that birds



**Figure 1** Two prisoners in Java with beriberi and needing assistance to walk. (Reproduced with permission from Vorderman (1897) Onderzoek naar het gevangenissen op Java en Madoera en het voorkomer van beri-beri order de geintemeerden. Batavia: Jav. Boekh & Drukkerij.)

became sick even when fed on meat that had been autoclaved. After further work, his statement in 1901 was perhaps the progenitor of the "vitamin era" in nutritional research: "there occur in various natural foods substances which cannot be absent without serious injury...they are easily disintegrated...and cannot be replaced by simple chemical compounds."

The work of Eijkman and Grijns was confirmed by British investigators in Malaysia and by Americans in the Philippines, and attempts began to extract the active material from rice polishings and to concentrate it. There are moving accounts of scientists in Manila being implored by local doctors to bring a few spoonfuls of extracted syrup to save the lives of infants with beriberi, and of the babies' spectacular recoveries. Women themselves seemed less susceptible to beriberi than men, but when mothers were receiving a diet of low thiamin content, their breast-fed babies were at a high risk of dying with acute infantile beriberi.

# **Isolation of Thiamin**

Isolation of the active factor proved very difficult. Each stage of extraction, and then further partitioning by reprecipitation involved biological assays with birds. Eventually, the next generation of Dutch workers in Indonesia was successful, obtaining

a few milligrams of active crystalline material after starting with one-third of a ton of rice polishings and going through at least 16 separation stages in which much of the vitamin was lost. It was found that adding just 2 ppm of the crystals to white rice was enough to keep birds healthy. In 1931, the crystals were found to contain sulfur as well as carbon, hydrogen, nitrogen, and oxygen, and the chloride salt was shown to have the empirical formula  $C_{12}H_{18}N_4SO_2Cl_2$ .

There were, of course, almost innumerable ways in which these atoms could be combined. By good fortune, Robert R. Williams in the USA found that adding sodium sulfite to a solution of the vitamin led to its division into two roughly equal halves. Further work at a number of centers showed that one of these compounds contained a pyrimidine and the other a sulfathiazole ring. By 1937, a synthesis of the active molecule was achieved. It was named "thiamin or thiamine" (i.e., the sulfur-containing vitamin) and soon began to be produced and marketed as a pharmaceutical.

# The Analysis of Foods

Thiamin can be oxidized to a highly fluorescent derivative, "thiochrome." This property is used to measure the thiamin contents of different foods, even at levels of less than 1 ppm. The procedure is specific, and no

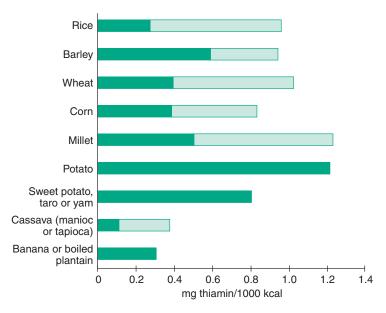


Figure 2 Representative analytical values for the thiamin content of different staple foods: (1) after husking only (■ □) and (2) after full processing (■), as explained in the text, and also for some staple root crops, etc. Sago meal is not shown, as it contains only an insignificant level of thiamin. (Reproduced with permission from Carpenter KJ (2000) *Beriberi, White Rice and Vitamin B.* Berkeley, CA: University of California Press.)

other naturally occurring compounds have been found that give the thiochrome reaction. However, thiamin can react with polyphenols and a compound present in garlic to give derivatives that are still biologically active (as will be referred to again) but do not give the thiochrome reaction. The analytical procedure may therefore underestimate the efficacy of a product. It is essential, therefore, to have a confirmatory bioassay before knowing for certain that any kind of processing has caused significant loss of thiamin.

# **Rice and Other Staples**

Figure 2 illustrates the thiamin levels in the world's major staple foods, both when fully milled and when minimally processed. In the case of grains, the latter means removal of the husk (or hull) but no more. It is clear that, for each grain, the full milling that removes both the bran and germ results in the loss of a major portion of the thiamin originally present.

White rice is not that much lower than white wheat flour in its content of the vitamin, but after the grains have been prepared for consumption, the difference is increased. White rice is normally washed several times, and this alone can remove half the thiamin present, and boiling in excess water can again halve the level of remaining vitamin. In contrast, white wheat flour is most commonly baked into bread with yeast as the raising agent, and this causes little loss of thiamin.

There is no evidence that cooked white rice has any positively harmful qualities, but if it is the major item

in a diet that contains only small amounts of foods that are richer in thiamin, so that the diet as a whole provides no more than about 0.25 mg per 1000 kcal, it is not surprising that beriberi should gradually develop.

The data in Figure 2 also explain the Japanese experience that serious problems with beriberi in their navy in the late 1800s disappeared when one-half of their rice ration was replaced by barley.

The same figure also shows the low thiamin content of tapioca prepared from cassava roots. This explains the existence of beriberi in Brazil at the same period among even well-off people whose favorite foods were tapioca and molasses. Their preferred protein supplement was dried, salted cod, which had to be soaked for several days to leach out most of the salt, which also removed most of the vitamin.

The very first reports of beriberi to reach Europe came from Portuguese priests working in the Molucca Islands (at the Eastern end of Indonesia) in the 1500s. Their staple was the locally produced sago meal, now realized to be almost pure starch, and the priests correctly attributed their weakness to a lack of "something" in this food and asked to be provided by their superiors with wheat flour.

Beriberi was also a serious problem in early spring in isolated communities in Newfoundland in the early years of the twentieth century. Their families, who would be cut off for the winter, had to buy 6 months of supplies, with white flour as their staple. They were also apparently not familiar with using yeast to leaven bread, but cooked their flour with baking soda, and it is known that much of the thiamin present would be destroyed under the alkaline conditions during this procedure.

# The Improvement of Rice

Once the association of beriberi with white rice in Asia had been established, attempts were made to replace it in some way with other foods. As already mentioned, barley was an economic and wellaccepted alternative in the diet of the Japanese armed forces.

In the Philippines, a proposal was made to enforce the use of brown rice by the local military. This, of course, is rice from which the husk has been removed

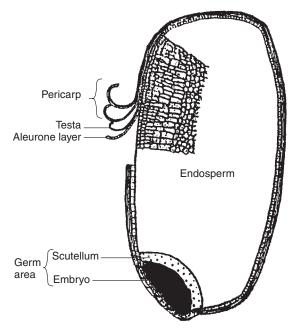


Figure 3 Dissection of a dehusked rice grain. (Reproduced with permission from Carpenter KJ (2000) Beriberi, White Rice and Vitamin B. Berkeley, CA: University of California Press.)

but not the entire bran layer and germ (embryo and scutellum) (Figure 3). This was the traditional staple of villagers in South-east Asia who had no access to mechanical rice mills. They would pound their paddy (i.e., rice still in the husk) in some kind of bowl and then winnow the product so that the lighter husks blew away, and the grains fell in a pile.

This procedure was time-consuming but created no problems when only enough was pounded for immediate use in the next 24 h. However, it was repeatedly found that in the tropics, brown rice on storage would become infested with insects of different kinds, and the oil in the bruised germ would become rancid. Since large organizations, or an army on the move, needed large-scale supplies ready for cooking, brown rice did not provide a practicable staple.

The early workers who discovered the association of beriberi with white rice had assumed that the important micronutrient was concentrated in the bran of the grain. However, it was later realized that more was present in the germ area (Table 1). Japanese millers then attempted to modify their machinery so as to remove the bran without removing the germ from the grain. The so-called "germ rice" that they were able to produce proved to be both palatable and an improved source of thiamin. However, millers were only able to produce it with certain varieties of rice, and not with the bulk of the rice favored in Japan.

A traditional method of processing rice common in Bengal is called parboiling. It had been found that if rice in the husk were to be steeped for a period in hot water and then allowed to dry in the sun, the husks cracked off more easily on pounding, and there was less breakage of the grains. Broken grains had a lower commercial value.

In Malaysia, where many immigrant groups were employed as laborers but living on their habitual diets, it was realized in ~1910 that Bengalis were remarkably free from beriberi, and studies in a mental hospital showed that replacing ordinary white rice with polished rice prepared from parboiled grains relieved

**Table 1** Thiamin contributed by the different parts of a sample of dehusked rice grains

Dissected parts of rice grain	Proportion by weight (%)	Thiamin		
		Concentration in fraction (mg per 100 g)	Contribution to 100 g grain (mg)	
Pericarp + testa + aleurone layers Germ area	6	3.1	0.186	
Embryo	1	5.9	0.059 0.248	
Scutellum	1	18.9	0.189	
Endosperm	92	0.05	0.046	
Total	100	_	0.480	

the inmates from the disease. After thiamin had been identified, analyses showed that the initial soaking of the grain caused thiamin to diffuse into the endosperm and to remain there as the grains dried out.

Again, this does not appear to be something that could be applied more widely. The traditional soaking and sun-drying leaves the rice with a characteristic musty flavor and slight discoloration, which is acceptable only to people who have grown up with it. The process can be modernized, with controlled autoclaving and vacuum-drying for so-called "conversion" of the rice, but this makes it too expensive for mass consumption in developing countries.

The final procedure for the production of "enriched" rice is to fortify it with the synthetic vitamin. For a flour, such as white wheat flour, this is relatively simple, requiring only very careful mixing of the traces of vitamin with a small quantity of flour, then the blending of the premix to larger batches. This is familiar, and indeed compulsory practice, in the USA and UK, together with other vitamins and trace minerals. As a powder, thiamin cannot be blended with grains of white rice. However, methods have been developed of preparing vitamin-rich granules with the size and appearance of rice grains, and blending these in the ratio of one granule to 200 grains, so that the mix has at least the thiamin content of brown rice. To reduce loss of the vitamin during washing and cooking, the granules are coated with a nontoxic resin at the final stage of their production. This method of enrichment has been tested in an area of the Philippines where beriberi was endemic and has proved successful in greatly reducing its incidence. Unfortunately, where widely separated villages each had a small electric mill, there were practical problems in persuading millers to pay for the premix when the product appeared unchanged, but the price had to be a little higher.

# **Supplementing Foods**

Unfortunately, there is no convenient food that is extremely rich in thiamin. Dried brewers yeast contains 15 mg per 100 g, but many people cannot tolerate it in more than extremely small regular doses. Of the meats, pork is richest with lean pork containing 1 mg per 100 g. Beef has only about one-tenth as much. Dry peas and beans contain about 0.5 mg per 100 g. Potatoes are another useful supplement — on a dry matter basis, they have slightly more thiamin than brown rice.

In practice, reaching a desirable intake of thiamin comes usually from eating a wide variety of foods. Nineteenth-century Japanese believed that consumptions of most foods other than rice during the summer months caused "heat." Hence, food consumption was almost entirely restricted to rice consequently leading to an increase in beriberi during these months.

### Is there an Antithiamin Problem?

There are many references in the literature to at least a suspicion that certain foods and beverages may be responsible for beriberi appearing in people whose intake of thiamin would otherwise be adequate.

#### **Thiaminases**

Many species of fish contain enzymes in their viscera that split thiamin molecules at the junction between its two ring structures. This was discovered when foxes, being reared for their fur and fed on a mix containing a large proportion of whole raw fish, developed a form of paralysis that responded to injections with thiamin. A similar condition was seen later in cats that had been fed on a canned food containing a large proportion of whole fish. It was believed that the thiamin in the mix had been largely destroyed after the mix had been prepared and was waiting to be autoclaved.

These experiences led to investigations as to whether humans could be similarly at risk, but it appears not. The enzymes are not present in fish muscles (i.e., fillets), and even where small fish are eaten whole, they are not ground up with other items of diet before being cooked. Lastly, it was found in animal studies that a subsequent meal with different constituents was not affected by thiaminases being present in an earlier meal.

Another source of thiaminases was found to be bracken, and their presence explained the condition known as "staggers" that occurs in horses that have been feeding on bracken. Cooked bracken, in which the thiaminase was inactivated, proved harmless to horses. The only known case of thiaminase poisoning in humans occurred in a group exploring the interior of Australia in the 1860s. Running out of provisions on their return journey, they lived on the sporocarps in the fronds of a particular fern that is now known to contain a high level of a particularly heat-resistant thiaminase. All four men developed leg weakness and lassitude; three died, and the survivor remained lame even after his safe return.

#### **Heat-Stable Antithiamins?**

It has been found that when the thiamin in a food comes into contact with polyphenols such as caffeic acid, it no longer gives a fluorescent product in the usual thiochrome procedure for the estimation of thiamin. This led some workers to suppose that drinking large quantities of tea or coffee, or chewing betel nut — all sources of polyphenols — might induce

a condition of thiamin deficiency. However, biological assays have indicated that the vitamin is still fully available.

When thiamin is incubated with garlic extracts, it undergoes a reaction with the allicin present in which the thiazole ring opens, and the sulfur atom in the ring links to the alkyl sulfide to form a disulfide compound. This is not measured in the thiochrome reaction, but in the body it is reduced to re-form the active vitamin. In fact, compounds of this type can be absorbed more efficiently by alcohol-damaged intestinal walls than ordinary thiamin. Thiamin tetra-hydrofurfuryl disulfide in particular is approved for this purpose in some countries.

Although there is no confirmed evidence of naturally occurring heat-stable compounds that inactivate thiamin, chemists have synthesized such materials. One, named "oxythiamin," has the amino group attached to the pyrimidine group in thiamin replaced by a hydroxy group. Giving it to animals results in the more rapid production of some of the signs of thiamin deficiency, although it differs from thiamin in being unable to pass the blood—brain barrier.

# **Acute Deficiency in the West**

With the discovery that autoclaving yeast powder would destroy the thiamin, whereas the other B-vitamins were retained, it was possible to place volunteers on an artificial diet essentially free of thiamin. To the surprise of investigators, some subjects had lost appetite within 2 weeks and became nauseated and dizzy, with other mental symptoms, by 6 weeks, but with no sign of peripheral nerve damage or cardiac abnormality, which are characteristic of classic beriberi.

Trials using pigeons and rats with very deficient diets also produced appetite loss and death before any sign of leg weakness had developed. It appeared that in both humans and animals, acute deficiency of thiamin resulted in damage to the central nervous system. With slightly higher intakes, the CNS had priority, whereas peripheral nerves gradually degenerated.

# **Deficiencies in Total Parenteral Nutrition**

There are many reports of people recovering from surgery of the gastrointestinal tract who have developed acute thiamin deficiency. They had been fed intravenously with a solution providing energy, amino acids, and minerals, but no vitamins. This is adequate for a short period, but thiamin is the first vitamin to become depleted.

In a number of cases where this type of parenteral feeding has continued for some weeks, a condition called "Wernicke's encephalopathy" has developed. Patients are confused and have characteristic involuntary eye movements. Where patients have died, autopsies have shown brain lesions analogous to those found in acutely deficient animals. The same outcome has been seen in subjects voluntarily fasting for long periods or being unable to take food because of persistent vomiting in pregnancy.

# **Alcoholism**

One material that can be responsible for the production of thiamin deficiency is ethanol (i.e., "alcohol" in everyday speech). In developed countries where nearly everyone can afford a well-balanced diet, most of those diagnosed as being thiamin-deficient are "alcoholics." The continued ingestion of high levels of alcoholic beverages has many undesirable effects. In the present context, two are relevant. First, the alcoholic typically no longer bothers to eat a normal range of foods, partly because the beverages provide a large portion of their calorie needs and partly from nothing but their next drink being of immediate interest. Second, the high level of alcohol ingestion damages the intestinal wall so that thiamin is absorbed less efficiently, and the requirement for the vitamin increases.

Unfortunately, a small proportion of such victims develop Wernicke's encephalopathy, which may lead in turn to Korsakoff's psychosis. Such people, sometimes referred to as suffering from the Wernicke–Korsakoff syndrome, are at present incurable and have to be maintained in a mental hospital for the rest of their life. The cost of this to the state is such that some specialists have argued that it would actually be cheaper to have all beer and wine fortified with thiamin as a preventive.

There may be a genetic factor making some Western people susceptible to the cerebral form of beriberi and the Wernicke–Korsakoff syndrome, since it was seen even in Western prisoners of the Japanese in the Second World War who had white rice but no alcohol, but apparently has not been seen in Asian subjects.

See also: Consumer Trends in Consumption. Cultural Differences in Processing and Consumption. Fortification of Grain-Based Foods. Grain, Morphology of Internal Structure. Grain and Plants, Morphology. Nutraceuticals from Grains. Nutrition: Guidelines for Grain-Based Foods; Effects of Food Processing; Vitamin Composition. Rice: Chinese Food Uses. Whole-Grain versus Refined Products. Appendix: Grain Composition Tables.

# **Further Reading**

Carpenter KJ (2000) Beriberi, White Rice and Vitamin B. Berkeley, CA: University of California Press.

Cook CC, Hallwood PM, and Thomson AD (1998) B vitamin deficiency and neuropsychiatric syndromes in alcohol misuse. Alcohol and Alcoholism 33: 317–336.

Eijkman C (1929) Antineuritic vitamin and beriberi. In: *Nobel Lectures: Physiology or Medicine*, pp. 1922–1941. Amsterdam: Elsevier.

Hinton JJC (1948) The distribution of vitamin B<sub>1</sub> in the rice grain. *British Journal of Nutrition* 2: 237–241.

Jansen BCP and Donath WF (1926) On the isolation of the anti-beriberi vitamin. *Koninklijke Akademie von Wetenschappen*, *Amsterdam*, *Proceedings* 29: 1390–1400.

Sauberlich HE, Herman YF, Stevens CO, and Herman RH (1979) The thiamin requirement of the adult human. American Journal of Clinical Nutrition 32: 2237–2248.

Shimazono N and Katsura E (1965) Review of Japanese Literature on Beriberi and Thiamine. Kyoto: Vitamin B Research Committee of Japan.

Takaki K (1906) The preservation of health amongst the personnel of the Japanese army and navy. *Lancet* i: 1369–1374.

Truswell AS and Apeagyei F (1982) Alcohol and cerebral thiamin deficiency. In: Jelliffe EF and Jelliffe DB (eds.) *Adverse Effects of Foods*, pp. 253–258. New York: Plenum Press.

Williams RR (1961) Toward the Conquest of Beriberi. Cambridge, MA: Harvard University Press.

Williams RR and Cline JK (1936) Synthesis of vitamin B<sub>1</sub>.

Journal of the American Chemical Society 58: 1504–1505.

Wuest HM (1962) The history of thiamine. Annals of the New York Academy of Sciences 98: 385-400.

# **Guidelines for Grain-Based Foods**

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# Introduction

Nutritional guidelines have been developed for many countries to recommend those foods that should be consumed as major or minor parts of the diet. Food guide diagrams derived from the guidelines visually represent these recommendations. A common factor in these various guidelines is the recommendation that grain-based foods, should form the greatest part of the diet, preferably as whole-grain products (Whole-Grain versus Refined Products). According to the food and nutrient composition table in Appendix: Grain Composition Tables, grains and grain-based foods, on the per serving basis, contribute many nutrients and other food components to make food intakes nutritionally adequate. Comparisons of the mineral and vitamin contents of various grains and grain-based foods on the per serving basis from Appendix: Grain Composition Tables are made and discussed in Nutrition: Mineral Composition; Vitamin Composition. By emphasizing use of grains in the diet, nutritional guidelines provide a major boost for the grain-growing and grain-processing industries of the world.

# The Many Pictorial Formats

Several food guide diagrams are pyramids (see *Dietetic Journal* and the Oldways Website) which represent the USA dietary guidelines. A pyramid diagram shows that foods at its base should be consumed in a correspondingly larger proportion of the diet than those near the apex. Pyramid formats used by the Philippines and Puerto Rico, a set of four "healthy eating pyramids," were developed by the private USA-based Oldways Preservation & Exchange Trust, the Harvard School of Public Health, and other organizations. The pyramid format was also adapted to suit the national architecture of China and Korea.

Food guide diagrams in a round format designed for other countries include circle, wheel, plate, or pie diagrams in which the sizes of the sectors or slices represent the relative importance of each food group. This format is used by Australia, Great Britain, Germany, Mexico, Portugal, and Sweden. Yet another format, a half rainbow diagram, is used by Canada. Other countries also use the USA pyramid whereas countries such as Japan provide no official illustrations of this type.

# **Common and Diverse Features of Dietary Guideline Diagrams**

The dietary guidelines diagrammed in these figures endorse the general recommendation of the increased consumption of plant products (grains, vegetables, and fruits), balanced by a moderate intake of animal products (meat and dairy foods), combined with

restricted amounts of "treat" foods, rich in fats and sugars. The pyramid message places plant foods, especially whole-grain foods, at the base of the pyramid or as the largest slice of the pie/plate diagram. Animal-based foods are placed nearer the apex of the pyramid, or as smaller slices, with treats near the apex or in the smallest slice. Grain legumes (pulses) and nuts are generally placed at an intermediate position together with fruits.

These guideline recommendations are broadly based on international research findings about the balance of food sources that are conducive to the maintenance of good health. Variations from one country to another reflect the differing traditional food types characteristic of each culture. For example, the foods illustrated in the diet pagoda of China include prominent representations of white steamed breads, plus rice and a cob of corn, whereas western-style foods are represented in the US, Canadian, and British illustrations (see *Dietetic Journal* and the Oldways Website).

The four Oldways pyramids emphasize regional diversity of traditional foods for the Mediterranean, Asian, and Latin American diets, as well as providing recommendations suited to a vegetarian diet. They all recommend a low intake of red meat, with plants as the major source of fats and oils, as well as carbohydrates from minimally processed forms, generally as whole-grain products.

# Food Information in Food Guide Diagrams

The amount of information about food that appears on food guide diagrams is variable. All of them include illustrations of some food items in each food group. Many countries have included food group names, or the recommended number of servings per day from each food group. Some provide word descriptors of how much to eat per day, such as "eat just enough, eat moderately, eat more, eat most," or

recommended eating frequency i.e., "at every meal, daily, weekly, monthly". Oldways pyramids include lists of desirable species of whole grains, types of grain-based foods, sources of plant oils, and specific nuts and seeds, thereby reinforcing the importance of grains in general in some food groups. Also relevant to products included as grain-based foods is the appearance of legumes and nuts in the grains group in some guidelines and food guide diagrams, as well as in the vegetables group, dairy group, or meat, and alternates group in others. The USA pyramid provides symbols to indicate addition of fat and sugars to foods.

### **Quantitative Recommendations**

To help consumers use food guide diagrams appropriately, many countries supplement their diagrams by adding quantitative information in a table or descriptive format. This information includes numbers of servings per day to eat from each food group, and serving sizes expressed as amounts for specific food types to eat per serving. These are listed in Table 1 for the grain-based foods only. It is important to make this information available to diagram users because many of them do not realize that the amount of food they eat is important. Determining the quantity of food to eat is a problem for many nutritional guideline and diagram users because they do not know/ understand the units of measurement used for food. Therefore, they cannot convert number of servings and units of measurement for a serving, e.g., cups, grams, ounces, into amounts of food to eat. Effective educational programs are needed to teach the public where to find and how to use nutritional guidelines, food guide diagrams, and quantitative information about food.

Differences in culture, in food habits, and in foods available determine types of food, serving sizes and servings per day in the various countries (Table 1). At first sight, there appears to be a great diversity of

Table 1 Grain-based foods recommended in the respective national dietary guidelines

Country	Grain serves/day	Examples of serve sizes for grain-based foods
Australia	3–11	2 slices bread/1\frac{1}{3} cup breakfast cereal/1 cup cooked rice or pasta
Britain	>5	30 g bread/30 g breakfast cereal/60 g rice, raw/cooked not specified
Canada	5-12	1 slice bread/30 g breakfast cereal/1/2 cup cooked rice or pasta
China	300-500 g	Total raw weight of any recommended grain-based foods altogether
Germany	250-350 g	Bread - 250-350 g, or cooked rice - 200-250 g, or potatoes - 250-300 g
Korea	4–5	3 slices bread (100 g)/90 g breakfast cereal/210 g cooked rice
USA	6-11	1 slice bread/1 oz breakfast cereal/ $\frac{1}{2}$ cup cooked rice or pasta

Adapted from Painter J, Rah J-H, and Lee Y-K (2002) Comparison of international food guide pictorial representations. *Journal of the American Dietetic Association* 102: 483–489, © American Dietetic Association.

recommendations when comparing the number of servings per day for different countries. However, as just described, servings per day must be interpreted together with the respective sizes of the servings, which in Table 1 range between one and three slices of bread, and from 30 to 90 g of breakfast cereal, to determine the amount to be eaten. Interestingly, potatoes are considered a substitute for grains in Germany.

# The USA Food Guide Pyramid -**Agreement and Controversy**

The current USA food guide pyramid, which was introduced in 1992, is presently being revised to match the concurrent revision of the dietary guidelines for Americans. Among the criticisms of this pyramid are opinions that it oversimplifies the nutrition message. According to Willett and Stampfer, writing in Scientific American in 2003, the pyramid is "grossly flawed." They wrote: "By promoting the consumption of all complex carbohydrates and eschewing fats and oils, the pyramid provides misleading guidance." They argued conversely, that not all fats are "bad," and that complex carbohydrates are not universally "good." Their suggested revision encouraged the consumption of "healthy fats" and avoidance of refined carbohydrates, butter, and red meat. Their revised pyramid retained whole-grain foods at the base, together with plant-derived oils, many of them from grains, e.g., from corn (maize), canola, sunflower, and peanut. The use of fats containing trans unsaturated fatty acids, which are formed in partially hydrogenated liquid vegetable oil when converted to a solid fat, and then used in firm margarines, baked products, and fried foods, is not recommended. Nuts and legumes are retained halfway up their pyramid. Foods high in complex carbohydrates such as white rice, white bread, pasta, and potatoes are placed at the apex, with the recommendation: "Use sparingly." These authors do not fully explain that fiber content and resistant starch are not greatly dissimilar for whole-grain and "white" (refined) foods. However, they do emphasize that "the best way to avoid obesity is to limit your total calories." Furthermore, they admit in conclusion that "uncertainties still cloud our understanding of the relation between diet and health."

The Oldways recommendations reinforce the concept of distinguishing between sources of fats and oils by recommending the greater consumption of oils from grains and nuts. However, the Oldways recommendations do not relegate the consumption of complex carbohydrates to the apex of the pyramid.

#### Conclusion

Despite some controversies, dietary guidelines generally emphasize the value of grain-based foods in the diet. This recommendation relates primarily to foods made from whole grains, because of their wider range of nutrients. In addition, the nutritional status of the diet is further enhanced by the inclusion of legumes and nuts, together with the variety of oils derived from grains such as maize and the oilseeds.

See also: Nutrition: Mineral Composition; Vitamin Composition. Whole-Grain versus Refined Products. Appendix: Grain Composition Tables.

# **Further Reading**

Henry RJ and Kettlewell PS (eds.) (1996) Cereal Grain Ouality. London: Chapman and Hall.

Juliano BO (ed.) (1985) Rice: Chemistry and Technology, 2nd edn. St. Paul, MN: American Association of Cereal

Nestle M (2002) Food Politics. Berkeley, CA: University of California Press.

Painter J, Rah J-H, and Lee Y-K (2002) Comparison of international food guide pictorial representations. Journal of the American Dietetic Association 102: 483-489.

Pomeranz Y (ed.) (1988) Wheat: Chemistry and Technology, 3rd edn. St. Paul, MN: American Association of Cereal Chemists.

United States Department of Agriculture, Human Nutrition Information Service (1992) The Food Guide Pyramid. Home and Garden Bulletin 252. Washington, DC: United States Department of Agriculture.

United States Department of Agriculture and United States Department of Health and Human Services (2000) Nutrition and Your Health: Dietary Guidelines for Americans, 5th edn., Home and Garden Bulletin No. 232. Washington, DC: United States Department of Agriculture.

Watson SA and Ramsted PE (eds.) (1987) Corn: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Webster FH (ed.) (1985) Oats: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Willett WC and Stampfer MJ (2003) Rebuilding the food pyramid. Scientific American 288(1): 64-71.

Wood PJ (ed.) (1993) Oat Bran. St. Paul, MN: American Association of Cereal Chemists.

### **Relevant Websites**

http://www.aaccnet.org - American Association of Cereal Chemists.

http://www.usda.gov - United States Department of Agriculture.

http://www.campden.co.uk - Campden & Chorleywood Food Research Association.

http://www.oldwayspt.org - Oldways Preservation and Exchange Trust.

http://www.gograins.grdc.com.au — GoGrains. http://www.wheatfoods.org — Wheat Foods Council.

# **Effects of Food Processing**

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# Introduction

In many foods, especially those from plants, a low level of an essential amino acid limits its nutritive value. These foods include cereals, which may be inadequate in the essential amino acids isoleucine, lysine, threonine, and tryptophan and legumes, which are often poor sources of methionine. These commodities are the principal sources of protein for much of the earth's rapidly growing population. The ratio of malnourished to adequately nourished humans will almost surely increase. For these reasons, and especially in view of the limited availability of high-quality (largely animal) protein to feed the present and future populations, improvement of food and feed quality is an important challenge to agricultural and nutritional sciences. Several approaches can be used to achieve such improvement. These include (1) fortification of foods with essential amino acids and good-quality protein supplements; (2) improvement of protein quality by plant breeding and genetic engineering; and (3) minimizing the damage to the nutritional value of proteins during food processing and storage. This brief article is largely limited to the last approach.

A variety of methods are used to process foods: if they are not edible, to render them so; to permit storage; to alter texture and flavor; to destroy microorganisms and other toxins. These methods include heating (baking, cooking, frying, microwaving), freezing, and employing high pH. It is a paradox of nature that the processing of foods can improve nutrition, quality, and safety; yet, occasionally these processing alternatives can lead to the formation of antinutritional and toxic compounds. These consequences of food processing result from molecular interactions among nutrients and with other food ingredients, both natural and added. Beneficial and

adverse effects of food processing are of increasing importance to food science, nutrition, and human health. A better understanding of the molecular changes during food processing and the resulting nutritional and safety consequences is needed to optimize beneficial effects such as bioavailability, food quality, and food safety, and to minimize the formation and facilitate the inactivation of deleterious compounds. Such an understanding will encompass multidisciplinary studies of the chemistry, biochemistry, nutrition, and toxicology of food ingredients. Possible approaches to prevent the formation of deleterious food ingredients are also addressed.

This article uses examples largely based on studies by the author to illustrate general concepts. It describes compositional changes and the nutritional impact of two major food-processing conditions: pH and heat. The discussion outlines the following aspects of processing-induced formation of novel food ingredients and the resulting consequences for nutrition: protein—carbohydrate nonenzymatic browning reactions; heat-induced formation of acrylamide; inactivation of soybean inhibitors of digestive enzymes; formation of lysinoalanine and D-amino acids in food proteins; the stability of phenolic compounds to high pH; and factors which influence the bioavailability of essential amino acids.

#### **Effect of Heat**

#### **Maillard Browning**

Amino-carbonyl and related reactions of food constituents involve those changes commonly termed browning reactions. Specifically, reactions of amines, amino acids, peptides, and proteins with reducing sugars and vitamin C (nonenzymatic browning, often called Maillard reactions) and quinones (enzymatic browning) cause deterioration of food during storage and processing. The loss of nutritional quality is attributed to the destruction of essential amino acids, a decrease in digestibility, and inhibition of proteolytic and glycolytic enzymes. The production of toxic compounds may further reduce the nutritional value and the safety of foods. Studies in this area include influence of damage to essential amino acids on nutrition and food safety, nutritional damage as a function of processing conditions, and simultaneous formation of deleterious and beneficial compounds. These compounds include kidney-damaging Maillard reaction products, mutagens, carcinogens, antimutagens, antioxidants, antibiotics, and antiallergens (Tables 1–4 and Figures 1–3).

Maillard reactions may also result in formation of desirable flavors and antimicrobial compounds against

**Table 1** Major sources of protein in the diet in developing and developed countries

Source	Developing (%)	Developed (%)
Cereals	58.8	29.1
Meat	8.6	26.4
Legumes	7.4	1.7
Milk and dairy	5.6	16.7
Fish, seafood	4.1	7.3
Oil crops	3.8	1.9
Vegetables	3.5	3.5
Starchy roots	3.1	3.2
Eggs	1.6	4.3
Offals	1.2	2.2
Fruit	1.0	1.1

**Table 2** Lysine content (g 100 g<sup>-1</sup> protein) of dry milled cereal flours

Flour	Lysine
Bulgur	2.12
Corn	2.20
Rice	2.25
Whole wheat	2.63
Soy	6.15

**Table 3** Effect of glucose and heat on lysine and arginine content of soy flour

Mole	Soy flour	Soy flour+ glucose	Soy flour+ glucose
(%)	control	(37°C, 10 days)	(95°C, 4 h)
Lys	5.67	4.15	2.65
Arg	5.74	5.50	2.51

Table 4 Loss of lysine after heat treatment at 205°C for 30 min

Protein	Lysine loss (%)
Gluten (commercial)	4.8
Gluten + 1% glucose	60.8

human pathogens. Since the discovery of browning reactions by Maillard  $\sim \! 100$  years ago, food scientists have been studying Maillard reactions and their effects on color, nutritional quality, and safety. In contrast, medical scientists have only been exploring relationships between *in vivo* browning and disease and aging for  $\sim \! 25$  years. From such broad-based crossfertilization of ideas, further progress that will benefit both food science and medicine can be expected.

Many individuals are sensitive to the antibrowning compound, sodium sulfite. Hence, the potential of sulfur amino acids to prevent browning was explored. The antioxidant and antitoxic effects of SH-containing

amino acids such as cysteine, cysteine ethyl ester, N-acetylcysteine, and glutathione are due to a number of mechanisms including their ability to act as (1) reducing agents; (2) scavengers of reactive oxygen (free-radical traps); (3) destroyers of fatty acid hydroperoxides; (4) strong nucleophiles which can trap electrophilic compounds and intermediates; and (5) inducers of cellular detoxification. Thus, positive results were expected from an evaluation of sulfur amino acids to prevent the formation of browning products. These expectations were realized since it was found that SH-containing amino acids were nearly as effective as sodium sulfite in preventing browning in apples, potatoes, fruit juices, and protein-containing foods such as nonfat dry milk and barley and soy flours.

Although extensive efforts have been made to elucidate the chemistry of both desirable and undesirable compositional changes during browning, parallel studies on the nutritional and toxicological consequences of browning are limited. This is understandable since, in principle, each combination of a specific amino acid or protein with a particular carbohydrate needs to be investigated to understand the scope of the problem. Reported studies in this area include (1) influence of damage to essential amino acids, especially lysine, on nutritional quality; (2) effects of fortifying browning products with essential amino acids on recovery of nutritional quality; (3) nutritional damage as a function of processing conditions; (4) biological utilization of characterized browning compounds, such as fructosyl-L-lysine; and (5) formation of mutagenic and clastogenic products.

A number of investigators have examined the effects of the Maillard browning reaction on digestibility and nutritional quality. Experimental evidence has shown that loss of nutritional quality of heat-treated casein is related to decreased nitrogen digestibility rather than to simple destruction of essential amino acids. The influence of glucose and starch was minimal compared to observed effects of heat on casein alone under the conditions used. Glucose and, perhaps starch, augment protein degradation and loss of nutritional quality under moderate, dry-heat conditions. Further studies are needed to explain the molecular basis for the extent and nature of the heat-induced destruction of essential amino acids and the formation of indigestible browned and crosslinked products. These changes impair intestinal absorption and nutritional quality in general. Toxic compounds formed under these conditions might also modulate nutritional quality. Thus, such studies should differentiate antinutritional and toxicological interrelationships and develop means for preventing or minimizing the formation of deleterious compounds in foods.

Figure 1 Pathways for Maillard nonenzymatic browning reactions of a protein with glucose and lactose.

Related studies showed that the phenolic compound chlorogenic acid decreased by  $\sim 100\%$  in the crust fraction and 65% in the crumb fraction of a conventional baked muffin. Microwave baking reduced the chlorogenic acid content by 77% of the original. These results demonstrate that varying degrees of thermal destruction of chlorogenic acid and possibly other phenolic compounds can occur in a typical flour mix at ordinary baking temperatures.

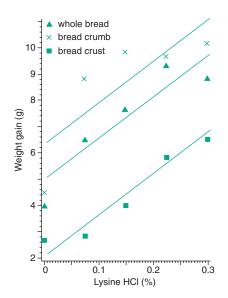
### Formation and Biological Effects of Acrylamide

Recent reports indicate that heat induces the formation of acrylamide ( $CH_2 = CH - CO - NH_2$ ) in food under conditions that also induce the formation of browning products. This observation stimulated interest in the underlying chemistry that may be

responsible for the formation of acrylamide as well as the chemical and biochemical basis of the toxicological effects of this conjugated vinyl compound: it has been reported to be a neurotoxin, reproductive toxin, and animal carcinogen.

It appears that the free amino acid asparagine and free glucose are major precursors of acrylamide. Selecting cultivars for food uses that contain low levels of asparagine and/or glucose may result in low-acrylamide processed foods.

In extensive studies, on the reactions of conjugated vinyl compounds including acrylamide, acrylonitrile, and methyl acrylate with wheat gluten, designed to prepare derivatives of potential industrial use, it was found that SH groups of cysteine residues, as well as, the -NH<sub>2</sub> group of lysine side chains has a strong avidity for the double bond of acrylamide (Figure 4).



**Figure 2** Weight gain in mice after 14 days fed whole bread, bread crumb, and bread crust supplemented with lysine before baking.

Seminal studies conducted in this area provide a chemical basis for the biological effects of acrylamide and its reactive epoxide metabolite, glycidamide, *in vivo*. The *in vivo* reactions involve biological alkylation reactions of proteins such as hemoglobin, enzymes, and DNA. Understanding the chemistry of formation of acrylamide during food processing and its reactions both *in vitro* and *in vivo* will make it possible to design effective means to prevent or arrest undesirable consequences of acrylamide in the diet. Research needs in this area include the following:

- 1. Does prevention of food browning by sulfur amino acids and peptides described above also prevent acrylamide formation?
- 2. Since acrylamide can react with lysine residues of proteins, do low-lysine proteins (wheat gluten, zein) produce more acrylamide than high-lysine proteins (casein, soy protein, meat proteins)?
- 3. Will replacement of corn meal with high-lysine corn meal result in less acrylamide in potato chips and tortillas?

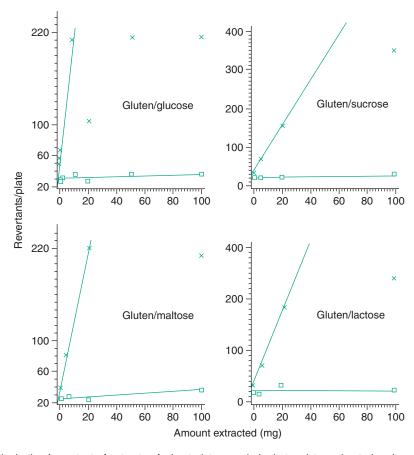
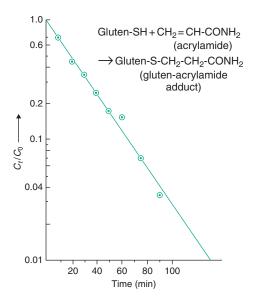


Figure 3 Mutagenicity in the Ames test of extracts of wheat gluten-carbohydrate mixtures heated under crust-baking conditions.  $\Box =$  unheated; x = heated.



**Figure 4** Reaction rate of wheat gluten SH groups with acrylamide at 30°C.  $C_0$  = initial concentration;  $C_t$  = concentration at time t

- 4. Do concurrent heat-induced Maillard browning products and other food ingredients affect the safety of acrylamide after consumption?
- 5. Will the use of wheat flour containing low levels of asparagine and/or glucose result in low-acrylamide baking products?

It is also relevant to note that in the course of studies on reactions of acrylamide and related compounds with proteins, it was found that vinylpyridine can be used to selectively modify protein SH groups. This reaction has been widely adopted and used to stabilize cereal proteins, facilitating their separation and isolation from extracts of wheat, corn, and barley by HPLC and related chromatographic methods.

#### **Ascorbate Browning**

When a nutritionally complete, low-protein basal diet containing 10% casein was supplemented with 20% protein from unheated casein, wheat gluten, or soybean, test mice exhibited a significantly increased weight gain. In contrast, weight gain was markedly reduced when the supplement was soy protein or gluten heated at 200°C or 215°C for 72 min in the dry state (simulated crust baking). Baked casein was non-nutritive. Adding carbohydrates to gluten during heating prevented subsequent growth inhibition. After heating with sodium ascorbate (but not L-ascorbic acid), soy protein (at 200°C) and gluten (at 215°C) completely prevented growth when added to the basal diet. Growth inhibition was also aggravated by a heated casein-ascorbate mixture, but less than

with the other proteins. The extent of nutritive damage increased sharply with heating temperature in the range 180–215°C, and with sodium ascorbate concentration in the range 1–20%. It was also found that sodium ascorbate heated with amino acids, especially tryptophan, results in the formation of antinutritional compounds.

The reduced weight gain of mice, fed a nutritionally adequate diet supplemented with these materials, suggests that heating induces the formation of nutritionally antagonistic or toxic compounds that interfere with essential metabolic pathways such as digestion, transport, absorption, and utilization of nutrients. Further studies of the chemical basis of these effects may be more conveniently performed with tryptophan or other amino acid/ascorbate mixtures than with the more complex protein/ascorbate blends, since the heat-induced products may be easier to isolate and characterize.

Heating experiments conducted by the author used proportionately much more sodium ascorbate than is used in thermal food processing to improve breaddough characteristics such as loaf volume and bread texture, and to inhibit nitrosamine formation in bacon. However, since results do not rule out possible cumulative biological effects, additional studies are needed to determine whether consumption of low levels of the heat-derived compounds can be a human health hazard.

The results suggest that deleterious material formed during heating of gluten or soy protein, and to a lesser extent casein, may represent the degradation of protein to nitrogenous materials without nutritional value. At the nominal protein level, such materials would represent a severe metabolic burden (toxic effect) when fed to the animal, which must then eliminate them. The protective effect of carbohydrates in diminishing the formation of toxic gluten is interpreted as a thermochemical volatilization of deleterious products, while sodium ascorbate appears to reduce vaporization.

These considerations suggest the need:

- to characterize the compound(s) in heated protein and amino acid—sodium ascorbate mixtures that may be responsible for the observed growth inhibition;
- 2. to determine the safety of the pure compounds in laboratory animals and measure their prevalence in commercial foods in order to define possible human risk;
- 3. to carry out studies with related food ingredients such as sodium citrate, sodium gluconate, and sodium glutamate in order to define the mechanism of this type of growth inhibition;

- 4. to devise processing conditions to prevent the formation of the growth inhibitors in food; and
- 5. to use ascorbic acid rather than sodium ascorbate in baking formulations.

# **Food Allergenicity**

As noted earlier, carbohydrates interact with proteins to form Maillard browning products. The effects of these transformations on the antigenicity of the Kunitz soybean trypsin inhibitor (KTI) with two monoclonal antibodies were studied. Solid mixtures of KTI and carbohydrates were heated in an oven at 120°C, dialyzed, freeze-dried, and analyzed by enzyme-linked immuno-sorbent assay (ELISA). Glucose, lactose, and maltose decreased the antigenicity of KTI to levels 60-80% lower than those observed in a control sample heated without carbohydrate. Starch was less effective than the three reducing sugars. The decrease was rapid, occurring within 10 min when glucose was heated with KTI, with retention of 60% of the chemically available lysine. Longer heating times increased browning and reduced the level of available lysine in KTI, without further reducing antigenicity. The results suggest that relatively mild conditions of heating food proteins with carbohydrates can reduce the antigenicity of the protein and possibly modify sites known to elicit allergenic responses. That these reactions can also introduce new antigenic determinants into a food protein should be noted.

# **Inactivation of Inhibitors of Digestive Enzymes**

Soy protein is increasingly important in the human diet. However, soy protein is not an ideal protein since it is deficient in the essential amino acid methionine. The content of another essential amino acid, lysine, is higher than that of wheat protein but lower than that of milk protein casein. Adverse nutritional and other effects following consumption of raw soybean meal have been attributed to the presence of endogenous inhibitors of digestive enzymes, lectins, and to poor digestibility. To improve the nutritional quality of soy foods, inhibitors are generally inactivated by heat treatment or eliminated by fractionation during food processing. Most commercially heated meals still retain up to 20% of the Bowman-Birk (BBI) and Kunitz (KTI) inhibitors of digestive enzymes.

The content and heat stability of protease inhibitors of a standard cultivar (Williams 82) and an isoline lacking the KTI were measured by using enzyme inhibition and ELISA. Steam heating of the isoline flour (121°C, 20 min) resulted in a near-zero level of trypsin inhibitory activity, while 20% remained in the Williams 82 sample. The raw soy flour prepared

from the isoline was nutritionally superior to the raw flour prepared from the standard variety, as measured by protein efficiency ratio (PER) and pancreatic weights. The increased PER was likely due to the lower level of trypsin inhibitor activity in the isoline. Steam heating the flours for up to 30 min at 121°C progressively increased the PER for both strains. Less heat was needed to inactivate the inhibitors in the isoline than in the standard cultivar.

Related studies showed that treating raw soy flour with cysteine, N-acetyl-L-cysteine, or reduced glutathione introduces new half-cystine residues into native proteins, with a corresponding improvement of nutritional quality and safety. The proteins are modified through formation of mixed disulfide bonds among added thiols, protease inhibitors, and structural protein molecules. This leads to decreased inhibitory activity and increased protein digestibility and nutritive value. The SH-containing amino acids also facilitate heat inactivation of hemagglutinins (lectins) in lima bean flour. Exposure of raw soy flour to sodium sulfite was also nutritionally beneficial.

Naturally occurring enzyme inhibitors, such as BBI in which every sixth amino acid residues is cystine, also have beneficial effects such as prevention of development of colon cancer in mice. Although the molecular basis for such beneficial effects needs to be ascertained, one possibility is that the inhibitors or inhibitor—protease complexes act as free radical traps, whereby the free electrons on damaging oxygen radicals are transferred or dissipated to the sulfur atoms of the sulfur-rich inhibitors or complexes. These considerations suggest the need for further studies to learn more about possible beneficial effects of plant protease inhibitors in relation to sulfur amino acids.

# Effect of pH

#### **General Aspects**

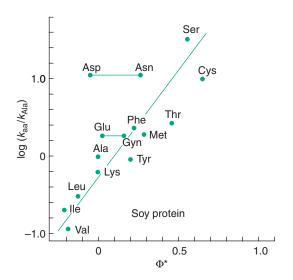
Exposure of food proteins to heat and high pH induces two major chemical changes: racemization of all L-amino acids to D-isomers and concurrent formation of lysinoalanine. Racemization of L-amino acids residues to their D-isomers in food and other proteins is pH-, time-, and temperature dependent. Although racemization rates of the 18 different L-amino acid residues in a protein vary, the relative rates in different proteins are similar. The diet contains both processing-induced and naturally formed D-amino acids. The latter include those found in microorganisms, plants, and marine invertebrates. Racemization impairs digestibility and nutritional quality. The nutritional utilization of different D-amino acids varies widely in animals and humans. In addition, some D-amino acids may be deleterious (Tables 5 and 6 and Figures 5-8).

Table 5 Effect of pH on the lysine and lysinoalanine (LAL) content of wheat gluten

Mole (%)	Control	pH 9.6	pH 10.6	pH 11.2	pH 12.5
Lys	1.33	1.40	1.16	0.96	0.94
LAL	0.00	0.00	0.26	0.42	0.76

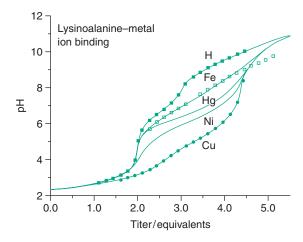
**Table 6** Digestibility and net protein utilization (NPU) of toasted and alkali-treated soy proteins in rats

Diet	Digestibility (%)	NPU (N retained/N intake) × 100
Casein	98.3	80.6
Toasted soy	97.0	62.4
Alkali-treated soy	83.2	28.3



**Figure 5** Linear relationship between the inductive constant  $(\Phi^*)$  of the amino acid side chain R in RCH(NH<sub>2</sub>)COOH and the racemization rate constant  $(k_{\rm aa})$  for amino acids in soy protein relative to Ala  $(k_{\rm Ala})$ .

Although proteins containing D-amino acids can be hydrolyzed at peptide bonds, the hydrolysis rates may be slower than those for corresponding native proteins. Such changes can impair the nutritional quality and safety of foods by generating nonmetabolizable and biologically nonutilizable forms of amino acids, creating D-D, D-L, and L-D peptide bonds partly or fully inaccessible to proteolytic enzymes, and forming nutritionally antagonistic as well as toxic compounds. Furthermore, these altered proteins may compete with proteins, which do not possess racemized amino acids, for the active site of digestive proteases in the gut and thus render the unracemized proteins also less nutritionally available. A need exists to develop a better understanding of the roles of D-amino acids in



**Figure 6** Relative affinities of metal ions to lysinoalanine determined by potentiometric titration.

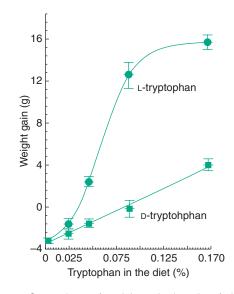


Figure 7 Comparison of weight gain in mice fed L- and D-tryptophan as part of an all-amino-acid diet.

human nutrition. For example, it is not known whether D-amino acids and peptides can change the microbial flora of the digestive tract.

### Racemization of L-Amino Acids to D-Isomers

Since the early 1900s, alkali and heat treatments have been known to racemize amino acids. As a result of food processing using these treatments, D-amino acids are continuously consumed by animals and man. Because all of the amino acid residues in a protein undergo racemization simultaneously, but at differing rates, assessment of the extent of racemization in a food protein requires quantitative measurement of at least 36 optical isomers, 18 L and 18 D. Analytically, this is a difficult problem.

**Figure 8** Structures of dehydro- and cross-linked amino acids formed during exposure of food proteins to heat and high pH. Asterisks indicate the number of asymmetric carbon atoms.

Racemization of an amino acid proceeds by removal of a proton from the  $\alpha$ -carbon atom to form a carbanion intermediate. The carbanion, having lost the original asymmetry of the  $\alpha$ -carbon, recombines with a proton from the environment to regenerate a tetrahedral structure. The reaction is written as

L-amino acid 
$$\xrightarrow{k_{\text{rac}}}$$
 D-amino acid

where  $k_{\rm rac}$  and  $k'_{\rm rac}$  are the first-order rate constants for the forward and reverse racemization of the stereoisomers. The product is racemic if recombination can take place equally well on either side of the carbanion, giving an equimolar mixture of L- and D-isomers.

Because the structural and electronic factors that facilitate the formation and stabilization of the carbanion intermediate are unique for each amino acid, it follows that the reaction rate for the isomerization of each amino acid is also unique. Thus, the inductive strengths of the R-substituents have been invoked to explain differing racemization rates in the various amino acids. Plotting racemization for individual amino acids in casein and soybean proteins against the inductive parameters clearly demonstrates strong correlations (Figure 5).

Two pathways are available for the biological utilization of D-amino acids: (1) racemases or epimerases may convert D-amino acids directly to L-isomers or to (DL) mixtures; or (2) D-amino-acid oxidases may catalyze oxidative deamination of the  $\alpha$ -amino group to form  $\alpha$ -keto acids, which can then be specifically reaminated to the L-form. Although both pathways

may operate in microorganisms, only the second has been demonstrated in mammals.

The amounts and specificities of D-amino acid oxidase are known to vary in different animal species. In some, the oxidase system may be rate limiting in the utilization of a D-amino acid as a source of the L-isomer. In this case, the kinetics of transamination of D-enantiomers would be too slow to support optimal growth. In addition, growth depression could result from nutritionally antagonistic or toxic manifestations of D-enantiomers exerting a metabolic burden on the organism.

The nutritional utilization of different D-amino acids varies widely, both in animals and humans. In addition, some D-amino acids may be deleterious. For example, although D-phenylalanine is nutritionally available as a source of L-phenylalanine, studies by the author have shown that high concentrations of D-tyrosine inhibit the growth of mice. The antimetabolic effect of D-tyrosine can be minimized by increasing the L-phenylalanine content (protein bound, or free) of the diet. Similarly, L-cysteine has a sparing effect on L-methionine when fed to mice; however, D-cysteine does not. The wide variation in the utilization of D-amino acids is exemplified by the fact that D-lysine is not utilized as a source of the L-isomer for growth. The utilization of methionine is dosedependent, reaching 76% of the value obtained with L-methionine. Both D-serine and the mixture of L-L and L-D isomers of lysinoalanine induce histological changes in the rat kidneys. D-tyrosine, D-serine, and lysinoalanine are produced in significant amounts under the influence of even short periods of alkaline treatment.

Whether the biological effects of D-amino acids vary depending on their consumption in the free state or as part of a food protein is unresolved. Indications are that L-D, D-L, and D-D peptide bonds in food proteins may not hydrolyze as readily as naturally occurring L-L peptide bonds. Possible metabolic interaction, antagonism, or synergism among Damino acids in vivo also merits further study. The described results with mice complement related studies with other species and contribute to the understanding of nutritional and toxicological consequences of ingesting D-amino acids. Such an understanding will make it possible to devise foodprocessing conditions to minimize or prevent the formation of undesirable D-amino acids in food proteins and to prepare better and safer foods.

# Lysinoalanine and Related Amino Acids

Heat and alkali treatment of foods, widely used in food processing, results in the formation of dehydroand cross-linked amino acids such as dehydroalanine, methyldehydroalanine,  $\beta$ -aminoalanine, lysinoalanine (LAL), ornithinoalanine, histidinoalanine (HAL), phenylethylaminoalanine, lanthionine (LAN), and methyl-lanthionine present in proteins (Figure 8). The presence of LAL residues along a protein chain decreases nutritional quality in rats (Table 6) and primates but enhances nutritional quality in ruminants. LAL, HAL, and LAN also occur in certain peptide antibiotics (cinnamycin, duramycin, nisin, and subtilin) and in body organs and tissues (aorta, bone, collagen, dentin, eye cataracts), where their formation may be a function of the aging process.

Detailed studies revealed that base-catalyzed synthesis of lysinoalanine proceeds by the addition of the  $\varepsilon$ -NH<sub>2</sub> group of lysine to the double bond of a dehydroalanine residue. This residue is derived from cysteine and/or serine (**Figure 9**). From a nutritional standpoint, lysinoalanine formation results in a decrease of the essential amino acid lysine, and the semiessential amino acid cystine, as well as in a decrease in digestibility of the modified protein.

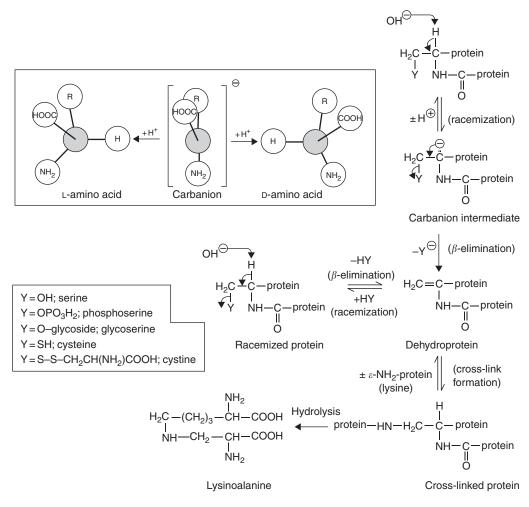


Figure 9 Mechanism of hydroxide ion-catalyzed racemization and lysinoalanine formation in food and other proteins.

In rats, studies have found histological changes in the kidneys related to dietary exposure to this substance, either isolated or as part of intact proteins. The lesions are located in the epithelial cells of the straight portion of the proximal renal tubules and are characterized by enlargement of the nucleus and cytoplasm, increased nucleoprotein content, and disturbances in DNA synthesis and mitosis.

Because of these observations, concern has arisen about the safety of foods that contain lysinoalanine and related dehydroalanine-derived amino acids known to produce similar lesions. However, since the mechanism by which these compounds damage the rat kidney is unknown, it is difficult to assess the risk to human health caused by their presence in the diet.

Lysinoalanine has two asymmetric carbon atoms, making possible four separate diastereoisomeric forms: LL, LD, DL, and DD. Its structure suggests that it should have excellent chelating potential for metal ions, a property that may be relevant to its toxic action. Accordingly, its affinity towards a series of metal ions, of which copper (II) was chelated the most strongly has been examined (Figure 6). On this basis, it was suggested that a possible mechanism for kidney damage in the rat involving lysinoalanine's interaction with copper within the epithelial cells of the proximal tubules.

The apparent direct relationship between the observed affinities of the two lysinoalanine isomers for copper (II) ions *in vitro* and their relative toxic manifestation in the rat kidney is consistent with the hypothesis that lysinoalanine exerts its biological effect through chelation of copper in body fluids and tissues. Limited studies on the binding of LL- and LD-lysinoalanines to cobalt (II), zinc (II), and other metal ions imply that lysinoalanine could also influence cobalt utilization *in vivo*.

Factors, which minimize lysinoalanine formation, include the presence of cysteine, N-acetylcysteine, and reduced glutathione, sodium sulfite, ammonia, biogenic amines, ascorbic, citric, and malic acids, glucose, dephosphorylation of O-phosphoryl esters, and acylation of  $\varepsilon$ -NH<sub>2</sub> groups of lysine.

#### **Stability of Plant Phenolic Compounds**

Phenolic compounds are secondary metabolites found in cereals, coffee beans, fruits, olives, vegetables, and tea leaves. A study on the stability of structurally different phenolic compounds in buffers in the pH range 3–11 revealed that caffeic, chlorogenic, and gallic acid were not stable to high pH. By contrast, catechin, epigallocatechin, ferulic acid, rutin, and cinnamic acid resisted pH-induced degradation. The

results suggest that if a specific phenolic compound is found to be unstable under food-processing conditions, it may not be effective as an antioxidant, anticarcinogen, or antibiotic when present in foods subjected to heat or high pH.

# **Bioavailability of Amino Acids**

#### Lysine and Derivatives

Wheat gluten, the major protein in many baking formulations, is considered a poor-quality protein, primarily because it has insufficient amounts of two essential amino acids: lysine, the first-limiting amino acid, and threonine, the second-limiting one. To compensate for the poor quality of most cereal proteins such as gluten, the minimum recommended daily allowance (RDA) for these proteins has been set at 65 g, compared to 45 g for good-quality proteins such as casein.

During baking, the mixture of protein, carbohydrate, and water plus additives in dough is exposed to two distinct transformations. Desiccation of the surface on exposure to temperatures reaching 215°C produces the crust. The crust encloses part of the dough in steam phase at  $\sim 100$ °C, resulting in the formation of the crumb.

Because lysine's  $\varepsilon$ -amino group interacts with food constituents to make it nutritionally less available, the baking process further reduces the dietary availability and utilization of lysine, especially in the crust, which makes up  $\sim$ 40% of the bread by weight. Many such interactions have been described including

- 1. the reaction of the amino group with carbonyl groups of sugars and fatty acids to form Maillard browning products;
- 2. the formation of cross-linked amino acids such as lanthionine, lysinoalanine, and glutamyllysine;
- 3. the interaction with tannins and quinones; and
- 4. steric blocking of the action of digestive enzymes by newly introduced cross-links, as well as native ones such as disulfide bonds.

Because these reactions of lysine with other dietary components may lead to protein damage and to the formation of physiologically active compounds, an important objective of food science and nutrition is to overcome these effects.

In principle, it is possible to enhance the nutritional quality of bread by amino acid fortification. A major problem encountered when free lysine is used to fortify foods is that the added amino acid can itself participate in browning and other side reactions.

To assess whether glutamyllysine, which undergoes less browning than does lysine, can serve as

a nutritional source of lysine, comparisons were made of the growth of mice fed (1) an amino acid diet in which lysine was replaced by four dietary levels of glutamyllysine; (2) wheat gluten diets fortified with lysine; (3) a wheat bread-based diet (10% protein) supplemented before feeding with lysine or glutamyllysine, not cobaked; and (4) bread diets baked with these levels of lysine or glutamyllysine. For the amino acid diet, the relative growth response to glutamyllysine was about half that of lysine. The effect of added lysine on the nutritional improvement of wheat gluten depended on both lysine and gluten concentrations in the diet. With 10% and 15% gluten, 0.37% lysine hydrochloride produced markedly increased weight gain. Further increase in lysine hydrochloride to 0.75% proved somewhat detrimental to weight gain. Lysine hydrochloride addition improved growth when 20-25% gluten was present in the diet and did not prove detrimental at 0.75% level. For whole bread, glutamyllysine served nearly as well as lysine to improve weight gain. The nutritive value of bread crust, fortified or not, was markedly less than that of crumb or whole bread (Figure 2). Other data showed that lysine or glutamyllysine at the highest level of fortification, 0.3%, improved the protein quality (PER) of crumb over that of either crust or whole bread, indicating a possible greater availability of the second-limiting amino acid, threonine, in crumb. These data and additional metabolic studies with [U-14C] glutamyllysine suggest that glutamyllysine, cobaked or not, is metabolized in the kidneys and utilized in vivo as a source of lysine; this and related peptides merit further study as sources of lysine in low-lysine foods.

Amino acids are used both metabolically, as building blocks for protein biosynthesis, and catabolically, as energy sources. Catabolism for most amino acids proceeds through transamination pathways; the exceptions are lysine and threonine. Specific enzymes catabolize these nutritionally limiting amino acids: threonine dehydratase acts on threonine and lysine ketoglutarate reductase on lysine. The concentrations of these enzymes in the liver of rats are subject to adaptive responses that control the utilization of these two amino acids. Although both enzymes are induced by feeding diets high in protein, rats differ in the mechanism of the adaptive response to highprotein diets and to diets whose threonine or lysine content is less than that needed for growth. Thus, reductase falls to very low levels in the liver of rats fed wheat gluten. This appears to be an adaptive response conserving body lysine. At the same time, catabolism of body proteins increases, producing endogenous lysine needed for survival. These considerations imply that as the level of wheat gluten in the diet decreases, lysine is no longer the limiting amino acid. Total protein or some other amino acid then becomes limiting.

In contrast to the apparent mechanism of lysine catabolism, threonine dehydratase does not appear to be substrate induced. Therefore, when lysine is the limiting amino acid, the catabolic enzyme falls to low levels and lysine is apparently conserved at the expense of body proteins. Loss of tissue proteins is much less when a diet low in threonine is fed, since the level of threonine dehydratase does not seem to be significantly affected by the protein or threonine content of the diet. Additional studies are needed to establish whether the catabolic enzyme patterns in mice parallel those of humans.

The results also show that mice provide a good animal model to study protein quality of native, fortified, and processed wheat proteins. Mouse bioassays have a major advantage in applications to label foods for protein nutritional quality. They require about one-fifth of the test material needed for rats and can be completed in 14 days instead of 28 days. They are especially useful to evaluate nutritional and safety impacts of new food ingredients formed during processing and of new plants and plant parts, when amount of material available for bioassays is limited.

#### **Methionine and Derivatives**

The low content of the essential amino acid L-methionine limits the nutritive value of many food proteins of plant origin. These include soybeans and other legumes. The problem is further compounded for two reasons. First, during food processing and storage L-methionine and other amino acids are chemically modified, further reducing nutritional quality. In the case of methionine, such modifications include oxidation to methionine sulfoxide and methionine sulfone, racemization to D-methionine, and degradation to compounds with undesirable flavors. Second, protein-bound methionine in some plant foods is poorly utilized, presumably because of poor digestibility.

A related aspect is the widespread use of L-methionine to fortify low-methionine foods in order to improve nutritional quality. Because of the reported antinutritional or toxic manifestations of high levels of free methionine in the diet, a need exists to find out whether methionine analogs and derivatives lack the apparent toxicity of L-methionine and whether they can be used as methionine substitutes in the diet.

As part of a program to evaluate the nutritional and toxicological potential of novel amino acids formed during food processing, weight gain in mice fed amino acid diets containing graded levels of L-methionine and 16 methionine derivatives, isomeric dipeptides,

and analogs was compared. Because the mice received no other source of sulfur amino acids, the results reflect the ability of each of the compounds to meet the animals' entire metabolic demand for dietary sulfur amino acids, relative to that for L-methionine. The results imply that some methionine dipeptides or analogs may be better candidates for fortifying foods than L-methionine.

The data for mice demonstrate that

- 1. the assay is highly reproducible, exhibits excellent dose-response characteristics, and yields useful estimates of relative potency for the 16 methionine analogs; and
- somewhat rigorous control of concentration may be required for dietary supplementation with Lmethionine in order to achieve maximum nutritional benefit while preventing toxicity problems.

This constraint may be alleviated or avoided by using one or more analogs as alternatives. Whether these compounds will also alleviate the reported adverse flavor aspects of sulfur amino acid supplementation associated with methionine when it is added to foods awaits further study.

# **Tryptophan and Derivatives**

The essential amino acid tryptophan contributes to normal growth and protein synthesis and participates in numerous biochemical processes. Since tryptophan is a nutritionally second-limiting amino acid in maize, and since cereals and processed foods are increasingly used to meet human dietary needs, it is of paramount importance to develop an understanding of thermally induced changes in tryptophan in order to improve the quality and safety of our food supply.

The stability of free or protein-bound tryptophan during processing and storage depends on temperature and the presence of oxygen or other oxidizing agents, especially lipid peroxides, and radiation. In the absence of oxidizing agents, tryptophan is a stable amino acid, even in strongly basic or acidic conditions. Free or bound tryptophan is relatively stable during heat treatments such as industrial or home cooking in the presence of air or steam sterilization. Only severe treatments cause a significant degradation of this amino acid. In the presence of carbonyl compounds and/or at high temperatures, however, carboline formation occurs. Both carbolines and tryptophan-derived nitroso compounds are potential carcinogens. Tryptophan losses during food processing cannot always be monitored because of the lack of reliable analytical methods.

The losses in tryptophan bioavailability during heat treatment such as home cooking or industrial steril-

ization appear less important than other detrimental effects, particularly on lysine or methionine. Some of the reported variabilities in the utilization of D-tryptophan could be due to the fact that the value (potency), of D-tryptophan as a nutritional source of L-tryptophan is strongly dose-dependent (Figure 7).

Possible consequences for nutrition, food safety, and human health of halogenated tryptophans, light-induced tryptophan adducts; tryptophanderived carbolines, and tryptophan-induced eosinophilia myalgia are not well understood.

#### **Conclusions**

This article shows that pH, heat, and oxygen have both beneficial and adverse effects on many nutrients. To maximize beneficial effects, future studies should emphasize the prevention of browning and the consequent antinutritional and toxicological manifestations of browning products and acrylamide in whole foods. Many of the safety concerns cited, especially those of genotoxic potential, are based on in vitro data that may not always be relevant to in vivo effects following the consumption of whole food products containing the browning-derived constituents. The presence of other dietary constituents in the food and the process of digestion and metabolism can be expected to decrease or increase the adverse manifestations of browning and heat-induced products. For nutrition and food safety, possible consequences of chelation of nutritionally essential trace materials to processing-induced food ingredients, beneficial effects of processing on food allergy, the immune system, and food microbiology and differentiating adverse and beneficial effects of heat and oxygen on lipids and vitamins also merit study.

A better understanding of the chemical changes during food processing will permit optimizing beneficial effects such as bioavailability, food quality, and safety, and to minimize the formation and facilitate removal of deleterious mutagens, carcinogens, allergens, pathogens, and other toxins. Future study should differentiate antinutritional and toxicological relationships, develop a relative biological potency scale of new food ingredients formed during food processing, define combined potencies of browning and other products, and develop means to prevent the formation of the most toxic compounds.

See also: Food Safety through the Production Chain. Fortification of Grain-Based Foods. Nutrition: Guidelines for Grain-Based Foods; Mineral Composition. Whole-Grain versus Refined Products.

# **Further Reading**

Borelli RC, Menella C, Barba F, et al. (2003) Characterization of coloured compounds obtained by enzymatic extraction of bakery products. Food and Chemical Toxicology 41: 1367-1374.

Friedman M (ed.) (1991) Nutritional and Toxicological Consequences of Food Processing. New York: Plenum.

Friedman M (1994) Improvement in the safety of foods by SH-containing amino acids and peptides. Journal of Agricultural and Food Chemistry 42: 3-20.

Friedman M (1996a) The nutritional value of proteins from different food sources. Journal of Agricultural and Food Chemistry 43:6-29.

Friedman M (1996b) Food browning and its prevention. Journal of Agricultural and Food Chemistry 44: 631 - 653.

Friedman M (1999a) Chemistry, biochemistry, nutrition, and microbiology of lysinoalanine, lanthionine, and histidinoalanine in food proteins. Journal of Agricultural and Food Chemistry 47: 1295-1319.

Friedman M (1999b) Chemistry, nutrition, and microbiology of D-amino acids. Journal of Agricultural and Food Chemistry 47: 3457-3479.

Friedman M (2003) Chemistry, biochemistry, and safety of acrylamide. Journal of Agricultural and Food Chemistry 51: 4504-4526.

Friedman M (2004) Application of the ninhydrin reaction for analysis of aminoacids, peptides, and proteins to agricultural and biomedical sciences. Journal of Agricultural and Food Chemistry 52: 385-406.

Friedman M and Brandon DL (2001) Nutritional and health benefits of soy proteins. Journal of Agricultural and Food Chemistry 49: 1069-1086.

Krajkovicova-Kudlackova M, Sebkova K, Schnitzel R, and Klvanova J (2002) Advanced glycation end products and nutrition. *Physiological Research* 51: 313–316.

Wenzel E, Tasto S, Erbersdobler HF, and Faist V (2002) Effect of heat-treated proteins on selected parameters of the biotransformation system in the rat. Annalen Nutrition and Metabolism 46: 9-16.

# **Mineral Composition**

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#### Introduction

Minerals are food components that are required to establish and maintain life processes in plants and humans. Whole grains, grain-based foods, legumes, and seeds are important dietary sources of minerals. In plants and the human body, minerals interact with one another and with other nutrients and components to provide beneficial effects. In grains, minerals enable plants to grow and produce mineral-rich kernels and seeds used as food by humans. When dietary intakes include grain-based foods, they supply minerals to maintain the health and wellness of the human body.

As quantitative information about the content of minerals, vitamins, and other nutrients in food became available with improved accuracy of analytical techniques and instruments, food composition tables have been developed to present the data. Such a table for minerals, vitamins, and other nutrients and food components in selected grains and grain-based foods is available in **Appendix**: Grain Composition Tables. The purpose of this article is to discuss and compare the mineral composition of grains and grain-based foods. Examples have been incorporated from the data provided in the above-mentioned appendix.

#### **Characteristics of Minerals**

Minerals are inorganic elements that are needed in very small amounts by plants, animals, and humans. For example, in plants, minerals function to promote growth. Magnesium is an important component of the green pigment, chlorophyll, involved in photosynthesis. Minerals give the human body structure; they are needed for growth and development, and are components of organs and fluids; and they serve as cofactors for enzymes. Minerals also participate in regulating nerve impulses, acid-base balance in fluids, and contraction and relaxation of muscles (Table 1). Mineral deficiencies or excesses in humans may result in major health problems. Long-term deficiency in calcium intake is known to be a major contributor to osteoporosis, often called "brittle bone" disease. Deficient intake of iron commonly results in iron-deficiency anemia. An excess of sodium intake has been associated with high blood pressure in some people.

Minerals are identified by their names or chemical abbreviations (Table 1 and Appendix: Grain Composition Tables). They are classified as major minerals and trace minerals, depending on the amounts required by the human body. Major minerals are essential elements that must be supplied in human food intakes. Their recommended intakes are >100 mg per day (Table 1). They are found in abundant amounts in foods, and their functions in the body are well understood. Trace minerals are also essential elements; however, their recommended intakes are <100 mg per day. They are found in small amounts in food sources. Overall, the importance of their functions has been identified primarily since the second half of the twentieth century, and the functions of some trace minerals are less well understood than

**Table 1** Characteristics of minerals in grains and grain-based foods<sup>a</sup>

Mineral name	Important functions	US recommended dietary allowances (RDA) <sup>b</sup> , adequate intake (AI) <sup>c</sup> , or minimum requirement <sup>d</sup>		
		Men	Women	
Major minerals				
Potassium (K)	Neuromuscular function	2000 mg <sup>b</sup>	2000 mg	
	Body-water balance			
	Acid-base balance	ar and a second and		
Sodium (Na)	Neuromuscular function	500 mg <sup>a</sup>	500 mg	
	Body-water balance			
	Acid-base balance	_		
Calcium (Ca)	Neuromuscular function	1000 $-$ 1200 mg $^c$	1000-1200 mg	
	Bone, tooth structure			
	Body-water balance			
	Growth, development	<b>b</b>		
Phosphorus (P)	Enzyme activity	700 mg <sup>b</sup>	700 mg	
	Bone, tooth structure			
	Growth, development			
	Body-water balance			
Magnesium (Mg)	Enzyme activity	420 mg <sup>b</sup>	320 mg	
	Neuromuscular function			
	Bone strength			
Trace minerals		- 6		
Iron (Fe)	Component of hemoglobin	8 mg <sup>b</sup>	8–18 mg	
	Carry oxygen to body			
	Enzyme activity			
	Growth, development	h		
Zinc (Zn)	Cofactor-enzyme activity	11 mg <sup>b</sup>	8 mg	
	Carbohydrate metabolism			
a (a)	Growth, development	h		
Copper (Cu)	Cofactor-enzyme activity	900 μg <sup>δ</sup>	900 µg	
	Energy release			
	Wound healing			

<sup>&</sup>lt;sup>a</sup> Adapted from Grodner M, Anderson SL, and De Young S (2000) Foundations and Clinical Applications of Nutrition – A Nursing Approach, 2nd edn. St. Louis: Mosby.

those of major minerals. Research continues to clarify functions of minerals in plants, animals, and humans.

Minerals are stable to heat and are not destroyed when foods are cooked. Although they are insoluble in water, when plant foods are cooked using liquid, minerals can be physically lost from cut surfaces of the tissue or leached from the food into the cooking liquid. These minerals remain intact and can be recovered by using the cooking liquid. Conversely, when cooked in already mineral-rich water, foods can absorb more minerals, thereby increasing their mineral contents.

# Grain Structure and Location of Minerals

Whole grains, legumes, and seeds make important contributions to the minerals in food intakes. Their

mineral contents are obtained from the soils in which they are grown. Dietary intake of minerals may not be fully absorbed by the body. Bioavailability of plant minerals, i.e., "the level of absorption of a consumed nutrient," is affected by how tightly they are bound in plant tissue. Such minerals are difficult to extract from plant tissue during digestion.

Cereal grain kernels have three major components: bran, germ, and endosperm. Bran, the outer covering of the kernel, is composed of several layers. The aleurone layer of the bran is an important layer next to the starchy endosperm, the largest portion of the kernel. The germ or embryo is the source of the new plant in a germinating kernel. All grains are similar in the types of tissues they contain and in the molecular and cellular organization of their tissues.

Minerals are concentrated in different parts of the kernel where they work together to activate a wide variety of reactions and interactions. Each compound in the grain is stored in a specific structural location.

<sup>&</sup>lt;sup>b</sup>RDA = recommended dietary allowance.

<sup>&</sup>lt;sup>c</sup>Al = adequate intake.

<sup>&</sup>lt;sup>d</sup>Recommended intake.

The bran and germ aleurone cells contain most of the minerals and other nutrients in the grain, and that is where metabolic activities occur. Wheat aleurone cells contain the highest proportion of the calcium, magnesium, potassium, sodium, and iron in the grain. The starchy endosperm contains the second largest proportion of minerals in the kernel. Whole cereal grains contain phytic acid in the bran layers, which binds minerals and thereby decreases their bioavailability during digestion. Other sources of phytic acid are pulses, oilseeds, and nuts.

# **Effects of Grain Processing on Minerals**

Grains are unique because they are seldom eaten in the raw state. For optimum benefits from their constituents, grains are processed in many ways to produce functional ingredients to make nutritious and palatable grain-based foods. Commercial processing of grains disrupts the organization of the kernels to release minerals and other constituents from storage in the aleurone cells and to make the starch of the endosperm available for digestion. Effects of processing differ for each process and grain combination. Processing techniques used include grinding or crushing the entire kernel of the grains into whole-grain flours, or milling to separate bran, germ, and endosperm of the kernel to obtain refined white flour. Different heating techniques used during processing, and the addition of other ingredients for specific products, create a wide variety of grain-based foods for the food marketplace.

Although minerals are not destroyed by processing procedures, mineral content is decreased when whole grains are milled into refined white flours. When processing losses are significant, mineral contents of flours may be adjusted by enrichment or fortification. Other products commonly enriched include breads, mixes, and other baked foods. Enrichment indicates that selected minerals that were present before processing are added back at appropriate levels to replace the losses resulting from processing. In US, iron is the only enrichment mineral added to replace that lost in processing. Other minerals lost from whole grains during processing are not replaced. Enrichment and fortification standards vary among different countries. Fortification indicates that selected nutrients, which may or may not have been present in the grain before processing, have been added to grain-based foods. Examples of several fortified grain-based foods, some highly fortified, are listed in the ready-to-eat cereal category (see Appendix: Grain Composition Tables).

# **Dietary Recommendations for Minerals**

Recommended mineral intakes for adult men and women are given in Table 1 for the eight minerals in grain-based foods found in Appendix: Grain Composition Tables. Concentrations of minerals in foods are also relatively low, but it is not difficult to meet daily needs by eating a variety of foods from each food group. Recommendations in Table 1 are based on new US dietary reference intakes (DRIs). The new recommended dietary allowances (RDAs) developed as a part of the DRIs are given in the table for most of the minerals. The recommended intake for calcium is expressed as adequate intake (AI), which indicates that the amount covers individual needs but insufficient data prevent setting an RDA level. The recommended minimum requirement given for sodium is representative of the amount that would be consumed if no salt were added and only unprocessed foods were eaten. Units of measurement for grain-based minerals are noted in Table 1 and listed in Appendix: Units of Grain Science.

# Mineral Composition of Grain-Based Foods

Names/descriptions of selected whole grains and grain-based foods from the wide variety available are classified into product categories (*see Appendix*: Grain Composition Tables). The table gives serving sizes and data for contents of minerals, vitamins and other nutrients, and food components. Table data are derived from the food and nutrient database developed and maintained by the Nutrition Coordinating Center, Division of Epidemiology, University of Minnesota, Minneapolis, MN, USA. Food product categories included in the table are listed below:

- grains, flours, and cooked cereals;
- pasta and rice;
- ready-to-eat cereal;
- baby food cereals;
- breads and other related products;
- crackers;
- cookies:
- cakes, pastries, and other desserts;
- granola and cereal bars;
- snacks and chips;
- legumes;
- meat substitutes;
- alcholic beverages; and
- ingredients used in grain products.

Some categories have examples of many different foods, while others have only a few similar foods. The uncooked, cooked, and processed foods in these

categories are discussed here based on their mineral contents per serving. Mineral composition comparisons for foods selected from Appendix: Grain Composition Tables are given in this article as examples of how to use and understand the data in the table. Appendix: Grain Composition Tables provides food names and data for weight of a serving in grams and mineral contents used in comparisons. Information in Appendix: Grain Composition Tables is summarized as below:

- abbreviations explained in table footnotes;
- food names alphabetized in each category;
- contents of minerals and other nutrients and food components given in amount per serving of the food;
- serving sizes specified in common US household units and weight in grams (see Appendix: Units of Grain Science for measurement abbreviations and equivalents);
- amount of each mineral per serving of each food calculated based on its serving size in grams; and
- nutrients added through commercial enrichment or fortification are shown in bold italics.

# **Mineral Content Comparisons**

Readers can use the following directions to make mineral content comparisons for selected foods using the serving weight and mineral data in Appendix: Grain Composition Tables. To compare mineral contents of foods, select foods, and then use contents of each mineral in each food (based on the same serving weight for each food) to classify the foods according to their mineral content. The serving weight must be the same for each food for a valid comparison. Foods within a category and from different categories can be included in a comparison as long as the weight of a serving for each food is the same. In this article, the mineral contents in foods are compared based on those with highest and lowest content.

#### **Grains, Flours, and Cooked Cereals**

This category includes selected unprocessed whole and refined grains and their products. Unprocessed whole grains and some processed grains are rarely eaten uncooked, but occasionally may be eaten cooked. Processed cereals in granulated and other forms are usually eaten cooked. Different types of flours used as ingredients in cereal-based foods are eaten cooked in further processed recipe products that have been baked, extruded, or had heat applied by other methods.

Minerals in whole grains - wheat, rye, barley, brown rice Comparisons of mineral contents (see Appendix: Grain Composition Tables) for 45 g servings of five classes of dry, unprocessed whole wheat: hard red spring, hard red winter, hard white, soft red winter, soft white; and three other dry, unprocessed grains: rye, barley, and brown rice, show differences among the grains.

Mineral contents highest:

- potassium, iron in soft white wheat;
- sodium, magnesium in brown rice;
- calcium in soft white wheat, rye, brown rice;
- phosphorus in soft red winter wheat;
- zinc in rye; and
- copper in hard red winter wheat, soft red winter wheat, rye.

Mineral contents lowest:

- potassium, iron, zinc, copper in brown rice;
- sodium in all wheats;
- calcium in hard red spring wheat; and
- phosphorus, magnesium in barley.

Minerals in whole wheat Comparisons of mineral contents (see Appendix: Grain Composition Tables) for 45 g servings of the five dry unprocessed whole wheats from above with each other show differences among them. Wheats include: hard red spring, hard red winter, hard white, soft red winter, and soft white.

Mineral contents highest:

- potassium, calcium, iron, zinc in soft white wheat;
- phosphorus in soft red winter wheat; and
- copper, magnesium in hard red winter wheat, soft red winter wheat.

Mineral contents lowest:

- potassium, calcium in hard red spring wheat;
- sodium in all wheats;
- iron in hard red winter wheat, soft red winter
- phosphorus in hard red winter wheat;
- zinc in soft red winter wheat;
- copper in hard white wheat; and
- magnesium in soft white wheat.

Minerals in whole rye, barley, and brown rice Comparisons of mineral contents (see Appendix: Grain Composition Tables) for 45 g servings of the three dry, unprocessed grains, rye, barley, and brown rice from above show differences in mineral contents among the grains.

Mineral contents highest:

- potassium in barley;
- sodium, calcium, magnesium in brown rice;

- calcium in rye and brown rice; and
- iron, phosphorus, zinc, copper in rye.

Mineral contents lowest:

- potassium, iron, zinc, copper in brown rice;
- sodium in rye; and
- calcium, phosphorus, magnesium in barley.

Minerals in whole spelt, triticale, wheat Comparisons of mineral contents (see Appendix: Grain Composition Tables) for 45 g servings of three similar dry, unprocessed whole grains, spelt, an ancient wheat, triticale, a cross between wheat and rye, and hard red spring wheat show differences among the grains.

Mineral contents highest:

- potassium, calcium, copper, magnesium in triticale;
- sodium in triticale and hard red spring wheat; and
- iron, phosphorus, zinc in spelt.

Mineral contents lowest:

- potassium, calcium, magnesium in hard red spring wheat:
- sodium, copper in spelt; and
- iron, phosphorus, zinc in triticale.

Minerals in processed wheat products Comparisons of mineral contents (see Appendix: Grain Composition Tables) for 45 g servings of whole-wheat flour ground from unprocessed whole-wheat and of white flour, wheat, all purpose, unenriched milled from unprocessed whole wheat show differences from effects of processing.

Mineral contents highest:

• All minerals in whole-wheat flour.

Mineral contents were lowest for all minerals in white unenriched wheat flour.

When the same serving size of enriched white allpurpose wheat flour is added to the comparison above, data for the three flours show the effect of enrichment on iron content.

Mineral contents highest:

- potassium, sodium, calcium, phosphorus, zinc, copper, magnesium in whole-wheat flour; and
- iron in enriched white flour.

Mineral contents were lowest for all minerals in white unenriched flour, and for all minerals, except iron, in enriched white flour.

Minerals in processed rice products Comparisons of mineral contents (see Appendix: Grain Composition

Tables) for 45 g servings of whole-grain brown rice flour and refined white rice flour show differences from effects of processing.

Mineral contents highest:

 Potassium, sodium, iron, phosphorus, zinc, copper, magnesium in brown rice flour.

Concentrations were lowest for minerals listed above in white rice flour, and were the same for calcium in both brown and white rice flours.

#### **Pasta and Rice**

This category consists of various types of cooked pasta products made from flours from different grains, and uncooked and cooked rice products.

Minerals in pasta Comparisons of the mineral contents (*see* Appendix: Grain Composition Tables) for 140 g servings of macaroni/spaghetti/noodles, whole wheat, cooked and macaroni/spaghetti/noodles, white, cooked show differences between the two pastas.

Mineral contents highest:

• All minerals in macaroni/spaghetti/noodles, whole wheat, cooked.

Mineral contents were lowest for all minerals in macaroni/spaghetti/noodles, white, cooked.

Minerals in uncooked brown and white rice Comparisons of mineral contents (see Appendix: Grain Composition Tables) for 45 g servings of uncooked brown and white rice show effects of removing bran and germ during processing.

Mineral contents highest:

• All minerals in uncooked brown rice.

Mineral contents were lowest for all minerals in uncooked white rice.

Minerals in cooked brown and white rice Comparisons of mineral contents (*see* Appendix: Grain Composition Tables) for 140 g servings of cooked brown and white rice show differences between the two rice products when cooked.

Mineral contents highest:

• Potassium, sodium iron, phosphorus, zinc, copper, magnesium in cooked brown rice.

Mineral contents were lowest for those listed above in cooked white rice. Calcium contents were the same in both cooked brown and white rice.

#### Ready-to-Eat Cereals

This category is composed of ready-to-eat cereals, each processed primarily from one type of grain (corn, oats, rice, or wheat), with other ingredients added for flavor, texture, and appearance, and fortified with a limited number of minerals. Fortified values are in bold italics in the table (see Appendix: Grain Composition Tables). None of the cereals are fortified with potassium, sodium, phosphorus, copper or magnesium.

Fortification of ready-to-eat cereals listed:

- Calcium, iron, zinc in unsweetened corn nuggets, puffed corn.
- Iron, zinc in bran flakes without raisins, unsweetened bran nuggets, oat flakes, unsweetened oat rings, wheat and barley nuggets.
- Iron only in unsweetened corn flakes, rice flakes, wheat flakes without raisins.
- Zinc only in wheat and barley flakes.
- No fortification in unsweetened rice nuggets, puffed rice, puffed wheat, unsweetened shredded wheat.

#### **Baby Food Cereals**

This category contains three representative baby food cereals in the highly processed dry, instant form. All are fortified with minerals and vitamins to increase nutrient levels (see Appendix: Grain Composition Tables).

#### **Breads and Other Related Products**

This category consists of many kinds of yeast bread, bread rolls, bagels, other assorted bread-based products and quick breads. These foods are made with a variety of flours and added ingredients for color, texture, flavor, and minerals. Effects of added ingredients on mineral contents are variable depending on the food item and amount added. Bread products made from white flour are usually enriched with iron. Enrichment does not include other minerals such as potassium, sodium, calcium, phosphorus, zinc, copper, or magnesium. Whole-wheat breadbased products are not enriched.

Whole-wheat and white pita breads are not enriched, and have the same serving sizes, therefore their mineral contents can be compared.

Minerals in pita bread Comparison of mineral contents (see Appendix: Grain Composition Tables) for 50 g servings of pita bread from whole-wheat flour and pita bread from unenriched white flour show differences between breads made from the two flours.

Mineral contents highest:

- Potassium, iron, phosphorus, zinc, copper, magnesium in whole-wheat pita bread.
- Sodium, calcium in white pita bread.

Mineral contents were lowest for all minerals except sodium and calcium in white pita bread.

#### **Crackers**

This category includes crackers that contain a variety of flours and added ingredients for color, texture, flavor, and minerals. Nearly all products are enriched with iron but not with other minerals. Mineral contents of whole-grain crackers can be compared because they are not enriched.

Minerals in whole-wheat and rye crackers Comparisons of mineral contents (see Appendix: Grain Composition Tables) for 30 g servings of three unenriched crackers: whole-wheat crackers, cheesefilled sandwich whole-wheat crackers, and rye wafer, plain, show differences from added ingredients and processing among these crackers.

Mineral contents highest:

- Potassium, iron, zinc, copper, magnesium in rye wafer, plain.
- Sodium, calcium, phosphorus in cheese-filled sandwich whole-wheat crackers.

Mineral contents lowest:

- Potassium, sodium, phosphorus in whole-wheat crackers.
- Iron, zinc, copper, magnesium in cheese-filled sandwich whole-wheat crackers.
- Calcium in rye wafer, plain.

#### Cookies

This category is composed of a variety of commonly consumed cookies (see Appendix: Grain Composition Tables). Most of them contain similar basic ingredients to which added ingredients provide different flavors, textures, appearance, and minerals. All but one kind of cookie are enriched with iron, but not the other minerals, and one cereal bar is fortified. Effect of minerals contributed by added ingredients is variable depending on food items and amount added.

#### Cakes, Pastries, and Other Desserts

This category contains a wide variety of popular cakes, desserts, pie crusts and a few complete pies (see Appendix: Grain Composition Tables). Mineral contents vary depending on kind and amount of added ingredients and weight of a serving. All but

a few foods are enriched with iron, but not the other minerals.

#### **Granola and Cereal Bars**

This category consists of two examples of these bars that are representative of those available in retail stores (*see* Appendix: Grain Composition Tables). Breakfast bars (intended as a meal replacement) are fortified with calcium, iron, and zinc, but not potassium, sodium, phosphorus, copper, or magnesium. Cereal bars are only fortified with iron, and at a lower level than breakfast bars.

#### **Snacks and Chips**

This category contains foods representative of the wide variety of available grain-based snack foods (see Appendix: Grain Composition Tables). Most snacks and chips are not enriched; none are fortified. Of the items listed, only bagel chips and pretzels are enriched with iron.

Minerals in snacks and chips Comparisons of mineral contents (see Appendix: Grain Composition Tables) for 30 g servings of the unenriched products show effects of kind of grain used, added ingredients, processing or method of preparation (popped popcorn).

Mineral contents highest:

- potassium, copper, magnesium in hot air popped popcorn;
- calcium, iron in taco or tortilla chips;
- sodium in cheese balls, puffs, or twists;
- zinc, copper in wheat nuts; and
- phosphorus, magnesium in rice cake.

Mineral contents lowest:

- potassium, calcium, phosphorus, zinc, copper, magnesium in corn chips;
- sodium in popcorn popped in hot air; popcorn popped in hot fat;
- iron in rice cake; and
- copper in taco or tortilla chips.

# Legumes

This category contains a collection of commonly used legumes. Overall, these cooked legumes are excellent sources of minerals, except sodium.

Minerals in legumes Comparisons of mineral contents (see Appendix: Grain Composition Tables) for 90 g servings show differences in minerals among cooked legumes.

Mineral contents highest:

- potassium in cowpeas, mung beans, northern beans:
- sodium in fava beans;
- calcium, iron, phosphorus, copper, magnesium in soybeans; and
- zinc in adzuki beans.

Mineral contents lowest:

- potassium, iron, zinc, magnesium in bayo beans;
- sodium in bayo beans, black beans, broadbeans, navy beans, soybeans;
- calcium in pigeonpeas, yellow or green split peas; and
- phosphorus and copper in fava beans.

#### **Meat Substitutes**

This category contains examples of soybeans processed into the meat substitutes "miso," "tempeh," and "tofu."

Minerals in meat substitutes Comparisons of mineral contents (*see* Appendix: Grain Composition Tables) for 85 g servings of these foods show differences in effects of processing among them.

Mineral contents highest:

- potassium, iron, phosphorus, zinc, copper, magnesium in tempeh;
- sodium in miso; and
- calcium in firm tofu.

Mineral contents lowest:

- potassium, calcium, iron, phosphorus, copper, magnesium in miso;
- zinc in silken tofu; and
- sodium in soft tofu.

#### **Alcoholic Beverages**

This category includes beverages fermented from grains. Overall, the beers have variable contents of minerals, but the scotch and whiskey have small serving sizes and very low contents of only iron, phosphorus, zinc.

Minerals in beer Comparisons of mineral contents (see Appendix: Grain Composition Tables) for 8 fluid ounce servings of the various beers show differences among them.

Mineral contents highest:

- iron, zinc, copper in light, low calorie beer; and
- potassium, sodium, phosphorus, magnesium in regular beer.

Calcium content was highest and did not differ among light, low calorie beer, low alcohol beer, and regular beer.

Mineral contents lowest:

- potassium, in low alcohol beer;
- iron in regular beer;
- phosphorus in low alcohol beer;
- magnesium in light, low calorie beer; and
- zinc, copper none in low alcohol beer.

Sodium content was lowest and did not differ between light, low calorie beer and low alcohol beer.

#### **Ingredients Used in Grain Products**

This category consists of several ingredients commonly added to various grain-based foods during preparation and processing to add variety in color, flavor, texture, and minerals to the grain-based foods. Some are excellent sources of minerals.

Minerals in ingredients used in grain-based foods Comparisons of mineral contents (see Appendix: Grain Composition Tables) for 30 g servings of these foods show wide differences among them.

Mineral contents highest:

- potassium in low fat peanut flour;
- sodium in dry roasted, salted peanuts;
- calcium, iron in poppy seeds;
- phosphorus, copper in sunflower seeds, dry roasted,
- zinc in sesame seeds, hulled kernels, dried; and
- magnesium in flax seeds.

Mineral contents lowest:

- potassium, phosphorus, iron, zinc, copper, magnesium in arrowroot flour;
- calcium in arrowroot flour, cashews, oil roasted;
- sodium none in raw almonds, raw filberts, low fat peanut flour, and raw pecans.

#### Summary

Grains and grain-based foods are important food sources of minerals. Mineral contents of grains are obtained from minerals in the soil in which they are grown. Minerals have metabolic functions in plants, animals, and humans. The mineral contents of various grains differ from one another. Processing does not destroy minerals, but they can be physically lost from cut surfaces, leached into cooking liquid, or by removing bran and germ that contain most of the minerals in the grain. The addition of ingredients to

produce grain-based foods can increase mineral contents. This article presents comparisons of the mineral composition of selected grains and grainbased foods.

# **Acknowledgments**

The author thanks Nancy Van Heel and Sally Schakel, both from the Nutrition Coordinating Center, Division of Epidemiology, University of Minnesota, for developing the food and nutrient data base in Appendix: Grain Composition Tables of this encyclopedia, and for their helpful suggestions on this manuscript, and Dr. Joan Gordon, retired faculty member, from the Department of Food Science and Nutrition, University of Minnesota, for her helpful suggestions on this manuscript.

See also: Cultural Differences in Processing and Consumption. Fortification of Grain-Based Foods. Grain, Morphology of Internal Structure. Grain and Plants, Morphology. Whole-Grain versus Refined Products.

# **Further Reading**

Barr SI, Murphy SP, and Poos MI (2002) Interpreting and using the dietary references intakes in dietary assessment of individual and groups. Journal of the American Dietetic Association 102(6): 780-788.

Duyfe RL (2002) American Dietetic Association Complete Food and Nutrition Guide, 2nd edn. Hoboken: Wiley.

Grodner M, Anderson SL, and DeYoung S (2000) Foundations and Clinical Applications of Nutrition -A Nursing Approach, 2nd edn. St. Louis: Mosby.

Hegarty V (1995) Nutrition Food and the Environment. St. Paul: Eagan Press.

Hoseney RC (1994) Principles of Cereal Science and Technology, 2nd edn. St. Paul: American Association of Cereal Chemists.

Marquart L, Slavin JL, and Fulcher RG (2002) Whole-Grain Foods in Health and Disease. St. Paul: American Association of Cereal Chemists.

Nestle M (2002) Food Politics. Berkeley: University of California Press.

Schakel S, Buzzard IM, and Gebhardt SE (1997) Procedures for estimating nutrient values for food composition databases. Journal of Food Composition and Analysis 10: 102-114.

Trumbo P, Schlicker S, Yates AA, and Poos M (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fattyacids, cholesterol, protein and amino acids. Journal of the American Dietetic Association 102(11): 1621-1630.

Wardlaw GM (1999) Perspectives in Nutrition, 4th edn. Boston: WCB/McGraw-Hill.

#### Relevant Websites

http://books.nap.edu - Food and Nutrition Board, Institute of Medicine-National Academy of Sciences. dietary reference intakes: recommended intakes for individuals, elements.

http://www.ncc.umn.edu - Nutrition Coordinating Center, Division of Epidemiology, University of Minnesota, Minneapolis, MN.

# Soy-Based Foods

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The soybean (Glycine max) is a native of China, where it has been part of the diet for several thousand years. It has also been important in Japan and Korea for almost as long. More recently, soybeans have become widely used in Western countries to produce a diverse range of foodstuffs. This article describes the use of soy in our food supply, the composition of soybeans, the role of soy in the diet, and its potential health benefits.

#### Use of Soy in Foods

Soybeans are perhaps the most versatile of plants, providing the source for a wide range of ingredients. In the Far East, soybeans are used to produce foods such as "tofu," "miso," "tempeh," and soy sauce. In Western countries, soybeans have become widely used in recent years in the production of a range of very diverse ingredients, e.g., soy flour, soy protein, soy oil, soy lecithin, and mono- and diglycerides of fatty acids. These ingredients are widely used in foods. For example, soy flour and soy protein are used in foods such as breads, pizza bases, other cereal products, and some meat products. Soy protein is often used to make vegetarian alternatives to meat products, e.g., textured vegetable protein (TVP) in vegetarian burgers, vegetarian sausages, etc. Soy oil is used as a vegetable oil both in domestic cooking and by the food industry. Soy oil is also used in the manufacture of spreadable fats. Soy lecithin and mono- and diglycerides of fatty acids are used as emulsifiers in many foods, e.g., breads, fat spreads, and ice cream. Vegan alternatives to cow's milk, cheese, and yogurt are produced from soy. Soy is also used to produce infant formulas for those infants who are intolerant to lactose or cows' milk protein.

#### Composition

Soybeans contain a wide range of nutrients. They contain some "antinutrients" and also other components, e.g., isoflavones, which may have beneficial effects on health.

#### **Nutrients**

Soybeans contain protein, carbohydrate, fat, vitamins, minerals, and fiber.

**Protein** Soybeans are a good source of protein, containing 14 g per 100 g. This is more than the protein content in most other vegetables and in most other legumes (typically 5-9 g per 100 g), the exception being peanuts which contain 26g of protein per 100 g. In terms of protein quality, soy contains substantial amounts of most essential amino acids. When compared with a reference protein (egg), soy protein, like other legumes, is deficient in sulfur-containing amino acids (the essential amino acid methionine and the nonessential amino acid cystine) and has a higher content of lysine. Combining legumes with cereals provides a meal with a high protein quality, as cereals have adequate amounts of sulfur-containing amino acids but are deficient in lysine.

Carbohydrate Soybeans contain 5 g of carbohydrate per 100 g. This is lower than that of many other legumes. Of the total carbohydrate content present, 37% is starch, 41% sugars, and 22% oligosaccharides. The sugars present are sucrose, fructose, and glucose.

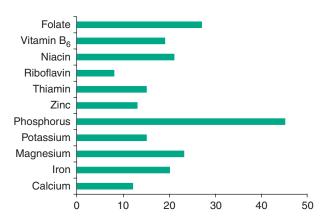
Fat Soybeans contain 7g of fat per 100g. This is more than the fat content in other legumes, most of which are low in fat. The exception to this is peanuts, which contain 46 g of fat per 100 g. The fatty acid composition of soy has a high ratio of unsaturates to saturates. Of the fatty acids, 16% are saturates, 24% are monounsaturates, and 60% are polyunsaturates. The polyunsaturated fatty acids present are the essential fatty acids – linoleic acid (18:2n-6)and  $\alpha$ -linolenic acid (18:3n-3).

Minerals Soybeans contain significant amounts of several minerals: calcium, iron, magnesium, potassium, phosphorus, and zinc (see Table 1). One hundred grams of soybeans provides  $\sim 12\%$  of the daily reference nutrient intake (RNI) (UK) for calcium, 20% for iron, 23% for magnesium, 15% for potassium, 45% for phosphorus, and 13% for zinc (see Figure 1).

Table 1 Vitamin and mineral composition of soybeans

	Amount per 100 g as consumed
Sodium	1 mg
Potassium	510 mg
Calcium	83 mg
Magnesium	63 mg
Phosphorus	250 mg
Iron	3.0 mg
Copper	0.32 mg
Zinc	0.9 mg
Manganese	0.7 mg
Selenium	5 μg
lodine	2 μg
Vitamin A (retinol equivalents)	1 μg
Vitamin D	0 μg
Vitamin E	1.13 mg
Thiamin	0.12 mg
Riboflavin	0.09 mg
Niacin equivalents	2.7 mg
Vitamin B <sub>6</sub>	0.23 mg
Vitamin B <sub>12</sub>	0 µg
Folate	54 µg
Pantothenate	0.18 mg
Biotin	25.0 μg
Vitamin C	Trace

Data from Royal Society of Chemistry and Ministry of Agriculture Fisheries and Food (1991) *McCance and Widdowson's The Composition of Foods*, 5th edn. Cambridge, UK: Royal Society of Chemistry. Crown copyright is reproduced with the permission of the Controller of Her Majesty's Stationery Office.



**Figure 1** Vitamin and mineral composition of soybeans — in relation to reference nutrient intakes (% of reference nutrient intake (RNI) provided by 100 g). (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 5393, Elsevier Ltd.)

Vitamins Soybeans contain a number of water-soluble vitamins: thiamin, riboflavin, niacin, vitamin  $B_6$ , and folate (see Table 1). One hundred grams of soybeans provides ~15% of the daily RNI (UK) for thiamin, 8% for riboflavin, 21% for niacin, 19% for vitamin  $B_6$ , and 27% for folate (see Figure 1). Soybeans also contain vitamin E: 1 mg per 100 g.

Fiber Soybeans are a valuable source of fiber in the diet: 6 g of nonstarch polysaccharides (NSP) per 100 g. Of this, 44% is soluble fiber and 56% insoluble fiber. One hundred grams of soybeans provides 33% of the daily "dietary reference value" (UK) for fiber.

#### **Antinutrients**

Raw soybeans contain a number of substances which may have detrimental effects on digestion and other metabolic processes (lectins, goitrogens, and digestive enzyme inhibitors). These substances are, however, inactivated by appropriate cooking of the beans or, in the case of goitrogens, counteracted by adequate iodine intake.

Lectins Lectins, also known as haemagglutinins, are present in raw soybeans and in other legumes. They are, however, heat labile and are inactivated when the beans are properly cooked. In experimental animals these polymeric proteins have been shown to cause damage to red blood cells and intestinal mucosa, and thereby impaired nutrient utilization and loss of body weight. In humans, these compounds can result in nausea, vomiting, diarrhoea, and abdominal pain.

Goitrogens Enlargement of the thyroid gland has been shown to occur in rats fed soybean meal. The goitrogenic agent in soybeans is unknown. However, the effect is counteracted by adequate iodine intake.

Digestive enzyme inhibitors Raw soybeans contain certain proteins (protease inhibitors) which react with digestive enzymes (trypsin, chymotrypsin or salivary, and pancreatic α-amylase), thereby interfering with the digestion of protein and starch. In humans, raw soy or isolated protease inhibitors increase levels of cholecystokinin (CCK) and pancreatic secretion. It is thought that chronic pancreatic stimulation may lead to pancreatic hypertrophy, hyperplasia, and possibly to cancer. In rats, raw soy has been shown to increase risk of pancreatic cancer. However, protease inhibitors present in raw soy are inactivated by heat and are therefore not a problem in cooked beans. For example, in countries such as Japan where soy foods are widely consumed, the incidence of pancreatic cancer is similar to or less than that for Western countries where soy forms a relatively small component of the total diet.

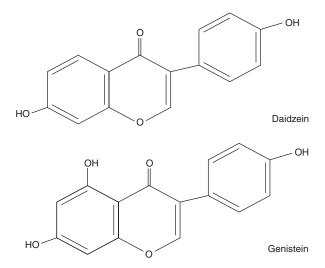
Phytic acid Phytic acid is present in soybeans and also in other fiber-containing foods. It has been suggested to reduce the absorption of calcium, iron, zinc, and vitamin D from the diet. However, these effects have been observed in *in vitro* experiments and not in *vivo* studies, even at fiber intakes at

the upper limit of the normal human consumption range. In addition, levels of micronutrients tend to be higher in fiber-rich foods than in fiber-poor foods. Thus, adverse effects of phytic acid on nutrient absorption are likely to be unimportant at the levels of fiber normally consumed in the human diet.

#### Isoflavones

Soybeans contain isoflavones, a group of compounds that are structurally similar to oestradiol (phyto-oestrogens). Soybeans contain higher amounts of the isoflavones – genistein and daidzein (see Figure 2) and smaller amounts of glycitein, primarily in the form of glycosides: genistin, daidzin, and glycitin (total isoflavone content of 180 mg per 100 g). The glycosides are hydrolyzed in the gut by a bacterial enzyme, glucose being removed to produce the aglycone forms (genistein, daidzein, and glycitein). Daidzein and genistein have been shown to have weak oestrogenic activity and are able to bind with low affinity to oestrogen receptors, the affinity being greater for  $\beta$ -receptors than for  $\alpha$ -receptors. Compared with  $17\beta$ -oestradiol (the main oestrogen produced by the body), daidzein and genistein have been reported to have much less potency in producing oestrogenic effects, by about 1000-fold or more.

In the 1940s, it was reported that phyto-oestrogens may exert adverse effects on uterine and ovarian function. This was because sheep grazing on pastures, containing a particular type of clover (*Trifolium* sp.) rich in formononetin, which is converted to daidzein in the rumen, developed a widespread infertility. An infertility syndrome has also been described in



**Figure 2** Chemical structure of the main isoflavones present in soy — daidzein and genistein. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 5394, Elsevier Ltd.)

captive cheetah, as a result of soybean use, the syndrome being reversed by removal of soy from the food. No adverse effects of soybeans on human fertility have been reported.

More recently, it has been recognized that isoflavones may act either as weak oestrogens or as antioestrogens, competing for oestradiol at the receptor
complex, yet failing to stimulate a full oestrogenic
response after binding to the nucleus. This fact has
stimulated much research into whether isoflavones
may have a protective role in hormone-related diseases such as breast cancer.

# Role of Soy in the Diet

Soy has been a staple in the diet of many Far Eastern countries for centuries, e.g., foods such as tofu, tempeh, and miso. These traditional soy foods are not very widely consumed in Western countries, but are sometimes used as an alternative to meat. As soybeans are extremely versatile, as described earlier, consumption of foods containing soy has increased in Western countries over the last 50 years. Nevertheless, the amount of soy consumed in Western countries such as the USA and UK is much less than in countries such as Japan and China. The Food and Agriculture Organisation (FAO) food balance sheets provide data for the amounts of various foods available for human consumption in different countries. These show that the amount of soybeans available for use in foods for humans is 8.5 kg per person per year in China and 7.9 kg per person per year in Japan. This compares with less than 1 kg per person per year in countries such as the USA and UK. In fact, of the soybeans grown worldwide, only ~10% of the total is used in foods for humans, the majority is used as feed for animals.

Soybeans can be an important component of a normal healthy diet as they are low in saturates, provide a source of fiber, and contain a wide range of other nutrients. Soybeans are also the main source of phyto-oestrogens in the diet. In addition, soybeans may have a number of potential health benefits and these are discussed in the following sections.

For vegetarians, and particularly for vegans who eat no animal products, soybeans play a very important role in the diet. They provide a valuable source of protein that can be of high quality if soybeans are combined with cereals, since the amino acid compositions are complementary. They also provide an important source of iron. Low iron intake can lead to iron-deficiency anemia. Ensuring adequate iron status can be difficult for vegetarians as, in general, iron from vegetable sources is less well absorbed than that from animal sources. However, soybeans provide

a source of iron that can be absorbed as well as that in meat.

For infants intolerant to cow's milk protein or to lactose, soy-based infant formulas can be an invaluable alternative to cow's milk-based formulas. For older children and adults who are allergic to cow's milk or intolerant to lactose, drinks and other foods made from soy can be very important components of the diet, helping to ensure that nutrient intakes are adequate for the maintenance of health.

In common with other legumes, soybeans are also of agronomic importance, as they increase the nitrogen content of the soil. Although green plants cannot utilize nitrogen in the atmosphere, there are several species of bacteria, fungi, and blue-green algae that are able to transform nitrogen in the air into a form that can be used by plants. An important genus of nitrogen-fixing bacteria is *Rhizobium*, which forms nodules in the roots of legumes. These bacteria live symbiotically with the legumes, the bacteria obtaining food from the green plant and the legumes obtaining abundant usable nitrogen compounds from the bacteria.

# **Soy and Coronary Heart Disease**

Coronary heart disease (CHD) is a major cause of death in many countries. For example, in the UK, CHD accounts for  $\sim$ 30% of male deaths and 23% of female deaths.

The level of cholesterol in the blood is a major risk factor for CHD. Soy protein substituted for animal protein in the diet results in reductions in total plasma cholesterol (by 9% on average), low-density

lipoprotein cholesterol (LDL-C) (by 13% on average), and triacylglycerols (by 11% on average). High-density lipoprotein cholesterol (HDL-C) is unchanged or may be slightly increased. The amount of reduction in blood lipids tends to be greater among those with the highest plasma cholesterol levels at baseline.

A great deal of research has been done to identify the component(s) of soy protein that is responsible for its effect on blood lipids. Studies using a mixture of amino acids that duplicate the amino acid profile of soy protein have found that this does not have the same effect on blood lipids as the intact protein. However, studies of soy protein with isoflavones intact have shown that this has a lipid lowering effect, whereas soy protein with isoflavones removed has no significant effect. It has therefore been suggested that the isoflavones present in soy protein are largely responsible for the lipid lowering effect of soy protein. The benefits of isoflavones present in soy protein on cholesterol lowering have been suggested to be mediated through upregulation of LDL-receptor activity. Nevertheless, studies of isolated and purified isoflavones have failed to show that these have a lipid lowering effect.

Soy may reduce risk of CHD through several mechanisms in addition to its ability to lower blood lipids (see Figure 3). For example, soy isoflavones are known to act as antioxidants and have been suggested to reduce oxidative damage to LDL-C. A decrease in oxidized LDL particles, which are considered atherogenic, may reduce the risk of atherosclerosis.

A study of young cynomolgus monkeys showed that the size of atherosclerotic lesions was 70% less in those fed on a diet containing soy protein with

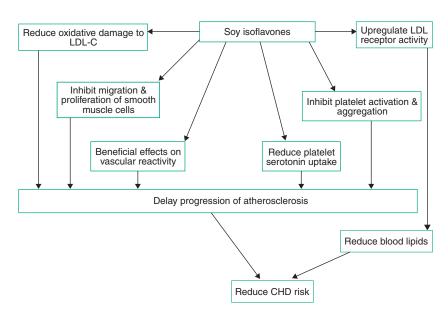


Figure 3 Mechanisms through which soy could reduce risk of coronary heart disease (CHD).

isoflavones present compared with those fed on a diet containing soy protein with isoflavones removed. This suggests that isoflavones may inhibit atherogenesis. In another study of surgically postmenopausal cynomolgus monkeys, soy protein with isoflavones intact was found to reduce the progression of atherosclerosis. The magnitude of the effect was reported to be comparable to that of postmenopausal oestrogen therapy.

Soybean isoflavones, in particular genistein, impact beneficially on vascular reactivity. Genistein has also been shown, *in vitro*, to have a number of other effects whereby the development of atherogenesis may be delayed: inhibition of the migration and proliferation of smooth muscle cells; inhibition of platelet activation and aggregation; and reduction in platelet serotonin uptake.

In 1999, in response to the available evidence on the benefits of soy protein, the USA Food and Drug Administration (FDA) announced that it would allow food manufacturers to label products containing 6.25 g of soy protein per serving as helping to reduce risk of heart disease, as part of a balanced diet low in fat and saturates.

# Soy and Cancer

Cancer is a major cause of morbidity and mortality. For example, in the UK about one in three people will develop cancer at some time during their life and cancer accounts for about one in four of all deaths. There are marked differences in death rates from several types of cancer between Asian countries and many Western countries. The differences are more striking for hormone-dependent cancers of the prostate, breast, and colon/rectum. For example, the risk of dying from prostate cancer among Japanese men is only one-fifth that of men in the USA and Japanese women have a breast cancer mortality rate that is only a quarter of that of women in the USA.

Countries where mortality from prostate and breast cancers is low have considerably higher intakes of soy than those countries where mortality from these cancers is high. In animal models, studies investigating the effects of soybeans on prostate cancer or breast cancer have shown reduced tumorigenesis. In humans however, evidence is inconclusive. Epidemiological studies of prostate cancer conducted in Japan and in Japanese migrants to Hawaii have shown no significant effect of soy consumption. However, a recent study in the USA reported that men who drank one cup of soy milk per day had a risk of developing prostate cancer that was 70% lower than that of controls. For breast cancer, although some studies have shown that soy can help prevent breast cancer, others have found no significant effect. An increase in menstrual cycle length has been observed in some studies, in response to soybean consumption, although no change was observed in others. Such an effect is of potential relevance to the hypothesis that soy may be protective against breast cancer, because some data indicate that longer cycles, which are typical of Asian women, are associated with a reduced risk of breast cancer.

For colo-rectal cancer data are also inconclusive: some studies showed no significant effect, some reported a protective effect of soy, and others observed an increased risk with soy consumption. For cancers at other sites, data tend to show that increased soybean consumption may be protective against lung and stomach cancer. Much more research is needed to confirm whether soy has a protective effect against cancer in humans.

Isoflavones appear to be the components of soy that would be most likely to account for any protective action of soy against cancer, especially in inhibiting the initiation stage of carcinogenesis (see Figure 4). Genistein has been shown to suppress the growth of a wide range of cancer cells. It inhibits certain enzymes that could affect the onset of cancer or the growth of tumors: tyrosine protein kinase, mitogenactivated protein (MAP) kinase, and ribosomal S6 kinase. Genistein also inhibits the DNA repair

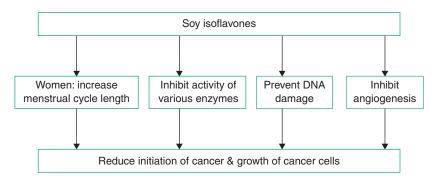


Figure 4 Mechanisms through which soy could reduce risk of cancer.

enzyme topoisomerase II and acts as an antioxidant, thus potentially preventing oxidative DNA damage. It increases *in vitro* concentrations of transforming growth factor  $\beta$ , which is thought to inhibit the growth of cancer cells. In addition, genistein has also been shown to inhibit angiogenesis, the formation of new blood vessels, an abnormal event that occurs as part of the growth and expansion of malignant tumors. However, many of these effects have been shown with very high concentrations of genistein and not in cells treated with levels likely to be achieved in plasma of human subjects eating soy foods.

# Soy and Bone Health

Osteoporosis is a clinical condition in which there is a reduced amount of bone per unit volume and an increased susceptibility to fractures, particularly fractures of the vertebrae, distal forearm (Colles fracture) and hip. Of these, hip fracture is the most severe, since patients require a prolonged hospital stay, there is a high mortality rate (about 20% within six months of the fracture) and of those who survive may suffer permanent disability and dependency. Osteoporosis is a major health problem in many Western countries, although not in Asian countries. A number of factors are known to increase risk of osteoporosis, including insufficient dietary calcium, low physical activity, and lack of oestrogen.

In women, bone mass reduces at a rapid rate in the first few years after the menopause, due to the reduction in oestrogen. Hormone replacement therapy (HRT) is well known to reduce bone loss in postmenopausal women. However, HRT is not appropriate for all women. Since soy may have oestrogenic effects, it has been hypothesized that it may provide an alternative to HRT.

Soy protein containing isoflavones has been reported to reduce bone loss due to oestrogen deficiency in ovariectomized rats. Soybean isoflavones have also shown a protective effect on bone loss in ovariectomized rats, suggesting that the beneficial effect of soy protein is due to isoflavones. The effect of isoflavones on bone loss in rats has been reported to be similar to that for oestrogen. The bone sparing effect of soy protein isoflavones has been suggested to be due to a reduction in bone resorption and/or to an increased osteoblast activity (increasing bone formation).

In humans, most but not all studies indicate that soy protein containing isoflavones favorably affects bone turnover and bone mineral density in the lumbar spine of perimenopausal and postmenopausal women. Treatment with ipriflavone, a synthetic isoflavone, has also been reported to have bone conserving effects

in postmenopausal women with low bone mass, confirming that the beneficial effect of soy protein is likely to be due to isoflavones.

In addition to the effect of soy protein on bone mineral density, it has also been suggested that soy protein, when substituted for animal protein, may indirectly enhance bone strength. Another effect of soy protein is that it helps to conserve calcium by reducing urinary calcium excretion. This is due to the lower sulfur amino acid content of soy protein.

Soy protein therefore appears to have modest beneficial effects on bone density. However, studies to date have been short term and have involved only small numbers of subjects. In addition, no study has investigated whether soy protein containing isoflavones has an effect on fracture risk.

# Soy and Menopausal Symptoms

Hot flushes (also known as hot flashes) are a common symptom among menopausal women in Western societies, but are reported to be much less common in Japan. Whether this difference is due to the higher consumption of soy in Japan is not clear. Hormone replacement therapy (HRT) generally alleviates hot flushes and other menopausal symptoms such as vaginitis. There is much research interest in the possibility that soy may provide an alternative to HRT in this regard.

Evidence of benefit of soy in menopausal women is conflicting. Some researchers have reported an improvement in the frequency of hot flushes in women taking soy protein daily. Others have reported no effect of soy protein on the number of hot flushes experienced but found a reduction in the severity of symptoms. Others have reported a reduction in both the incidence and severity of hot flushes with soy protein or an isoflavone extract compared with a control group. Others have reported no difference in either the frequency or severity of hot flushes in the intervention group compared with the control group.

Two studies have investigated the effects of phytooestrogen supplements on vaginal cytology and found an increase in cell proliferation (an indication of oestrogenic activity) and reversal of menopausal atrophy. Others have reported no significant effect.

There are difficulties in interpreting the results of these studies. This is because there are differences in the amounts of soy protein and isoflavones used and differences in the duration of the studies. Interpretation is also complicated by the fact that reported frequency and severity of symptoms tends to reduce in the control group as well as the intervention group. Thus, much more research needs to be done

before soy protein can be proposed as a potential alternative to HRT for the control of menopausal symptoms.

See also: Beans. Genetically Modified Grains and the Consumer. Nutraceuticals from Grains. Nutrition: Guidelines for Grain-Based Foods; Effects of Food Processing. Pulses, Overview. Soybean: Agronomy; Processing; Soy Concentrates and Isolates; Soy-Based Fermented Foods; Soymilk, Tofu, and Okara.

# **Further Reading**

Anderson JJB, Anthony M, Messina M, and Garner SC (1999) Effects of phyto-oestrogens on tissues. *Nutrition Research Reviews* 12: 75–116.

Anderson JW, Johnstone BM, and Cook-Newell ME (1995) Meta-analysis of the effects of soy protein intake on serum lipids. New England Journal of Medicine 333: 276–282.

Bingham SA, Atkinson C, Liggins J, Bluck L, and Coward A (1998) Phyto-oestrogens: where are we now? *British Journal of Nutrition* 79: 393–406.

Lissin LW and Cooke JP (2000) Phytoestrogens and cardiovascular health. *Journal of American College of Cardiology* 35: 1403–1410.

Messina MJ (1999) Legumes and soybeans: overview of their nutritional profiles and health effects. *American Journal of Clinical Nutrition* 70(suppl.): 439S–450S.

Messina M and Messina V (2000) Soyfoods, soybean isoflavones and bone health: a brief overview. *Journal of Renal Nutrition* 10: 63–68.

Messina M, Persky V, Setchell KDR, and Barnes S (1994) Soy intake and cancer risk: a review of the *in vitro* and *in vivo* data. *Nutrition and Cancer* 21: 113–131.

Potter SM (2000) Soy – new health benefits associated with an ancient food. *Nutrition Today* 35: 53–60.

Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food (1991). *McCance and Widdowson's The Composition of Foods*, 5th edn. Cambridge, UK: Royal Society of Chemistry.

#### **Relevant Websites**

http://www.nutrition.org.uk - British Nutrition Foundation.

http://www.food.gov.uk - Food Standards Agriculture Organisation.

http://apps.fao.org – Food and Agriculture Organisation (FAO).

http://www.ajcn.org - American Journal of Clinical Nutrition.

# **Vitamin Composition**

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#### Introduction

When grains and grain-based foods are used for humans, they provide essential vitamins and other nutrients for health and wellness. Worldwide, grains are a low-cost staple food in people's food intakes in most cultural groups. Kinds of grains include wheat, corn, rice, oats, barley, rye, and others listed in **Appendix**: Grain Composition Tables. The staple grain used in a culture is determined by the available type, which in turn depends on growing conditions, and, therefore, differs from place to place. Examples of staple grains include: wheat in North America, some parts of Europe, and Australia; corn in Mexico, Central and South America; rice in Asian countries; and rye in northern European countries.

Vitamins are grain components that are synthesized and used by the plants during growth, stored in grain kernels during ripening, and needed during kernel germination for development of new plants. The discovery of the human need for vitamins occurred in Asia in the 1890s when the deficiency disease beriberi caused by eating the staple, polished white rice, was cured and prevented by eating brown rice. Later, the B-vitamin (thiamin) in the outer bran coating of rice kernels was identified as the vitamin that prevented beriberi.

The discovery of specific vitamins began in the early twentieth century. Grains and grain-based foods were identified as important natural sources of six B-vitamins. Research continues today on vitamin synthesis, functions, and storage mechanisms in maturing grain kernels. Study of vitamins includes their functions as biologically active compounds in the human body where they prevent deficiency diseases and decrease risk for chronic diseases. Early dietary studies on vitamins emphasized determining optimal amounts to prevent deficiency diseases and identifying toxic effects of excessive consumption.

As quantitative information about vitamins, minerals, and other nutrients in food became available with improved accuracy of analytical methods and instruments, food composition tables have been developed to present the data. Such a table for vitamins, minerals, and other nutrients and food components in selected grains and grain-based foods are presented in **Appendix**: Grain Composition Tables. Examples of vitamin composition of grains and grain-based foods

have been incorporated from the data provided in **Appendix**: Grain Composition Tables.

#### **Characteristics of Vitamins**

Vitamins are "essential organic molecules needed in very small amounts for cellular metabolism." Each vitamin has one or more specific metabolic effects in plants and humans. Of the vitamins in cereals, all are essential for humans except niacin, i.e., they must be provided by food because they cannot be synthesized by the body. Niacin, a B-vitamin, is not classified as essential, because the body can synthesize some of its niacin needs from tryptophan, an amino acid derived from protein. No foods or group of foods provides all essential vitamins, therefore food intakes must include a variety of foods to satisfy human needs for vitamins.

Vitamins are identified by their formal biochemical name, a letter, or a combination of a letter and a number. The names and forms of six B-vitamins

and vitamin A found in grains and grain-based foods are listed in Table 1.

Vitamins are classified according to their solubility in water or fat (Table 1). These characteristics affect their processing losses and retention in the body after being eaten. Water-soluble vitamins are not stored in the body. When water is used in processing, preparation, and/or cooking, these vitamins are dissolved out of cut surfaces and leached from other plant tissue. Fat-soluble vitamins are stored in the body if intake exceeds daily needs. Toxicity seldom occurs from food intakes, but is possible if large supplemental doses are routinely ingested.

Stability of vitamins to heat, light, and air affects losses from foods during processing and/or cooking (Table 1). B-vitamins except niacin in grain-based foods are affected by applying heat, e.g., cooking in hot liquid, baking, and extrusion cooking. Riboflavin, pyridoxine (B<sub>6</sub>), folate, and vitamin A losses occur when foods are exposed to light. Thiamin, folate, and vitamin A levels are reduced by exposure of food to air. When food-processing losses from heat,

**Table 1** Characteristics of vitamins in grains and grain-based foods<sup>a</sup>

Vitamin name	Major metabolic functions	Solubility	Stable to			US recommended dietary	
			Heat	Light	Air	allowances (RDA) <sup>b</sup> or adequate intake (AI) <sup>c</sup>	
						Men	Women
Thiamin (B <sub>1</sub> )	Energy release Neuromuscular function	Water	No	Yes	No	1.2 mg <sup>b,d</sup>	1.1 mg
Riboflavin (B <sub>2</sub> )	Energy release Healthy skin, mouth Healthy tongue	Water	No	No	Yes	1.3 mg <sup>b,d</sup>	1.1 mg
Niacin (B <sub>3</sub> ) Nicotinic acid Niacinamide	Energy release Neuromuscular function Maintain skin	Water	Yes	Yes	Yes	16 mg <sup>b,d</sup>	14 mg
Pyridoxine (B <sub>6</sub> ) Pyridoxamine Pyridoxal	Energy release Blood formation Neuromuscular function Maintain skin	Water	No	No	Yes	1.3 mg <sup><i>b</i>,<i>d</i></sup>	1.3 mg
Folate, folic acid Folacin Pteroyl-glutamic acid	Blood formation Prevent neural tube defects DNA synthesis	Water	No	No	No	400 μg <sup>b,e</sup>	400 μg
Pantothenic acid (B <sub>5</sub> )	Energy release Neuromuscular function Fat synthesis	Water	No	Yes	Yes	5.0 mg Al <sup>c,d</sup>	5.0 mg Al
Vitamin A Precursor: carotenoids preformed retinol	Eye function, vision Antioxidant	Fat	No	No	No	900 RE <sup>f</sup>	700 RE

<sup>&</sup>lt;sup>a</sup> Adapted from Grodner M, Anderson SL, and DeYoung S (2000) Foundations and Clinical Applications of Nutrition – A Nursing Approach, 2nd edn. St. Louis: Mosby.

<sup>&</sup>lt;sup>b</sup>Recommended dietary allowances.

<sup>&</sup>lt;sup>c</sup>Al = adequate intake.

 $<sup>^{</sup>d}$ mg = milligram.

 $<sup>^{</sup>e}\mu g = microgram.$ 

<sup>&</sup>lt;sup>f</sup>RE = retinol equivalent.

light, and air are significant, foods may be enriched or fortified to increase the vitamins that are lost.

Vitamins in grain-based foods, Table 1, have an important role in major metabolic functions. Prevention of deficiency diseases is an important resulting health benefit. These diseases include beriberi linked to thiamin deficiency, pellagra caused by niacin deficiency, xerophthalmia – night blindness and keratomalacia leading to complete blindness resulting from vitamin A deficiency. Neural tube birth defects, i.e., spina bifida, the most common birth defect in the US and other countries, and possibly oral clefts and congenital heart defects, have been linked to folic acid deficiency based on research in recent years. More research on folic acid benefits is needed. Overall, vitamin deficiencies are less prevalent now, but can be found in populations where food is scarce, or illnesses, or addictions cause poor diets. Nutrients added to the diet from increased use of cereals and cereal-based foods can help reduce these deficiencies.

Recommended intakes in the US for six B-vitamins and vitamin A for adults are very small. Concentrations of these vitamins in food are also very small, but it is not difficult to meet daily needs by eating a variety of foods. The recommended intakes for individuals, vitamins in the US, Table 1, are expressed as recommended dietary allowances (RDAs) based on new US dietary reference intakes (DRIs). The recommended intake for pantothenic acid is expressed as adequate intake (AI), which indicates the amount covers individual needs, but lack of data prevents setting an RDA level. Recommendations for vitamin A are given in retinol equivalents (RE). Units of measurement for grain-based vitamins are footnoted in Table 1, in Appendix: Grain Composition Tables, and listed in Appendix: Units of Grain Science.

# Grain Kernel Structure and Location of Vitamins

Grain kernels have three major components: bran, germ, and endosperm (*see* Grain, Morphology of Internal Structure. Grain and Plants, Morphology). Kernels are covered by several layers of bran. The aleurone layer of bran is next to the endosperm. The largest portion of the kernel is the starchy endosperm. The germ or embryo is the source of the new plant in a germinating kernel. All grains are similar in the types of tissues they contain and in the molecular and cellular organization of their tissues.

Vitamins and other biologically active constituents of grains are synthesized and stored in specific structural locations in the kernel, where they activate a variety of reactions and interactions. The extractability of these constituents from storage within grains varies.

Bran and germ aleurone cells contain most of the important nutrients and other bioactive components in the grain, and are where metabolic activities take place. These cells contain the maximum amount of thiamin, riboflavin, niacin, and pantothenic acid in the kernel. Niacin in the aleurone cells is complexed with protein or carbohydrate making these vitamin deposits difficult to separate from the cells during processing and digestion. It is not known whether thiamin and riboflavin in aleurone cells occur in the niacin complexes. Variable amounts of B-vitamins also are stored in the germ, from which a new plant originates, and in some parts of the starchy endosperm.

# **Grain-Processing Effects on Vitamins**

Grains are unique because they are seldom eaten in the raw state. For optimum benefits from their constituents, grains are processed to produce ingredients for nutritious and palatable grain-based foods. Commercial processing disrupts the organization of the kernels to release vitamins and other constituents from storage in the aleurone cells and elsewhere, and to make the endosperm starch available for digestion. Effects of processing differ for each process and grain combination. Processing techniques include grinding or crushing the entire kernel of the grains into whole grain flours or meals that have minimal vitamin losses, or milling to separate bran, germ, and endosperm of the kernel to obtain refined white flour with significant vitamin losses. Different heating techniques used during processing affect vitamin losses, and the addition of other ingredients for specific products can contribute to the vitamin content and also provide a wide variety of grain-based foods for the food marketplace.

When significant losses occur during processing, nutrient levels in grain-based foods are increased by enrichment or fortification. Products commonly enriched in the US include refined flours, breads, mixes, and other baked foods. "Enriched" indicates that selected nutrients present in grain-based foods before processing or refining are added back to replace nutrient losses resulting from processing. Nutrients added in the US through enrichment are thiamin, riboflavin, niacin, folate, and iron. Enrichment and fortification standards vary among different countries. "Fortified" indicates that specified nutrients are added to grain-based foods that may or may not have been present in the grain before processing. Vitamin A, for example, is added to fortify

grain-based foods that originally contain little or no vitamin A, e.g., ready-to-eat breakfast cereals, making them important grain food sources of vitamin A (see Appendix: Grain Composition Tables). Vitamin A content of grain-based foods is also increased without fortification, when ingredients high in vitamin A or its precursors are added, such as pumpkin or carrots.

Beginning in 1998, the US Food and Drug Administration required manufacturers of cereal grain products (breads, flours, corn meals, rice, pastas, breakfast cereals, and other grain products) to enrich/fortify them with 140 µg per 100 g of folic acid, the most bioavailable form of the vitamin. These enriched/fortified products are permitted to claim on the label that adequate intake of folic acid may reduce the risk of neural tube-birth defects. Most of these cereals and cereal products already contain some naturally occurring folate, the less bioavailable form of the vitamin that must be digested to yield folic acid. Also, some imported foods, may not meet the US enrichment/fortification standards for folic acid or folate content, or may not be enriched/fortified at all.

# Vitamin Composition of Grain-Based Foods

Names/descriptions of selected whole grains and grain-based foods are classified into product categories in Appendix: Grain Composition Tables. The table gives serving sizes and data for vitamins, minerals, and other nutrients and food components. Table data are derived from the food and nutrient database developed and maintained by the Nutrition Coordinating Center, Division of Epidemiology, University of Minnesota, Minneapolis, MN, USA. Food product categories included in the table are listed below. Some categories have examples of many different foods, while others have only a few similar foods.

- grains, flours, and cooked cereals;
- pasta and rice;
- ready-to-eat cereal;
- baby food cereals;
- breads and other related products;
- crackers;
- cookies:
- cakes, pastries, and other desserts;
- granola and cereal bars;
- snacks and chips;
- legumes;
- meat substitutes;
- alcoholic beverages; and
- ingredients used in grain products.

Vitamin composition comparisons for foods selected from **Appendix**: Grain Composition Tables are given in this article as examples of how to use and understand the data in the table. The table also gives food names and data for weight of a serving in grams and vitamin contents used in comparisons.

Information in **Appendix**: Grain Composition Tables includes:

- abbreviations explained in table footnotes;
- food names alphabetized in each category;
- contents of vitamins and other nutrients and food components given in amount per serving of the food;
- serving sizes specified in common US household units and weight in grams (see Appendix: Units of Grain Science for measurement abbreviations and equivalents);
- amount of each vitamin per serving of each food, calculated based on its serving size in grams; and
- nutrients added through commercial enrichment or fortification in bold italics.

# Vitamin Content Comparisons

Readers can use the following directions to make their own vitamin content comparisons for selected foods using the serving weight and vitamin data in Appendix: Grain Composition Tables. To compare and classify foods on the basis of their vitamin contents, select foods and then use contents of each vitamin in each food (based on the same serving weight for each food). For a valid comparison, the serving weight must be the same for each food. Foods within a category and from different categories can be included in a comparison as long as the weight of a serving for each food is the same.

Information is presented in Table 2, which gives the highest amount of each vitamin that can be expected on the basis of how it is consumed, e.g., cooked, in an unenriched and enriched/fortified grain food, and in another food source highest in a given vitamin. This table enables readers to compare vitamin contents of grain foods relative to the values for other grains and for nongrain food sources.

#### Grains, Flours, and Cooked Cereals

This category includes selected unprocessed whole grains, and processed whole and refined grain-based foods, in uncooked and cooked forms. Unprocessed whole grains and some processed grains are rarely eaten uncooked, but are sometimes eaten cooked. Processed cereals in granulated and other forms are usually eaten cooked. Different types of

**Table 2** Highest vitamin contents per serving for foods, as eaten basis<sup>a</sup>

Vitamin name	Unenriched grain foods	Enriched/fortified grain foods	Other food sources of the vitamin
Thiamin (B <sub>1</sub> )	0.281 mg <sup>b</sup>	0.527 mg	0.54 mg
	Oatmeal, regular, cooked	Rice flakes, ready-to-eat cereal	Pork chop, fresh, loin, lean, cooked
Riboflavin (B <sub>2</sub> )	0.154 mg <sup>b</sup>	0.589 mg	0.40 mg
	Quinoa, cooked	Rice flakes, ready-to-eat cereal	Milk, fluid, 2% fat
Niacin (B <sub>3</sub> )	3.340 mg <sup>b</sup>	7.130 mg	4.55 mg
	Cracked wheat, cooked	Rice flakes, ready-to-eat cereal	Pork chop, fresh, loin, lean, cooked
Pyridoxine (B <sub>6</sub> )	0.515 mg <sup>b</sup>	2.030 mg	0.34 mg
	Oatmeal, instant cooking, flavored or plain, cooked	Bran flakes without raisins, ready-to-eat cereal	Pork chop, fresh, loin, lean, cooked
Folate	38 μg <sup>c</sup>	404 μg	179 μg
	Millet, cooked	Bran nuggets, unsweetened, ready-to-eat cereal	Lentils, cooked
			97 μg
			Spinach, cooked
			44 μg
			Broccoli, cooked
Pantothenic acid (B <sub>5</sub> )	$0.920{ m mg}^b$	Foods are not usually enriched or	Many foods contain this vitamin
	Corn flakes, unsweetened, ready-to-eat cereal	fortified with this vitamin	
Vitamin A	8 RE <sup>d</sup>	381 RE	1900 RE
	Cornmeal, cooked	Oatmeal, instant cooking, flavored or plain, cooked	Sweet potato, boiled, mashed 1596 RE
			Carrots, boiled, drained

<sup>&</sup>lt;sup>a</sup> Values from **Appendix**: Composition Tables for unenriched and enriched/fortified foods, and from Version 4 of the Nutrition Coordinating Center Nutrient Data Base for other food sources of the vitamins.

grains and flours are combined with other foods as ingredients in cereal-based foods that are eaten after being cooked by baking, extrusion, or other methods of applying heat. In these foods, grains are the base ingredient and other ingredients add nutrients, flavor, and color, and/or are needed for structure of the food.

The following first comparison of vitamin contents in unprocessed whole grains shows how the food and nutrient data in **Appendix**: Grain Composition Tables can be used to obtain the amounts of vitamins in these products. The range of vitamin contents among the grains (shown numerically as an example of the data for each vitamin and each product) gives the highest and lowest contents, and also provides an indication of how much these foods differ from one another in vitamin content. This information can be obtained from **Appendix**: Grain Composition Tables for the vitamin content of each food for every comparison presented in this article.

Vitamins in whole grains — wheat, rye, barley, brown rice Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 45 g servings of five classes of dry, unprocessed whole wheat: hard red spring, hard red winter, hard white, soft red winter, soft white, and three other dry, unprocessed

grains: rye, barley, and brown rice, show differences among the grains.

Vitamin contents highest:

- thiamin (0.227 mg), niacin (2.569 mg) in hard red spring wheat;
- riboflavin (0.113 mg), folate (27 μg), pantothenic acid (0.657 mg) in rye; and
- vitamin B<sub>6</sub> (0.196 mg) in brown rice.

Vitamin contents lowest:

- thiamin (0.086 mg), pantothenic acid (0.126 mg), vitamin B<sub>6</sub> (0.117 mg) in barley;
- riboflavin (0.028 mg), folate (6 μg) in brown rice;
- niacin (1.921 mg) in rye; and
- vitamin A (5 IU (International Units)) in rye. None in the other grains.

Vitamins in whole wheat Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 45 g servings of the five dry unprocessed whole wheat listed above, with each other show differences. Vitamin contents highest:

- thiamin, niacin, folate in hard red spring wheat;
- riboflavin in hard red winter wheat;
- pantothenic acid the same in hard red winter wheat and hard white wheat; and
- vitamin B<sub>6</sub> in soft white wheat.

<sup>&</sup>lt;sup>b</sup>mg = milligram.

<sup>&</sup>lt;sup>c</sup>μg = microgram. <sup>d</sup>RE = retinol equivalent.

Vitamin contents lowest:

- thiamin in hard red winter wheat;
- riboflavin, pantothenic acid, vitamin B<sub>6</sub> in soft red winter wheat;
- folate the same in hard red winter wheat and hard white wheat;
- niacin in hard white wheat;
- pantothenic acid in soft white wheat; and
- vitamin A none in these wheat.

Vitamins in whole rye, barley, brown rice Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 45 g servings of the three dry, unprocessed grains, rye, barley, and brown rice from above show differences in vitamin contents among the grains.

Vitamin contents highest:

- thiamin, niacin, vitamin B<sub>6</sub> in brown rice;
- riboflavin, folate, pantothenic acid in rye; and
- vitamin A in rye.

Vitamin contents lowest:

- thiamin, riboflavin, pantothenic acid, vitamin B<sub>6</sub> in barley;
- niacin in rye;
- folate in brown rice; and
- vitamin A none in barley or brown rice.

Vitamins in whole spelt, triticale, wheat Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 45 g servings of three similar dry, unprocessed whole grains, spelt, a predecessor of wheat, triticale, a cross between wheat and rye, and hard red spring wheat show differences among the grains.

Vitamin contents highest:

- thiamin in hard red spring wheat;
- riboflavin, niacin in spelt;
- folate, pantothenic acid, vitamin B<sub>6</sub> in triticale; and
- vitamin A in spelt and triticale.

Vitamin contents lowest:

- thiamin in spelt;
- riboflavin, folate, pantothenic acid, vitamin B<sub>6</sub> in hard red spring wheat;
- niacin in triticale; and
- vitamin A none in hard red spring wheat.

Vitamins in processed wheat products Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 45 g servings of whole wheat flour ground from unprocessed whole wheat and of white flour, wheat, all-purpose, unenriched milled from

unprocessed whole wheat show differences from effects of processing.

Vitamin contents highest:

All vitamins in whole wheat flour.

Vitamin contents were lowest for all vitamins in white unenriched flour.

When 45 g servings of white flour, wheat, all-purpose, enriched are added to the comparison, data for the three flours show vitamin contents are not always highest with enrichment. Flour enrichment includes thiamin, riboflavin, niacin, folate, iron, but not pantothenic acid, vitamin B<sub>6</sub> or Vitamin A.

Vitamin contents highest:

- thiamin, riboflavin, folate in white flour, wheat, all-purpose, enriched; and
- niacin, pantothenic acid, vitamin B<sub>6</sub>, vitamin A in whole wheat flour.

Vitamin contents lowest:

• thiamin, riboflavin, niacin, folate in white flour, wheat, all purpose, unenriched.

Vitamin contents of pantothenic acid, vitamin B<sub>6</sub>, and vitamin A in unenriched and enriched white flours are the same or similar because enrichment does not include these vitamins.

Vitamins in processed rice products Comparison of vitamin contents, in Appendix: Grain Composition Tables, for 45 g servings of whole grain brown rice flour and refined white rice flour show differences from effects of processing.

Vitamin contents highest:

• All B-vitamins in whole grain brown rice flour.

Vitamin contents were lowest for all B-vitamins, and vitamin A is not found in either whole grain brown rice flour or in refined white rice flour.

# **Pasta and Rice**

This category consists of various types of cooked pasta products made from flours from different grains, and uncooked and cooked rice products.

Vitamins in pasta Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 140 g servings of macaroni/spaghetti/noodles, whole wheat, cooked and macaroni/spaghetti/noodles, white, cooked show differences between the two pastas.

Vitamin contents highest:

- thiamin, riboflavin, niacin, pantothenic acid, vitamin B<sub>6</sub> in whole wheat, cooked pasta; and
- folate and vitamin A in white, cooked pasta.

Vitamin contents were lowest for thiamin, riboflavin, niacin, pantothenic acid, vitamin B<sub>6</sub> in white cooked pasta. However, folate and vitamin A were lowest in whole wheat cooked pasta.

Vitamins in uncooked brown and white rice Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 45 g servings of uncooked brown and white rice show effects of removing bran and germ during processing.

Vitamin contents highest:

- thiamin, riboflavin, niacin, folate, vitamin B<sub>6</sub> in uncooked brown rice; and
- pantothenic acid in uncooked white rice.

Vitamin contents were lowest for thiamin, riboflavin, niacin, folate, vitamin B<sub>6</sub> in uncooked white rice. Pantothenic acid was lowest in uncooked brown rice, and no vitamin A was found in either uncooked brown or white rice.

Vitamins in cooked brown and white rice Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 140 g servings of cooked brown and white rice show effects of water solubility and heat sensitivity of the vitamins in the two types of rice during cooking.

 The same vitamins were highest and lowest for cooked brown and white rice as for uncooked brown and white rice in the comparisons above.

#### Cereal, Ready-to-Eat

This category is composed of ready-to-eat cereals, each processed primarily from one type of grain (corn, oats, rice, wheat), with other ingredients added for flavor, texture, and vitamins and by fortifying most of the cereals listed with vitamins except pantothenic acid (*see* **Appendix**: Grain Composition Tables). Cereals fortified with vitamin A makes them important sources of the vitamin, with several cereals at higher levels of added vitamin A than the RDAs for men and women (Table 1). Highest levels of folate in the fortified cereals are at or above the RDA level, 400 µg per serving (Table 1). Fortification levels for rice flakes make this food highest in contents for thiamin, riboflavin, and niacin, one of the four cereals highest in folate, and third highest in vitamin A.

#### **Baby Food Cereals**

This category contains three representative baby food cereals in the highly processed dry, instant form. All are fortified with vitamins and minerals to increase nutrient levels (see Appendix: Grain Composition Tables).

#### **Breads and Other Related Products**

This category consists of many kinds of yeast bread, bread rolls, bagels, other assorted bread-based products and quick breads. Nearly all these foods are enriched with thiamin, riboflavin, niacin, folate, and iron, but not with pantothenic acid and vitamin B<sub>6</sub> (see Appendix: Grain Composition Tables). Most of these products contain little or no vitamin A. Higher vitamin A levels in some products are from added ingredients such as eggs and/or milk. Whole wheat products, taco shell, corn tortilla, and white pita bread are not enriched.

Vitamins in pita bread Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 50 g servings of pita bread from whole wheat flour and pita bread from unenriched white flour show differences between breads made from the two flours.

Vitamin contents highest:

- thiamin, niacin, folate, pantothenic acid, vitamin B<sub>6</sub>, vitamin A in whole wheat flour pita bread; and
- riboflavin in white flour pita bread.

Vitamin contents were lowest for thiamin, niacin, folate, pantothenic acid, vitamin B<sub>6</sub>, and no vitamin A was found in white flour pita bread. Riboflavin was lowest in whole wheat flour pita bread.

#### **Crackers**

This category includes crackers that contain a variety of flours and added ingredients for color, texture, flavor, and vitamins. Nearly all products are enriched with thiamin, riboflavin, niacin, folate, and iron but not pantothenic acid and vitamin B<sub>6</sub>.

Vitamins in whole wheat and rye crackers Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 30 g servings of three unenriched crackers: whole wheat crackers, cheese-filled sandwich whole wheat crackers, and rye wafer, plain, show differences from added ingredients and processing among these crackers.

Vitamin contents highest:

- thiamin, vitamin B<sub>6</sub> in rye wafer, plain;
- riboflavin, folate, vitamin A in cheese-filled sandwich whole wheat crackers; and
- niacin, pantothenic acid in whole wheat crackers.

Vitamin contents lowest:

 thiamin, riboflavin, folate, vitamin B<sub>6</sub> in whole wheat crackers;

- niacin, pantothenic acid in rye wafers, plain; and
- vitamin A none in rye wafers, plain, and whole wheat crackers.

#### Cookies

This category is composed of a variety of commonly consumed cookies. Most of them contain similar basic ingredients to which added ingredients provide flavors, textures, appearance, and vitamins. All but one kind of cookie are enriched with thiamin, riboflavin, niacin, folate and iron, but not pantothenic acid, vitamin B<sub>6</sub> or vitamin A, and one cereal bar is fortified (*see* Appendix: Grain Composition Tables). Vitamin A contributed by added ingredients increased vitamin A content in nearly all cookies listed.

#### Cakes, Pastries, and Other Desserts

This category contains a wide variety of popular cakes, desserts, piecrusts and a few complete pies. All foods are enriched with thiamin, riboflavin, niacin, folate, and iron, but not pantothenic acid and vitamin B<sub>6</sub> (see Appendix: Grain Composition Tables). Vitamin A content is high in some foods listed, and low or none in others, depending on vitamin A in added ingredients. Carrots in carrot cake and pumpkin in pumpkin pie greatly increased vitamin A contents in these foods.

# Granola and Cereal Bars

This category consists of two examples of these bars that are representative of those available in retail stores. Breakfast bars are fortified at higher levels than cereal bars with thiamin, riboflavin, niacin, folate, vitamin B<sub>6</sub>, and vitamin A, but no pantothenic acid is added, (*see Appendix*: Grain Composition Tables).

#### **Snacks and Chips**

This category contains foods representative of the wide variety of available grain-based snack foods. Most snacks and chips are not enriched, and none listed are fortified (*see* **Appendix**: Grain Composition Tables). Of the items listed, only bagel chips and both kinds of pretzels are enriched with thiamin, riboflavin, niacin, folate, but not pantothenic acid or vitamin B<sub>6</sub> or vitamin A.

Vitamins in snacks and chips Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 30 g servings of the unenriched products show effects of kind of grain used, added ingredients, processing, and cooking (popcorn).

Vitamin contents highest:

- thiamin, riboflavin, vitamin A in taco or tortilla chips;
- niacin, pantothenic acid in rice cake; and
- folate, vitamin B<sub>6</sub> in wheat nuts.

Vitamin contents lowest:

- thiamin, riboflavin, folate in corn nuts;
- niacin in microwave popped popcorn;
- pantothenic acid in corn chips;
- vitamin B<sub>6</sub> in microwave popped popcorn; popcorn popped in fat; rice cake; and
- vitamin A none in rice cake or pretzels.

# Legumes

This category contains a collection of commonly used legumes. Overall, these cooked legumes are excellent sources of the B-vitamins, but not vitamin A.

**Vitamins in legumes** Comparisons of vitamin contents (*see* in **Appendix**: Grain Composition Tables) for 90 g servings of these cooked foods show differences among legumes.

Vitamin contents highest:

- thiamin in black beans, broadbeans, cowpeas, navy beans;
- riboflavin, vitamin B<sub>6</sub> in soybeans;
- niacin, vitamin A in fava beans; and
- folate, pantothenic acid in lentils.

Vitamin contents lowest:

- thiamin in tepary beans;
- riboflavin, folate in bayo beans;
- niacin in brown beans, mung beans, northern beans;
- pantothenic acid in fava beans;
- vitamin B<sub>6</sub> in pigeonpeas, yellow or green split peas; and
- vitamin A none in several types of legumes.

#### **Meat Substitutes**

This category contains examples of soybeans processed into the meat substitutes "miso," "tempeh," and "tofu."

Vitamins in meat substitutes Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 85 g servings of these foods show differences in effects of processing among them.

Vitamin contents highest:

- thiamin in silken tofu;
- riboflavin, pantothenic acid, vitamin B<sub>6</sub>, vitamin A in tempeh; and
- niacin, folate in firm tofu.

Vitamin contents lowest:

- thiamin, vitamin B<sub>6</sub> in extra firm tofu;
- riboflavin in extra firm tofu, silken tofu, soft tofu;
- niacin in silken tofu;
- folate in tempeh;
- pantothenic acid in silken tofu, soft tofu; and
- vitamin A none in firm tofu, silken tofu, soft tofu.

# **Alcoholic Beverages**

This category includes beverages fermented from grains. Overall, the 8 fluid ounce servings of beers contain B-vitamins, but 1.5 fluid ounce servings of scotch and whiskey contain practically none.

Vitamins in beer Comparisons of vitamin B contents, in Appendix: Grain Composition Tables, for 8 fluid ounce servings of the various beers show differences among them.

Vitamin contents highest:

- thiamin, riboflavin in light, low calorie beer;
- thiamin, riboflavin, niacin, folate in regular beer;
- pantothenic acid in low alcohol beer; and
- vitamin B<sub>6</sub> in regular beer, low alcohol beer.

Vitamin contents lowest:

- thiamin, riboflavin, niacin, folate in low alcohol beer:
- pantothenic acid and vitamin B<sub>6</sub> in light, low calorie beer; and
- vitamin A none in alcoholic beverages.

#### **Ingredients Used in Grain Products**

This category consists of several ingredients commonly added to various grain-based foods during preparation and processing to add color, flavor, texture, and vitamins. Some are excellent sources of B-vitamins.

Vitamins in ingredients used in grain-based foods Comparisons of vitamin contents, in Appendix: Grain Composition Tables, for 30 g servings of these foods show wide differences among them.

Vitamin contents highest:

- thiamin in raw sunflower seeds;
- riboflavin raw almonds;
- niacin and folate in defatted peanut flour;
- pantothenic acid in dry roasted, salted sunflower seeds:
- vitamin B<sub>6</sub> in flax seeds; and
- vitamin A in arrowroot flour.

Vitamin contents were lowest in arrowroot flour for all vitamins except vitamin A. Vitamin A was not found in several ingredients.

# **Summary**

Grains and grain-based foods are important food sources of B-vitamins, but not vitamin A unless fortified, or contain added ingredients high in vitamin A content. B-vitamins, which are synthesized in grain plants and stored in the kernels, have metabolic functions in the plant and in the human body when eaten by people. Different grains differ from one another in vitamin composition. Changes in vitamin composition occur during processing.

# **Acknowledgments**

The author thanks Nancy Van Heel and Sally Schakel, both from the Nutrition Coordinating Center, Division of Epidemiology, University of Minnesota for developing the food and nutrient data base in **Appendix**: Grain Composition Tables, and for their helpful suggestions on this manuscript. Thanks also to Dr. Joan Gordon, retired faculty member, Department of Food Science and Nutrition, University of Minnesota for her helpful suggestions on this manuscript.

See also: Cultural Differences in Processing and Consumption. Fortification of Grain-Based Foods. Grain, Morphology of Internal Structure. Grain and Plants, Morphology. Nutrition: Beriberi, A Deficiency Related to Grains; Guidelines for Grain-Based Foods. Whole-Grain versus Refined Products. Appendix: Grain Composition Tables; Units of Grain Science.

#### **Further Reading**

Barr SI, Murphy SP, and Poos MI (2002) Interpreting and using the dietary references intakes in dietary assessment of individuals and groups. *Journal of the American Dietetic Association* 102(6): 780–788.

Duyfe RL (2002) American Dietetic Association Complete Food and Nutrition Guide, 2nd edn. Hoboken: Wiley.

Grodner M, Anderson SL, and De Young S (2000) Foundations and Clinical Applications of Nutrition – A Nursing Approach, 2nd edn. St. Louis: Mosby.

Hegarty V (1995) Nutrition Food and the Environment. St. Paul: Eagan Press.

Hoseney RC (1994) *Principles of Cereal Science and Technology*, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Marquart L, Slavin JL, and Fulcher RG (2002) Whole-Grain Foods in Health and Disease. St. Paul, MN: American Association of Cereal Chemists. Nestle M (2002) *Food Politics*. Berkeley: University of California Press.

Schakel S, Buzzard IM, and Gebhardt SE (1997) Procedures for estimating nutrient values for food composition databases. *Journal of Food Composition and Analysis* 10: 102–114.

Trumbo P, Schlicker S, Yates AA, and Poos M (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *Journal of The American Dietetic Association* 102(11): 1621–1630.

Wardlaw GM (1999) Perspectives in Nutrition, 4th edn. Boston: WCB/McGraw-Hill.

#### **Relevant Websites**

http://www.folicacid.net — Association of Schools of Public Health and March of Dimes, advocating for folic acid: a guide for health professionals.

http://books.nap.edu — Food and Nutrition Board, Institute of Medicine-National Academy of Sciences, dietary reference intakes: recommended vitamins intakes for individuals.

http://www.ncc.umn.edu — Nutrition Coordinating Center, Division of Epidemiology, University of Minnesota, Minneapolis, MN.

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# OATS

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#### Introduction

Oats have played a significant role in farming systems from domestication to present due to the versatile uses of the grain and plant (Figure 1). Oats currently rank sixth in world production of cereals after maize, rice, wheat, barley, and sorghum. World oat production was similar to millet and exceeded rye, and triticale.

Oats are primarily grown in cool temperate climates with  $\sim$ 67% of world production occurring in the northern hemisphere. The Russian Federation, Canada, United States of America, Finland, and Poland were ranked as the top five countries for

world oat production. Oats are also grown in the southern hemisphere with Australia ranked first in production while Argentina, Chile, and Brazil are also significant producers.

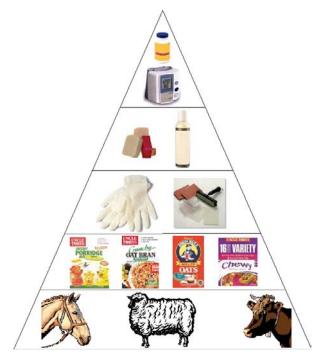
There are two types of oats — husked oats, with hulls surrounding the kernel or groat after harvest and naked oats, where the hull is removed when the crop is harvested. Naked oats have the free threshing character similar to wheat. Husked oats represent the majority of oat production, but naked oats are gaining prominence for specialist markets as improved varieties are being developed.

Oats are used for animal feed, human consumption, and nonfood uses. Figure 2 shows the uses of oats relative to the proportions utilized.

Oats were a traditional feed on farms for centuries and powered workhorses until the introduction of



Figure 1 Oat plants pictured at heading.



**Figure 2** The main end uses of oats are shown with the largest proportion used for animal feed, the second major use for human consumption, and minor uses for industrial, cosmetic, and pharmaceuticals.

machinery powered by fossil fuels. Between 50% and 90% of the world oat production is used as animal feed for horses, cattle, and sheep. Naked oats are particularly suited for poultry, pigs, racehorses, and birds. Oats as an animal fodder are also important for domestic and export markets. Oats can be grazed as a green feed, made into silage, utilized as straw, or baled for hay.

Oats for human consumption are used to produce traditional, functional, and medicinal products. Oats are differentiated from other cereal grains by using the entire kernel after the hull is removed for many food products. Porridge or oatmeal, hot cereals, bread, biscuits, infant food, and muesli or granola bars are a few examples of food products produced from oats. Nondairy food uses have been developed resulting in oat milk, yogurt, and ice cream. Oats have been shown to have health benefits for lowering blood cholesterol, normalizing blood glucose levels, and reducing the risk of colorectal cancer. Although pharmacological properties are reported in the literature, no products have been commercialized.

Nonfood and industrial oat products vary from cosmetics to the production of cardboard products to the manufacture of furfural and furnan compounds used for solvents, adhesives, filtering aids, and the construction of board material and cellulose pulp.

Although oat production is declining globally, specialization for animal feed, hay, food, industrial, and pharmaceutical products is growing and provides opportunities for adding value to the oat crop.

This article will briefly outline the significance of oats as a cereal crop including origin, domestication, plant morphology, production, end uses, and variety improvement.

#### Classification

The genus of oat is *Avena* L.(Poaceae) and belongs to the tribe Aveneae of the family Gramineae. The primary species cultivated is *Avena sativa*. However, *Avena byzantina* and *Avena strigosa* are also grown in some regions for animal feed and fodder.

The species described in *Avena* form a polyploid series varying from one to three chromosome sets with the basic chromosome number (n) seven. Each chromosome set has a genome designation A, B, C, D donated by a different oat species in the evolution of the genus. The different species with varying ploidy levels evolved over time and will be discussed below. The diploid species have one set of chromosomes and are designated as 2n = 2x = 14. This designation indicates the diploid is composed of one genome with 14 chromosomes. The tetraploid species have two sets of chromosomes and are designated

2n = 4x = 28. This designation indicates the tetraploid has two different genomes each with 14 chromosomes to total 28. The hexaploid species include cultivated oats and have three sets of chromosomes designated 2n = 6x = 42. This designation shows that the hexaploid species are composed of three genomes each with 14 chromosomes to total 42 chromosomes.

#### **History of Oats**

#### **Speciation**

Three distinct diploid genomes A, C, and D hybridized in the development of cultivated oats. Each genome was composed of 14 chromosomes. The specific species that hybridized to form cultivated oats has been difficult to ascertain. Several species have been described with the A and C genomes, but no species have been identified with the D genome. Nonetheless, domesticated oats evolved from a hexaploid wild oat species composed of A, C, and D genomes with *Avena sterilis*, *Avena fatua*, or *Avena hybrida* all hypothesized as the progenitors of domesticated oats.

# **Domestication and Early Cultivation**

Although it is not certain where the center of origin for oats is located, the greatest genetic diversity encompasses the Canary Islands, the Mediterranean, the Middle East, and the Himalayan region.

Avena species were identified at several sites in the Near East dating from 10 500 to 5750 BC. The Neolithic revolution spread from the Near East to the European Continent, Great Britain, and east to Asia. By 2000 BC, farming and trading prospered in Europe. Wheat and barley seed moved into these regions bringing oats and rye as weed contaminants. Although the historical record is limited, cultivated oats were identified in northern regions of Western Europe between 4500 and 400 BC when the region experienced cool and wet climatic changes. Oats and rye were favored in these conditions compared to wheat and barley. There are references about oats for fodder, animal feed, human food, and medicinal properties by Greek and Roman authors ~AD 23-79.

Oat cultivation continued during the Dark Ages and by the Renaissance, oats was ranked fourth in importance after wheat, barley, and rye. Oat was the dominant crop in Scotland by the thirteenth century. Oats could flourish in areas where wheat and barley produced marginal yield and became known as a crop that could be produced on less productive land. Although the primary use of oats was for animal feed, by 1500–1700 it was the principal grain crop for human consumption in Scotland, Wales, Ireland, and Britain. Oats also continued to be an important

feed for horses, cattle, and sheep. During the potato crop failure in Ireland during 1740–1741, oats were used in soup to sustain the hungry.

Immigrants and explorers from Great Britain and Spain introduced oats into North America. The English introduced oats into Canada, New England, and eastern USA in 1500-1600 as an animal feed. Scottish immigrants to North America continued to use oats for porridge and other foods. The Spanish brought oats to the Pacific Coast, the southwestern, and southeastern USA in the early 1800s to feed their horses. The general public continued to use oats for the sick, purchasing the product at chemists. Oat production moved west to the Upper Mississippi Valley and into Canada by the 1880s. Oatmeal as a breakfast food began to flourish by 1900 in USA because local mills began milling oats for breakfast cereal and the product was now sold in grocery stores instead of pharmacies or chemists.

Governor Phillip introduced oats into New South Wales, Australia in 1791. The primary use of oats was green fodder and hay for horses, dairy cattle, and pigs. Oat varieties, introduced from Europe, were late maturing in Australia, so successful production was primarily in Tasmania and the elevated regions of mainland Australia until the early maturing variety Algerian was introduced for lower rainfall regions. Human consumption of rolled oats was associated with immigrants from Great Britain. Leonard and George Parsons emigrated from England to Australia in 1861 and by the late 1880s, began manufacturing the John Bull brand of rolled oats. In 1854, Harry Clifford Love emigrated from Dublin to Australia. After several businesses, Harry and his son Joseph formed the Imperial Manufacturing Company, which began producing the Uncle Toby's brand of rolled oats in 1893.

#### **Plant Growth and Morphology**

Oats are an annual crop with tall and short stature depending on the presence of dwarfing alleles. Each plant produces about five stems depending on the growing season and each stem produces about five to six leaves on dwarf stature plants and eight to ten leaves on tall plants (Figure 3). Each stem or culm produces a terminal panicle where the seeds develop. Plant height will vary with growing season and the presence or absence of dwarfing alleles. Varieties with dwarfing alleles will vary in height between 45 and 70 cm in Australia. Tall varieties without dwarfing alleles will vary in height between 70 and 135 cm in Australia. Root development is fibrous and will vary according to aboveground growth as well as maturity, but is ~1 m deep.

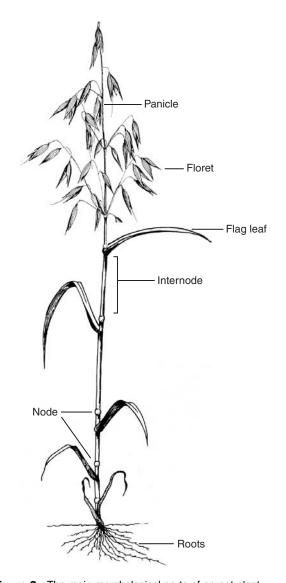


Figure 3 The main morphological parts of an oat plant.

Temperature and daylength influence the length of the growing period for producing oat grain. Winter and spring oats differ by vernalization requirements. Winter oats require cold temperatures to produce grain whereas spring oats will produce grain without the vernalization period. Due to mild winter temperatures in the Mediterranean climates of the southern and northern hemisphere, spring oats can be sown in the winter season.

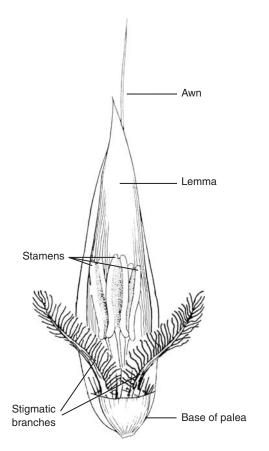
The major developmental stages of plant growth are germination, leaf production, tiller production, stem elongation, panicle development and emergence, anthesis, grain filling, and ripening. When the seed germinates, starch reserves in the seed provide energy for root and leaf development until the plant begins to photosynthesize. Leaves develop at regular intervals until panicle emergence. Tiller production begins

when the seedling has three to four leaves. The number of tillers that live to produce grain is dependent on environmental conditions as well as the age of the tiller. Internodes of the plant begin to elongate when about four to seven leaves are present, resulting in increased height of the plant. Preanthesis panicle development occurs when the growing point is less than 1 mm. As the internodes elongate the developing panicle grows upward. Unlike wheat, barley, and rye, oats form a panicle composed of branches with the seed produced at the tip. Flowering or anthesis occurs when pollen is shed on the feathery stigmas enclosed by the lemma and palea with outer tissues called glumes (Figure 4). This stage occurs when the panicle has fully emerged from the flag leaf in tall varieties, but may occur while the head is still contained in the flag leaf for dwarf varieties. Fertilization occurs within 24 h. Grain size and weight increase as sugars are converted to starch. As the seed matures, the plant begins to lose moisture and senesces.

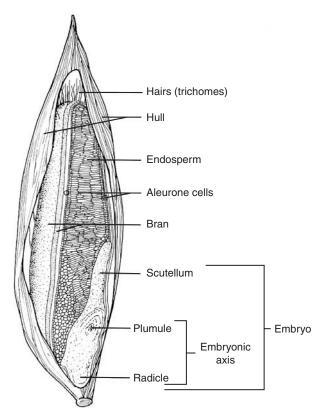
The mature oat grain consists of a groat or caryopsis tightly covered by a hull or husk previously the

lemma and palea (Figure 5). The hull represents  $\sim 30-40\%$  of the total grain weight. It is comprised of cellulose, hemicellulose, and lignin. Compared to other cereals, the oat groat is slender and covered with hairs or trichomes under the hull. There is a groove on the inner surface of the groat.

The groat is composed of three major fractions, bran, endosperm, and germ. Several layers of compressed tissue, and aleurone cells constitute the bran located in the outer layers of the groat (Figure 5). The aleurone cells represent the largest component of the bran and play a role in seed germination. The endosperm represents from 55% to 80% of the groat. It is composed of starch, protein, lipids, and the major concentration of  $\beta$ -glucans. The endosperm provides nutrients for the growing embryo. The germ or embryo has three structures, the scutellum, plumule, and radicle. The scutellum is located between the embryo and endosperm and is involved in germination and food transfer. The plumule has two to three leaf primordial and the radicle has two to three root primordial.



**Figure 4** The plant parts that comprise an oat floret. (Reproduced with permission from Marshall HG and Sorrells ME (eds.) (1992) *Oat Science and Technology*, Agronomy number 33, p. 72. Madison, Wisconsin: American Society of Agronomy.)



**Figure 5** Composition of an oat kernel. (Reproduced with permission from Youngs VL (1986) Oat lipids and lipid-related enzymes. In: Webster FH (ed.) *Oats: Chemistry and Technology.* St. Paul, MN: The American Association of Cereal Chemists.)

# **Growing Oats – Agronomy**

Good management practices are necessary to realize the genetic potential of oat varieties. Sowing reliable seed, using optimum sowing and fertilizer rates, and timely disease and pest control constitute good management practices.

#### **Soil Factors**

Oats tolerate a wide range of soil types. The crop will tolerate acid soils to a pH of 4.5 and alkaline soils to a pH of 8.5, but optimum productivity occurs with pH range from 5 to 6. Acid soils can be associated with aluminum toxicity in oats. Oats are not as tolerant of salt as wheat, barley, and rye, but are more tolerant than sorghum. The crop is also not as tolerant of high boron levels as wheat or barley, but is similar to sorghum. Oats are generally tolerant of high manganese levels in the soil.

#### **Climate**

The most significant climatic factors affecting oat productivity are temperature and moisture. Oats flourish in cool moist environments and require more moisture to produce a unit of dry matter than other cereals. Oat-producing regions in North America, Europe, and Asia are primarily located between 40°N and 60°N latitudes. Maritime climates in northern Europe are also oat-producing regions. The length of the growing season in the northern hemisphere varies from 90 to 110 days with decreasing daylength as the growing season progresses. Prime oat-growing regions in the southern hemisphere occur within the latitudes 20°S and 45°S. Oats are grown between 30°S and 40°S latitudes in Australia, between 25°S and 45°S latitudes in New Zealand, and between 20°S and 30°S latitude in South America. The growing season in Australia and South America can vary from 150 to 180 days with increasing daylength as the growing season progresses.

Although oats tolerate cold in seedling and tillering stages, yield loss can result once the panicle emerges. However, oats tolerate frost better than wheat and barley. Hot dry weather can also reduce grain yield and quality especially from anthesis to grain filling.

# **Agronomy**

Spring varieties are primarily sown in the southern hemisphere during the winter months. Both spring and winter varieties are grown in the northern hemisphere and time of sowing will vary with latitude. Sowing rates are dependent on a combination of climate, soil conditions, and use of the crop. Sowing rates are higher for fodder production when compared to grain production. Seed size also varies extensively in oats. Hence, sowing rates need to be calculated as number of seeds m<sup>-2</sup> rather than weight of seeds m<sup>-2</sup> to achieve optimum plant density.

Adequate nutrition of oats is essential to achieve maximum yields. Inputs such as nitrogen, phosphorus, potassium, and sulfur are applied based on soil tests. Micronutrient deficiencies for copper, zinc, manganese, molybdenum, and iron occur in some soils. Micronutrient toxicity for boron, aluminum, and manganese also occurs in some soils.

Oats generally tend to be more sensitive to herbicides than wheat or barley. Sensitivity is also variety dependent.

A harvester is used to cut standing plants and threshes the grain from the plant. Wind rowing is another option for harvest and requires that plants are cut and put into rows for grain threshing at a later date.

# **Growing Oats - Diseases and Pests**

A wide range of pests and diseases caused by fungi, bacteria, viruses, nematodes, and insects affect all stages of plant growth, resulting in reduced grain yield, dry-matter production, and decreased grain quality. Control measures include genetic resistance, chemical control, and management practices. This brief overview summarizes the major diseases and pests in oats. (see Cereals: Grain Diseases) for a more comprehensive summary of cereal diseases.

# **Diseases Caused by Fungi**

Powdery mildew caused by *Erysiphe graminis* D.C. ex Marat f. sp. *avenae* Marshal is an important disease in cool humid climates such as Northwest Europe. Stem rust caused by *Puccinia graminis* Pers. f. sp *avenae* Erikss. & Henn. (Figure 6) and crown or leaf rust



Figure 6 Oat stem rust.

caused by *Puccinia coronata* Cda f.sp. *avenae* Erikss. (Figure 7) are the two most devastating foliar diseases in oats. Significant grain yield and quality losses result from infection. It occurs in both the northern and southern hemispheres where oats are sown. Genetic



Figure 7 Oat leaf rust.



Figure 8 Pyrenophora leaf blotch of oats.

resistance is the preferred means of control for these diseases.

Although septoria leaf blotch (caused by Septoria avenae f.sp. avenae Frank.) is considered a minor disease compared to stem and leaf rust, the disease causes significant yield losses when it occurs. The disease is reported in eastern Canada, USA, Australia, Europe, Great Britain, and Israel. Pyrenophora leaf blotch caused by Pyrenophora avenae Ito & Kuribayashi apud Ito occurs worldwide with varying economic significance (Figure 8). Pyrenophora was the third most important disease in Germany, significant in Brazil, and common in Scandinavia. Red leather leaf caused by Spermospora avenae Sprague & A.G. Johnson is a minor disease identified in northwestern USA, Turkey, and Australia.

# **Diseases Caused by Bacteria**

Halo (*Psuedomonas syringae* pv. *coronafaciens*) and stripe blight (*P. syringae* pv. *striafaciens*) are collectively called bacterial blight (*Figure 9*). The disease is common while cool, moist conditions persist. Severe foliar symptoms develop on susceptible varieties. Dry conditions limit the spread of bacterial blight.

#### **Diseases Caused by Viruses**

Barley yellow dwarf virus is the most yield-limiting viral disease of oats and is an economic threat world-wide (Figure 10). The virus is transmitted by a number of aphid species.

#### **Nematodes**

Cereal cyst nematode (CCN), *Heterodera avenae* Wollenweber, causes a serious economic threat to oat production worldwide (Figure 11). In regions where CCN is abundant, genetic resistance reduces CCN population sizes and oat varieties with genetic



Figure 9 Bacterial blight in oats.

tolerance ensure maximum productivity. Genetic resistance and tolerance are independently inherited.

Stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, limits oat production in cool moist climatic conditions (Figure 12). Winter oats sown where mild winters occur and spring oats sown in the winter (Mediterranean climates) are affected by the nematode. Resistance and tolerance appear to be more closely associated for stem nematode than CCN.



Figure 10 Barley yellow dwarf virus in oats.

Root lesion nematode, *Pratylenchus neglectus*, affects oat crops in Europe, Iran, USA, and Australia. Yield losses up to 37% were recently demonstrated in South Australian trials where the nemtode population was high. Resistance and tolerance mechanisms are inherited similar to CCN.

#### **Insect Pests**

Russian wheat aphid (*Diuraphis noxia*), greenbug (*Schizaphis graminum*), bird cherry aphid (*Rhopalosiphum padi*), grain aphid (*Macrosiphum avenae*), and rose grain aphid (*Metopolophium dirhodum*) can attack oats with varying effects on productivity.

Army worm (*Phalaenidae* spp.), fruit fly (*Oscinella frit* (L.)), and wireworms (*Agriotes* spp.) are insect pests that affect oat production in varying capacities worldwide.

# **Processing Oats**

In order for oats to be processed, the hull must be removed from the groat, contaminants removed, and a product produced with quality appearance and taste. There are specifications for milling oats that include hull to groat ratio, color, and flavor.

Milling and processing operations consist of cleaning, grading, hulling, hull and fine separation, groat separation, and kilning (Figure 13). Grading occurs when groats are separated into two or three streams based on groat length or thickness. The hulling process is the next step when groats are separated from the hull using either impact- or stone-hulling systems. Impact hulling is more common than stone hulling.



Figure 11 Cereal cyst nematode affected plants are yellow and poorly developed.



Figure 12 Symptoms of stem nematode in an oat plant.

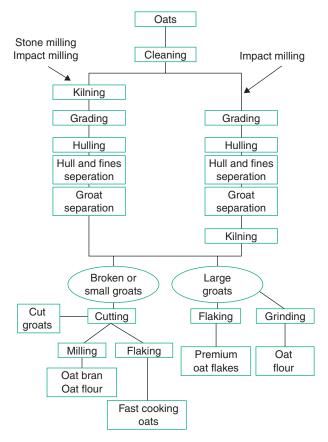


Figure 13 The milling process of an oat kernel.

Hulls and fines are separated from whole groats, broken groats, and unhulled oats. Further refinement occurs when groats are separated based on physical characters, such as groat size and weight. Because oat groats have a high oil content, a heat treatment is required to inactivate enzymes that cause rancidity and bitterness in the final product. Kilning is a process that heats the groat at a certain temperature and

moisture content to inactivate the enzymes. Kilning can occur before grading or after groat separation (Figure 13).

Commercial processors generally can produce 100 kg of product from 175 kg of oats. Milling efficiency varies according to the variety and the milloperating efficiency. Products produced include steel-cut groats, rolled oats, quick oats, baby oats, instant oat flakes, oat flour, and oat bran.

# **Using Oats**

Oats are a versatile crop used as grain and fodder for animal feed, human foods, industrial products, cosmetics, and pharmaceuticals. Figure 2 shows the diversity of oat products relative to the proportion used. Animal feed is the largest use of oats; cereal products and other human food the second most common use. Although pharmaceuticals represent the smallest group of products, they have the greatest potential for future growth and value.

#### Oats for Feed Grain

Oats have been a traditional feed grain for centuries. Recent advances in corn, wheat, barley, soybean, and canola as feed grains have resulted in a worldwide decline of oat production for feed. Despite the decline, the primary use of oats remain as a feed grain. Prior to mechanization oats were the primary feed for horses that powered farm equipment. Oats are a suitable feed for dairy and beef cattle, sheep, and horses. Although not as prevalent, oats can also be used for poultry, pigs, cats, dogs, birds, rabbits, bison, deer, and fish. In recent years, naked oats are being developed as a feed grain with improved nutritional value for markets such as weaner and grower pigs, poultry, racehorses, and birds. The following discussion centers on the traditional oat grain possessing a hull.

The nutrient value for animal feed is based on the proportion of groat to hull. The ratio varies with variety and environment. Oat groats have a higher oil or lipid content than other cereals, varying between 3% and 11%. The oil is comprised primarily of unsaturated fatty acids, which can alter the fatty acid composition of the animal fat. Protein content in oat groats varies from 9% to 15% with higher lysine content than corn, wheat, and barley. Lignin is the primary fiber fraction of the hull and reduces grain digestibility in animals. Lignin content also varies in different varieties. Although hulls with high lignin reduce digestibility, varieties with lower-hull-lignin content can have a beneficial affect for horses, cattle, and sheep. The hull reduces digestive problems in these animals.

The hull is a major constraint as a feed grain for poultry and pigs. It reduces digestibility resulting in low protein and poor energy. Because naked oats do not have a hull, the grain provides a good source of energy for grower and weaner pigs, broilers, and laying hens.

Overall, oats are a favorable feed for ruminants such as cattle and sheep. Oats are also the preferred feed for horses due to the palatability, digestibility, and nutritive value of the grain. Naked oats are also used for racehorses, due to the limited requirement of grain intake and the need for a good source of energy.

#### **Oat Grain for Human Consumption**

Products produced from oats are generally made with the whole grain after the hull is removed, whereas other cereal products are produced from grain with the germ and bran removed. Whole oats have the highest protein and a favorable ratio of unsaturated to saturated fatty acids compared to other cereals. Whole oats also provide vitamins, minerals, and antioxidants. The heat treatment used to stop rancidity also enhances the unique sensory characters of oats.

The unique composition of whole oats results in healthy products.  $\beta$ -glucan content in the groat varies between 1.8% and 7.5% on a dry matter basis. The high level of water-soluble dietary fiber of which  $\beta$ -glucans are the main component, helps to reduce high blood cholesterol and normalize blood glucose levels. The fiber also reduces the risk of colorectal cancer. The groat also contains vitamins and antioxidants. Lactose intolerant individuals can now purchase nondairy oat products.

The main product produced from oats is porridge or oatmeal. Other food products processed from oats are cold cereals, infant foods, muesli or granola bars, breads, biscuits or cookies, thickeners, and specialty flour. New processes were developed recently to manufacture nondairy products such as milk, ice cream, and yogurt using oats. Antioxidants in oat flour can be used to stabilize some milk and meat products sensitive to fat oxidation during storage. Oat gum primarily composed of  $\beta$ -glucans is used to stabilize ice cream. Oat proteins have been used in many food products including heat resistant chocolate, because of viscosity and emulsification properties.

#### **Oat Grain for Industrial and Nonfood Uses**

There are many potential uses of oat grain fractions and hulls in products such as adhesives, cosmetic products, pharmaceuticals, and oil-spill cleanup. Some of the uses are proposed, whereas the technology for other uses has been developed and implemented.

The unique chemical composition of the oat groat is responsible for oat starch gels having more elastic, adhesive, and translucent properties than wheat or maize starches. Oat starch could replace products produced from wheat or maize with the waxy starches the exception. Products currently produced from starch include brown paper and cardboard products, and coating agents for tablets in the pharmaceutical industry.

Oats have been used in cosmetic products for some time. The starch characteristics make oats an useful ingredient in bath products to relieve itching and nappy rash. Other products produced from oats include cleansers to replace soap in facial masks, soap for cleansing dry and oily skin, shampoo, lotions, bath additives, and skin care.

Pharmaceutical products have great potential, but are not currently tapped. The health benefits of oat fractions were discussed in the section above.

The hull can also be used to produce industrial products. Oat hulls are used to produce furfural and furan compounds. These compounds are used in the manufacturing process of: crude petroleum; nylons; formaldehyde furfural resins; solvents for dyes, resins, lacquers, paints and varnish; elastomers and thermosplastics; phenolic resin glues and plywood adhesives; construction board material; and cellulose pulp. A very different aspect of hull utilization is the cariostatic properties of tooth protection.

#### **Oats for Fodder**

Fodder production is also an important component of animal feed shown in Figure 1. Several types of oat fodder, hay, silage, grazing, and straw, are produced as an essential component of animal production systems worldwide.

Oat hay is produced for dairy and beef cattle, horses, and sheep. Hay is an important commodity domestically, but is also traded internationally. A significant market is the Asian dairy and beef cattle industry. The oat hay crop is cut at the early milk growth stage for optimum hay quality. Hay-quality characters that may be associated with palatability are digestibility, water-soluble carbohydrates, acid detergent fiber, and neutral detergent fiber, and stem diameter. Sensory quality characters are also important and include hay color and aroma.

Cattle and sheep graze oats during the vegetative stage when the plant height is from 20 to 40 cm. Animals are removed from the oat crop before the growing point of the plants is damaged, so the crop produces grain. Grazing is important in countries worldwide including New Zealand, Australia, South America, northern Africa, Nepal, and the USA.

Silage production using oats is used in the dairy and beef cattle industry worldwide. Oats are cut in the early heading stage, chopped and ensiled.

# **Breeding Improved Oat Varieties**

#### History

Early oat improvement occurred when farmers selected the best performing plants in their paddocks. The first attempt to introduce genetic variation by crossing different oat genotypes was reported in England and accomplished by Shirreff in the 1860s. The first variety developed from hybridizing oats, Abundance, was released from Gartons Ltd. of Warrington, England.

# **Breeding Priorities**

Breeding priorities determine selection criteria that eventually result with the release of oat varieties with improved traits. Understanding production and processing constraints of oats is the initial step in identifying breeding priorities for improving varieties. These priorities will differ or change in emphasis depending on the growing environment and end use of the oat plant or grain. Breeding priorities are based on gross margin for growers and processors, resulting in a superior product for the consumer and processor. Breeding priorities set to achieve long-term goals are based on developing varieties that offer new products or increased value for both growers and industry. The long-term breeding priorities are particularly important for oats due to the global decline of production.

Although there are many different growing environments and end uses globally, the primary breeding priorities deal with productivity, disease resistance, agronomy, and quality.

Increased grain and hay yields are the primary aims of increased productivity. Improved disease resistance increases yield and enhances quality.

The significance of oat diseases vary with region and continent and are prioritized by breeding programs. Diseases such as stem and leaf or crown rust are two global diseases that cause significant losses to the oat industry. Barley yellow dwarf virus, smut, and septoria also reduce yield and quality in certain production areas of the world. Cereal cyst, stem, and root lesion nematodes when present in the production environment are equally as devastating, but more regionally localized in the world.

Important agronomic characters are standing ability, maturity of the plant, and the ability of the plant to resist shattering or loss of seed at maturity. Winterhardiness is also an important agronomic

trait of the varieties that require a cold period to produce seed.

Grain-quality characters that are essential for the industry are – high groat to hull ratio and groat color. Weather and disease can cause groat discoloration. Other grain-quality characters of importance for variety development are protein, oil,  $\beta$ -glucan content, hectoliter weight, and screenings percent. The most important aspect of fodder quality is palatability. The traits that comprise palatability are being researched, but are most likely a combination of digestibility, water-soluble carbohydrates, neutral detergent, and acid detergent fibers, color, and smell.

#### **Germplasm Collections**

Oat-germplasm collections are maintained in many countries and are a source of new genetic variation for improving oats. Without these collections and the exchange of advanced germplasm from oat-breeding programs, oat-variety improvement would not advance. Several collections of notable importance are located in the United Kingdom, Canada, USA, Israel, the Russian Federation, and China.

See also: Animal Feed. Cereals: Overview; Grain Defects; Grain Diseases; Grain-Quality Attributes; Protein Chemistry; Evolution of Species. Plants: Diseases and Pests. Taxonomic Classification of Grain Species. Variety Registration and Breeders Rights.

#### **Further Reading**

Baum BR (1977) Oats: Wild and Cultivated. A Monograph of the Genus Avena L. (Poaceae), pp. 463. Monograph 14. Ottawa, ON, Canada: Department of Agricultural Supply and Services Canada.

Bowden CW (1987) Oats. In: Lazenby A and Matheson EM (eds.) Australian Field Crops: Wheat and Other Temperate Cereals, vol. 1, pp. 481–507. North Ryde, New South Wales, Australia: Angus and Robertson.

Bonnett OT (1961) Morphology and Development. In: Coffman FA (ed.) Oats and Oat Improvement, vol. 8, pp. 41–74. Madison, Wisconsin: American Society of Agronomy.

Cross RJ (ed.) (2000) 6th International Oat Conference: Proceedings at Lincoln University, Lincoln, NZ 13–16 November 2000. New Zealand Institute for Crop and Food Research Limited, Christchurch, New Zealand.

Jensen NF (1961) Genetics and Inheritance in Oats. In: Coffman FA (ed.) Oats and Oat Improvement, vol. 8, pp. 125–206. Madison, Wisconsin: American Society of Agronomy.

Marshall HG and Sorrells ME (eds.) (1992) Oat Science and Technology. Agronomy number 33, 820pp. Madison, Wisconsin: American Society of Agronomy.

Scoles G and Rossnagel R (eds.) (1996) 5th International Oat conference and 7th International Barley Genetics Symposium Proceedings. Invited papers, vol. 1 & 2. University of Saskatchewan, Saskatoon 30 Jul-6 Aug 1996. University Extension Press, University of Saskatchewan, Saskatoon, Canada.

Webster FH (ed.) (1986) Oats Chemistry and Technology, pp. 1–426. St. Paul, MN: American Society of Cereal Chemists.

Welch RW (ed.) (1995) The Oat Crop Production and Utilization, pp. 1–560. London, UK: Chapman and Hall.

Youngs VL (1986) Oat lipids and lipid-related enzymes. In: Webster FH (ed.) Oats: Chemistry and Technology. St. Paul, MN: The American Association of Cereal Chemists.

#### **Relevant Website**

http://www.grdc.com.au - This website gives research information in Australia and Worldwide.

# OIL FROM RICE AND MAIZE

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#### Introduction

The oils from rice and maize, hereafter referred to as corn, have both similarities and dissimilarities. They are similar in that each one of them would be considered a specialty oil derived from less commonly used sources and possess unique functional/nutritional components that distinguish them, such as high levels of natural antioxidants. They are dissimilar primarily due to their fatty acid composition. Corn oil has higher linoleic acid content, whereas rice oil contains higher levels of oleic acid. This article discusses the two oils from the viewpoint of their production, processing, and utilization.

#### **Production**

# Rice Oil

The level of rice production is second only to wheat, among all the cereal grains produced worldwide, yet it is a minor commodity in the US. Rice oil is obtained from the bran composite that is removed during the milling of brown rice to produce white rice. In actuality, the oil is found primarily in the germ, which represents  $\sim\!2\%$  of the rice kernel (Figure 1). However, in typical rice milling operations it is not possible to separate the germ from the bran, so the resulting

product, commonly called rice bran, contains the germ as well. Rice bran, which represents  $\sim 8\%$  of the whole grain weight, contains  $\sim 20\%$  lipid, with a range from 15% to 25%. Thus, it represents a considerable resource when considering the world production of rice. However, only  $\sim 8\%$  of the world's potential rice oil production is realized, amounting to  $\sim 7$  million metric ton (Mt) in 1998. There are two primary reasons that a greater proportion of the potential rice oil production fails to be attained. The first is that in most of the countries that produce large quantities of rice, such as China and India, rice is milled in ways that do not separate the outer hull from the bran and germ layers. This

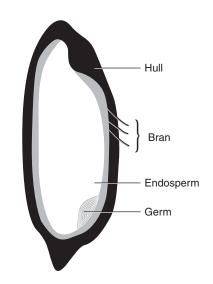


Figure 1 Rice grain.

makes the extraction of oil more difficult and not as economical. The second reason is that, even in mills that possess the capability of separating hull from bran and germ, the bran fraction is highly unstable and if not stabilized or extracted immediately, would yield oil of such low quality that it would not be suitable for human consumption. The reason for this instability is that the rice grain possesses highly active lipolytic enzymes that cause both oxidative and hydrolytic degradation of the lipids. Lipases tend to be the main culprit, causing a very rapid rise in free fatty acids that result in off-flavors, and are more susceptible to oxidative reactions and increase refining costs. Lipoxygenases can accelerate lipid oxidation, which primarily results in offflavors, but can also degrade fat-soluble vitamins and generate toxic compounds.

Rice oil is generally produced from stabilized bran. Bran is stabilized in a number of ways, but usually involves the elevation of temperature that denatures the enzymes responsible for lipid degradation. A popular means to stabilize rice bran is to pass it through an extruder so the frictional energy generated through the process of forcing the bran through the small orifice of the extruder plate causes an increase in the temperature. Temperatures required for adequate stabilization are generally believed to be more than 120°C. Other means of stabilization include pH adjustment using weak acids, microwave heating, and irradiation; however, no other practical methods have been utilized commercially.

Until recently, rice oil was primarily produced only in Japan. India has also begun a program to increase the utilization of rice oil. In 1993, the Riceland Foods Cooperative(Stuttgart, Arkansas) incollaboration with a Japanese company, Itochu Corporation, began producing rice oil for commercial distribution in the US.

It should be noted that defatted rice bran, which would become a by-product of rice oil production, does not have the full health benefits such as hypocholesterolemic properties associated with full-fat rice bran. This is due to the removal of functional components such as vitamin E vitamers (tocopherol and tocotrienol homologues) and oryzanol. However, defatted rice bran is more stable for human food processing and possesses high levels of fiber and other potential health-promoting compounds.

#### Corn Oil

Corn is one of the three major cereal grains grown in the world, along with rice and wheat. Like rice, it is a major staple in regions of the world outside of the US, yet is a minor food commodity in the US; rather, it is primarily used as animal feed. Corn oil is primarily a by-product of the corn milling industry, which processes  $\sim \! 10\%$  of the total world corn crop. As with rice oil production, the primary source of oil in corn is from the germ. Although it is possible to extract oil from corn bran that is separated from the germ, the amount of oil extracted would be much lower and its composition would be different from the corn oil that is typically extracted from milled corn.

The germ of corn is relatively larger than in most cereal grains, comprising  $\sim 10-14\%$  of the seed weight (Figure 2). The bran consists of the first few layers of tissue and comprises  $\sim 5\%$  of the seed weight. The germ contains  $\sim 35\%$  oil, although varieties of corn have been developed in which the level of oil in the germ can approach 50%, whereas the bran would contain  $\sim 1\%$  oil.

There are two types of milling, wet and dry, each of which produces different products and from which oil quality and yields vary according to the processing methods employed. The primary goal in dry milling is to produce prime endosperm products such as corn meal, grits, and flour. The primary goal of wet milling is to produce corn starch, which could also be processed further into corn sweeteners or ethanol. With the increasing importance of modifying starch or converting it to high-fructose corn syrup for applications in the food industry, wet milling has become predominant. Oil yields tend to be higher from wet milling (4%) compared to those from dry milling (2%).

Corn germ from dry milling is similar to rice bran in that it tends to be unstable due to the presence of lipolytic enzymes, which must be inactivated by cooking. Both dry- and wet-milled germs are generally dried to a moisture content of 3% and are then flaked prior to extraction.

The history of corn oil manufacturing in the US is much longer than that of rice oil. The reader is referred to the Mazola corn oil website for an interesting depiction of the history of corn oil in the US.

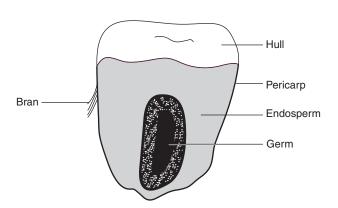


Figure 2 Corn grain.

# **Processing**

#### Rice Oil

**Extraction** Rice oil is typically extracted with solvents. The traditional solvent of choice has been hexane, although concern for impending, regulatory scrutiny of hexane for environmental and toxicological reasons has prompted exploration of alternative approaches to extraction. Although it is possible to extract oil from bran through mechanical means via pressing, oil recovery is lower and costs higher compared with solvent extraction. It has been found that pelletizing the fine rice-bran particles aids in the percolation of solvent through the bran during solvent extraction and increases yield. Alternative solvents have been considered, including ethyl acetate, isopropyl alcohol, and acetone. However, currently these are not as economical as hexane, although if regulatory action is taken with regard to hexane, these solvents may become the only viable option. At an academic level, supercritical fluids have been evaluated as a potential means to extract oil from rice bran. In principle, this approach offers interesting advantages such as ease of solvent removal and lack of toxicity. Also, it is possible to obtain fractionation of oil during the extraction process, which could facilitate refining or production of nutraceuticals. The major obstacles to the use of supercritical fluids are the large capitalization cost of initial startup and safety concerns due to the high pressures involved. Again, regulatory actions may necessitate such approaches in the future.

**Refining** Following extraction, several processes are undertaken that are collectively referred to as "refining" in order to improve the quality of the oil. The refining process may also cause a reduction in nutritionally active compounds such as vitamin E and oryzanol, which may be recovered as by-products of the refining process. Generally, the first step in the refining process is dewaxing. Crude rice oil can have up to 8% wax, depending on the extraction method. Such high levels of wax can have an adverse effect on subsequent refining steps causing reduced yields, and can cause problems with refining machinery. A common practice is to allow crude oil to sit in settling tanks to allow the higher-melting-point waxes to settle out. After removal of the oil, these waxes can be recovered and may have value as a by-product. The next step is the removal of the phosphatides by water washing, which is referred to as degumming. Again, this step is carried out early in the refining process so as to avoid problems with subsequent refining steps and machinery. A by-product of this step would be lecithin.

Perhaps the most critical step in the refining of rice oil is the neutralization step. This is because of the propensity for rice bran to undergo hydrolytic rancidity with the accumulation of free fatty acids. High levels of free fatty acids can lead to quality problems related to color and other physical properties. Free fatty acid neutralization is normally accomplished using sodium hydroxide or other caustic compounds such as sodium carbonate. The amount of caustic material used is dependent on initial free fatty acid levels. In crude oils with high levels of free fatty acids, it may be necessary to include physical refining by distillation of free fatty acids in order to increase yields to an acceptable level. A by-product of the neutralization process is soap stock, which is obtained after water washing. In addition to its obvious potential for the production of industrial soaps, soap stock can be an excellent source of a class of compounds called oryzanols, which may have unique health benefits. Furthermore, oryzanol can be maintained at much higher levels in the oil by only physically refining, rather than caustically refining, the oil. Because of the health benefits associated with oryzanol, this approach has been advocated as a way to maximize the potential health benefits of rice oil.

Bleaching using clay or diatomaceous earth can be done to remove color, and deodorization by counter-current steam is employed to remove volatile substances. By-products of these steps include tocopherols, tocotrienols, and carotenoids, each of which has potential health benefits. Other finishing steps could include hydrogenation to reduce unsaturated fat and hardening of the oil, and winterization to remove the remaining high-melting-point glycerides and waxes.

#### Corn Oil

Removal of oil from the wet-milled corn generally involves a combination of expelling and extraction. Initially, expelling is employed to reduce the oil content from  $\sim 50\%$  to  $\sim 20\%$ , after which solvent extraction is used. Dry-milled germ, which has generally been cooked in order to stabilize it, is then flaked and extracted with solvents. Refining steps for corn oil production are similar to those for rice oil. However, crude corn oil does not have the high levels of waxes found in crude rice oil, which simplifies the refining process, although it must also be degummed to remove the phospholipids prior to further refining. Also, because corn oil is promoted as a salad oil, the winterization process is critical in order to greatly reduce high-melting-point glycerides and waxes. Like rice oil, corn oil tends to have high levels of vitamin E vitamers, especially tocopherols, which could be an

important by-product of oil refining. Also, corn has higher levels of carotenoids such as lutein and zeax-anthin, which are receiving considerable attention as compounds that could reduce the eye disorder called macular degeneration. Thus, recovery of these carotenoids from the refining process could also be valuable.

# Utilization

#### Rice Oil

In the US, rice oil has not been produced in large quantities, and is therefore more of a specialty item found primarily in international food stores and some health or gourmet food shops. In Japan, rice oil is a preferred cooking oil due to its delicate flavor and textural properties. Also, it has been suggested that food cooked in rice oil has a longer shelf life because of the natural abundance of antioxidant compounds. Additionally, recent evidence that rice oil may possess health-promoting properties has increased interest in developing commercial applications. A patent has been granted that describes the use of rice oil in blends with less stable oils, such as soybean oil, in order to increase overall stability. Another patent has been granted for the incorporation of rice oil into mayonnaise in order to reduce cholesterol absorption from mayonnaise-containing food. Rice oil may also be ideal for use in margarine. Because of its lower level of unsaturated fatty acids, it would require less hydrogenation, which would reduce trans-fatty acids. Also, rice oil has a natural tendency to produce more stable beta prime crystals, which would give rise to a smoother, creamier texture. Rice-oil-based margarine could be an alternative to recently released cholesterol-lowering margarines that contain phytosterols or stanols. Rice oil has a naturally high abundance of these compounds, whereas some of the currently available products have incorporated phytosterols from other sources such as pine tar.

The combination of unique sensory properties and functional applications for rice oil, along with potential health benefits, has increased the utility of rice oil as a viable commercial entity. In addition, rice oil possesses a unique abundance of components that have commercial potential as valuable by-products. As previously mentioned, these include waxes, lecithin, oryzanols, tocopherols, tocotrienols, and carotenoids.

Waxes tend to be difficult to isolate and purify; however, the high concentrations of wax in rice oil could be potentially valuable if more efficient methods of purification could be developed. Combining supercritical fluid extraction with fractionation offers some hope in this regard. Waxes are primarily used in

nonfood applications; however, recent evidence suggests that certain long-chain alcohols found in wax could have health benefits. Crude rice oil also tends to be high in gums, which could have commercialization potential if appropriate recovery processes were developed. Currently, much of the lecithin production comes from the soy-oil-processing industry, where economies of scale make it a more economical source. However, the reputation of rice as a nonhypoallergenic food could provide an alternative to soy lecithin. Oryzanols are an interesting class of compounds made up of triterpene alcohols, including sterols and stanols, esterified to the phenolic compound ferulic acid. Crude oil can have up to 2% oryzanol, which can be concentrated during processing. However, oryzanol does not currently have Food and Drug Administration (FDA) additive status, which limits its commercial application beyond its normal existence in rice oil. As such, it has been touted as an antioxidant and for its potential to lower serum cholesterol. Tocopherols and tocotrienols are considered vitamin E vitamers (i.e., possess vitamin E activity), but tocotrienols may have unique health benefits such as lowering serum cholesterol and exhibiting anticancer activity toward certain types of cancer such as breast and colon cancer. Rice bran oil is one of the richest sources of tocotrienols, especially the gamma homologue, for which the greatest health benefits have been suggested.

#### Corn Oil

Corn oil has been promoted traditionally, based on its light color and texture, as an ideal salad and cooking oil. It also tends to have good flavor stability and a high smoke point, which makes it a good choice for fried foods. Corn oil margarine is another product, well known for its perceived health benefits related to low saturated fat and high linoleic acid content. As previously mentioned, by-products from refining corn oil include the tocopherols from deodorizer distillate and carotenoids from bleaching. Recent research has revealed that the oil obtained from corn fiber generated during wet milling is very high in ferulate esters of sterols and stanols, similar to the oryzanol component of rice bran.

# **Quality Factors**

The fatty acid composition of rice and corn oil is depicted in **Table 1**. Rice oil contains more oleic and palmitic acids than does corn oil; the linoleic acid content is, however, less in rice oil. Both are very low in linolenic acid, which in part contributes to their oxidative stability and utility as frying oils.

Table 1 Composition of major fatty acids in rice and corn oil

Fatty acid (common name)	Rice	Corn
Myristic (C14:0) Palmitic (C16:0) Stearic (C18:0) Oleic (C18:1)	<1.0 12-18 <3.0 40-50	<1.0 9-14 0.5-4 24-42
Linoleric (C18:2) Linoleric (C18:3)	29-42 <1.0	32–62 <2.0

Table 2 Quality factors for rice and corn oil

Oil	FFA (%)	Nonsaponi- fiables (%)	Peroxide value (meq kg <sup>-1</sup> )	lodine value
Corn	1.5-4.0	<2.0	0.3	103-133
Rice	3-15	6-8	0.6	92-115

As stated previously, rice and corn oils are rich sources of a variety of compounds that may have health benefits. Both are unique in their high concentrations of vitamin E vitamers. Corn oil tends to be higher in tocopherols, whereas rice oil is higher in tocotrienols; in both cases there is an especially large concentration of the gamma homologue. Both also contain relatively high levels of ferulic acid esters, although rice oil has higher levels of dimethyl triterpene alcohol forms and corn oil is higher in sterol forms.

Typical oil quality standards are shown in **Table 2**. Rice oil has a much higher level of free fatty acids than corn oil, which is a reflection of the susceptibility of rice oil to hydrolytic cleavage by active lipases. Also, the nonsaponifiable fraction of rice bran oil is quite high compared with corn and most other grains. This is due to the high concentration of tocopherols, tocotrienols, oryzanols, and especially phytosterols. Rice and corn oils have very low peroxide values, which is a reflection of their excellent oxidative stability. The higher degree of unsaturated fatty acids in corn oil is seen in its higher iodine value.

#### Conclusion

Rice and corn oils have distinctive characteristics that lend themselves to commercial utilization, but both tend to be underutilized relative to total world production of the cereals from which they are derived. Also, both could potentially generate a wide variety and abundance of valuable by-products during their production and processing. Since both are byproducts of the milling of cereals to produce other primary products, increasing their utilization would depend on changes in the milling process itself. In the case of rice, more efficient milling processes that at least separate bran from hulls and possibly germ from bran would be needed. Also, processing systems would be needed that could rapidly stabilize and/or extract the bran in order to prevent lipid degradation. In the case of corn, the primary limitation is the low level of milling currently practiced in the corn-production and corn-processing industry. Increasing applications for milled corn products in the food industry will most likely increase the percentage of the crop that is milled. Also, the continuing consideration of corn as an alternative fuel source through ethanol production could increase the amount of milled corn available for oil extraction.

It is likely that the production and utilization of rice and corn oil will increase, especially as the population becomes more aware of their health benefits, but it is unlikely that either will ever become a major commodity.

See also: Lipid Chemistry. Maize: Dry Milling; Wet Milling. Nutraceuticals from Grains. Oilseeds, Overview. Rice: Chinese Food Uses.

# **Further Reading**

Chakraverty A and Singh RP (2001) Postharvest Technology: Cereals, Pulses, Fruits and Vegetables, pp. 274–283. Enfield, NH: Science Publishers.

De Greyt W and Kellens M (2000) Refining practice. In: Hamm W and Hamilton RJ (eds.) *Edible Oil Processing*, pp. 79–128. Sheffield, England: Sheffield Academic Press.

Fils J (2000) The production of oils. In: Hamm W and Hamilton RJ (eds.) *Edible Oil Processing*, pp. 45–78. Sheffield, England: Sheffield Academic Press.

Godber JS and Juliano BO (2004) Rice lipids.
 In: Champagne E (ed.) Rice Chemistry and Technology,
 3rd edn., pp. 163–190. St. Paul, MN: American Association of Cereal Chemists.

Johnson LA (1991) Corn: production, processing, and utilization. In: Lorenz KJ and Kulp K (eds.) *Handbook of Cereal Science and Technology*, pp. 55–131. New York: Marcel Dekker.

Juliano BO (1985) Polysaccharides, proteins and lipids of rice. In: Juliano BO (ed.) *Rice Chemistry and Technol*ogy, 2nd edn., pp. 142–160. St. Paul, MN: American Association of Cereal Chemists.

McCaskill DR and Zhang F (1999) Use of rice bran oil in foods, *Food Technology* 53(2): 50–53.

Patterson HBW (1989) Handling and Storage of Oilseeds, Oils, Fats and Meal, pp. 114–117, 140–143. New York: Elsevier.

Salunkhe DK, Chavan JK, Adsule RN, and Kadam SS (1991) World oilseeds: chemistry, technology, and utilization, pp. 403–448. New York: Van Nostrand.

Sharp RN (1991) Rice: production, processing, and utilization. In: Lorenz KJ and Kulp K (eds.) *Handbook of Cereal Science and Technology*, pp. 55–131. New York: Marcel Dekker.

Taylor JB, Richard TM, Wilheim CL, et al. (1996) Rice bran oil antioxidant. US Patent #5,552,167.

#### **Relevant Website**

http://www.corn.org/web/cornoil.htm - Corn Oil Refiners Association.

# **OILSEEDS, OVERVIEW**

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# Introduction

Oilseed crops are generally grown for the oil in their seeds and vary considerably in oil content, quality, and composition – factors that rely heavily on the crop species or cultivar and upon the environmental conditions in which the crop is grown. The seed meal left after the oil has been extracted can provide a high-protein product for use in either animal or human food. In other crops, the seed meal or some other factor is more important than the oil and the oil is a by-product. For example, soybean is primarily grown for its high-protein meal and cotton for its fiber. Consequently, in oil-producing crops, plant-breeding programs are aimed both at increasing oil production and quality with the additional aim of increasing meal quality. In some oil crops, antinutritional components are present (such as gossypol in cottonseed and glucosinolates in rapeseed) that also need to be considered that may limit the use of the meal.

#### Oilseeds and Their Uses

The major use of oilseed crops is the oil, which, in many cases accounts for up to 80% of the crop value. The oil-free meal provides additional value along with various by-products such as lecithin, and a range of extracts from both meal and oil.

Vegetable oils contain 95–98% triacylglycerols (or triglycerides). The remaining fraction consists of phospholipids, mono and diacylglycerols, and unsaponifiable components including sterols and tocopherols.

The oils consist of long-chain fatty acids from C14 to C24 in length and the proportion of these fatty acids in the oil has a significant contribution to its nutritional value. In recent years, there has been a trend in human diets towards reduction of saturated fats such as palmitic acid (C16:0) and an increase in polyunsaturated (C18:2 and C18:3), and monounsaturated (C18:1) fats. Plant breeders have successfully developed more nutritionally favorable products from traditional oilseed crops by altering their fatty acid profiles and other constituents.

World consumption of oils is forecast by FAO to exceed 122 million tons (Mt) in 2005. Although demand is slowing down in developed countries as they reach saturation levels, consumption in developing countries is steadily increasing. Nonfood uses of vegetable oils in developed countries are estimated to account for ~22% by 2005 due to developments in the oleochemical industries with a shift away from petroleum-based products to environmentally friendly oleochemicals. In addition, there is an increase in technological developments, particularly with the genetic modification of oilseeds to create a new range of products. For cooking oils and salad dressings, plant breeders have selected cultivars with lower levels of polyunsaturated and saturated fats and an increase in monounsaturates for increased oxidative stability. New developments include oils with increased stearic acid to provide margarine type fats without the need for hydrogenation and the subsequent production of trans-fatty acids. The uses of vegetable oils in paints, lubricants, cosmetics, and pharmaceuticals have been well documented.

World consumption of oilseed meal is expected to reach 28 Mt by 2005. Soybean meal dominates the market, forecast to account for  $\sim$ 63% of the global meal production by 2005. It is expected, however,

that future meal production from sunflower and rapeseed will increase relative to soybean. Much of the meal is utilized as stock feed and the production of livestock products, in particular pork and poultry and more recently, aquaculture. Meal products, such as protein extracts, are used for edible purposes and consumption of whole seed, including roasted soybeans, peanuts, and sesame seed is common. The meal also has industrial applications in cosmetics, paints, and adhesives.

# Oil Analysis

The traditional methods of oilseed analysis made plant breeding and selection a slow and tedious process. The Goldfische or Soxhlet methods of oil extraction required several hours to complete and involved the use of hazardous flammable solvents. In addition, only a few samples could be processed at a time reducing the capacity of the laboratories to service the needs of plant-breeding programs. Kjeldahl nitrogen analysis to determine the nitrogen content of the meal also required the use of dangerous chemicals and strong acids. More recent advances in oil content determination include nondestructive seed analysis, such as the use of nuclear magnetic resonance (NMR) spectroscopy. The rapid and accurate nondestructive testing of seed for oil content allows the breeder to retain the seed of potentially suitable lines. The introduction of near-infra-red reflectance (NIR) spectroscopy has further enhanced the ability of laboratories to determine a wider range of seed components including oil, protein, moisture, fiber, and numerous others on relatively small samples in a short time. Consequently, hundreds of seed samples can now be analyzed each day and the intact seed returned to the breeder for further growth and evaluation.

# **New Variations on Old Crops**

The major oilseed crops of the world include soybean, cottonseed, rapeseed, sunflower, groundnut (or peanut), sesame seed, linseed (from which the name linolenic acid is derived), safflower, and mustard seed. Many other crops can be used for oilseed production including castor beans, grape seed, tobacco seed, flax, corn oil, tung beans, and okra. The oil and protein content of the major oilseed crops is shown in Table 1.

From these crops, breeders have developed specialty oil types within the different species. Sunflower oil, for example, is traditionally high in linoleic (polyunsaturated) acid and is promoted as such for its health benefits. With further plant breeding, there are now several grades of sunflower oil, from high linoleic acid to high oleic (monounsaturated) as well as intermediate types. Oleic acid, being

**Table 1** Approximate oil content of seed and protein content of oil-free meal and the main uses of selected oilseed crops

Oilseed	Oil (%)	Protein (%)	Main use
Soybeans	20	46	Food
Cottonseed	16	37	Fiber
Peanut	41	46	Food
Rapeseed	41	34	Oil
Sunflower seed	40	28	Oil
Sesame seed	40-60	42	Food
Linseed	40	32-34	Oil
Safflower seed	34	23	Oil
Mustard seed	20-50	35	Condiment

Adapted from Australian Oilseed Federation Technical and Quality Standards - 2002 and other literature values.

monounsaturated, has higher oxidative stability. In addition, the reduction in linoleic acid means that the need for hydrogenation to reduce polyunsaturated oils and the formation of trans-fatty acids is reduced. Trans-fatty acids are nutritionally undesirable and similar to saturated fats in their effects. Examples of plant species, which have been bred with increased oleic acid, include sunflowers (high oleic and Nusun<sup>TM</sup>), linseed (solin, Linola<sup>TM</sup>), cottonseed, soybean, rapeseed (canola, Monola<sup>TM</sup>), safflower, and mustard.

Other changes of significance to oilseed crops have been the development of genetically modified plants with specific applications other than for edible oils. The most obvious example of these is soybean in which large numbers of products are in development including those containing antibodies, altered amino acid profiles, zero lipoxygenase and others. Rapeseed is also in the process of undergoing numerous transformations to produce products for polymers and detergents, inks, cosmetics, pharmaceuticals, lubricants, plasticizers, and resins.

# The Crops

# Soybean Seed

The seeds are rich in protein, mainly globulins, which make up 90% of the total proteins and 36% of the seed weight. Ten percent of the seed weight is carbohydrate, mainly sugars, and 3% is starch. The seed is relatively low in oil compared to other oilseed crops at ~20%. Following oil extraction, heat treatment is used to inactivate enzymes in the high-protein meal that otherwise reduce the digestibility of the stock feed. Traditional soybean oil is polyunsaturated with 48–59% linoleic acid (C18:2) and 4–11% linolenic acid (C18:3) (Table 2). Breeding has produced cultivars with various fatty acid profiles; in particular, high oleic soybean oil, which contains up to 84% oleic, has less saturated (palmitic) acid, and is more stable for cooking purposes. The meal may also be

Table 2 The fatty acid components and quantities in the major oilseed crops (amounts in rounded percentages)

Fatty acid	Name Symbol	Palmitic C16:0	Palmitoleic C16:1	Stearic C18:0	Oleic C18:1	Linoleic C18:2	Linolenic C18:3	Arachidic C20:0	Gadoleic C20:1	Behenic C22:0	Erucic C22:1
Oilseed	Туре										
Soybean	Normal	8-14	< 0.2	2-6	17-30	48-59	4-11	< 0.6	< 0.5	< 0.7	< 0.3
Soybean	High oleic	6		3	84	2	4				
Cottonseed	Normal	21-26	< 1.2	2-3	15-22	47 - 58	< 0.5	0.2 - 0.5	< 0.1	< 0.6	< 0.3
Peanuts	Normal	8.3		3.1	56	26		2			
Rapeseed	Low erucic	2-7	< 0.6	1-3	51-70	15-30	5-14	0.2 - 1.5	0.1 - 5	< 0.6	< 2.0
	High erucic	1.5-6	<3	0.5 - 3	8-60	11-23	5-13	<3	3-15	<2	2-60
	High oleic	3-4		2-3	63 - 76	13-25	2-3		1-2	< 0.6	< 0.2
Sunflower	Linoleic	5-8	< 0.3	2-7	14 - 40	48 - 74	< 0.3	0.1 - 0.5	< 0.3	0.3 - 1.5	< 0.3
	Mid-oleic	4-5		3-4	50-75	20 - 30	<1				
	Oleic	2-5	< 0.1	3-7	75-91	2-17	< 0.3	0.2 - 0.5	0.1 - 0.5	0.5 - 1.5	< 0.3
Sesame seed	Normal	8-12	< 0.2	5-6	36 - 42	42 - 48	0.3 - 0.4	0.3 - 0.6	< 0.3	< 0.3	
Linseed	Normal	5-7	< 0.3	3-4	19-20	14-17	52-61	< 0.5	< 0.6		
	Low linolenic	6		3-4	15	73	2-3				
Safflower	Linoleic	5-8	< 0.2	2-3	8-22	68 - 33	< 0.1	0.2 - 0.4	< 0.3	< 1.0	< 1.8
	Oleic	3-6	< 0.2	1-3	70-84	9 - 20	<1	0.3 - 0.6	< 0.5	< 0.4	< 0.3
Mustard seed	B. carinata	4 - 10		<2	8-23	15-22	18-27		<2		20-50
	B. nigra	2-7		<2	10-27	15-22	11-27				33-45
	B. juncea	3-10		1-3	15-64	14-28	9-24		1-3		<40

Adapted from Codex Alimentarius Commission, Alinorm 03/17, 2003 and other literature values.

used for industrial purposes such as cosmetics, paints, and adhesives as well as various edible purposes.

#### Cottonseed

Cotton is basically grown for its fiber but the seed has several important components consisting of oil (16%), protein (37%), hull (37%), and linters (10%). Also, within the kernel is a phenolic compound called gossypol, ~1% of the seed weight: toxic to humans and monogastric animals. Cottonseed oil, prior to refining, is red due to the residual gossypol and associated products. Increased seed oil content has been a secondary objective to fiber production although the value of the oil is significant. Cottonseed oil contains  $\sim$ 47–58% linoleic acid. To make the oil more stable for cooking and to reduce the need for hydrogenation, the Commonwealth Scientific and Industrial Organization (CSIRO) in Australia have developed an alternative cottonseed oil. By "switching off" genes that convert oleic acid to linoleic they have been able to produce high oleic (73%), low linoleic (5%) oil with increased oxidative stability. Additionally, they have found it possible to alter the proportions of saturated fatty acids, palmitic and stearic, which provide the solid properties necessary for making margarine.

#### **Groundnut or Peanut**

Peanuts are a valuable source of oil and also produce a high-protein meal for stock feed. Increased yields are an important breeding priority, particularly in developing countries where yield is poor. Increased oil content is also desirable as peanuts have a wide range from 40% to greater than 60% in wild types. Reduction in linoleic acid produces oil with a better oxidative stability. Meal protein is high at ~46% but the amino acid profile is lacking in sulfur-containing amino acids particularly methionine. The components of whole peanuts are protein (26%), oil (41%), and carbohydrates (24%). The main product is the oil that has  $\sim 80\%$  unsaturated fatty acids making it a nutritionally favorable oil. Additionally, the level of oleic acid and palmitic acid makes it stable with good keeping qualities for cooking and in food mixtures. Plant selection has seen the development of new cultivars with high oleic acid levels of up to 60% and a subsequent reduction in linoleic acid content. Fatty acid profiles are influenced significantly by environmental conditions with high temperatures favoring high oleic acid contents.

# Rapeseed

Traditional rapeseed oil characteristically contains high levels of erucic acid, found to have detrimental effects on the myocardial muscle of rats fed the oil. The value of the meal was also low due to the presence of sulfur-containing compounds called glucosinolates, found to have deleterious effects on the thyroid gland of mono-gastric animals. Through plant breeding, major changes have been achieved to traditional rapeseed by increasing yield, oil, and simultaneously

increasing meal protein content. The development of cultivars with very low levels of glucosinolate in the meal has also been achieved. With the dramatic change in rapeseed from traditional types to those grown today, new terms have been used to discriminate between the types. Canola is recognized under ISO 5725, Codex Alimentarius and ISTA as cultivars of rapeseed with less than 2% erucic acid in the oil and less than 30 µmol of aliphatic glucosinolates in the meal. European nomenclature to describe cultivars of rapeseed include low erucic acid rapeseed (LEAR) and Colza which is the French name for B. napus rapeseed in general. The seed oil is the main value of the crop yielding 42% oil, while the meal contains 35% protein. Current canola cultivars have very low erucic acid and saturated fatty acids levels and a good proportion of oleic (C18:1), linoleic (C18:2), and  $\omega$ -3 linolenic (C18:3) fatty acids. The balanced fatty acid profile makes rapeseed ideal for mayonnaise and salad dressings as well as a wide range of applications in the bakery and confectionary industry. New high oleic/low linolenic types have improved oxidative stability for cooking and longer shelf life. Alternatively, high erucic oil is utilized for industrial purposes such as cutting oils. Erucic acid is extracted from high erucic rapeseed and converted to erucamide for plastic manufacture.

#### Sunflower

Sunflower is grown for the seed oil, which is  $\sim 80\%$  of the seed value. The de-hulled meal has 28–42% protein. The oil is highly considered due to its low linolenic acid value and therefore oxidative stability for cooking, salad oil, and margarines. Heritability of high oil is reliable and it has been possible to increase oil contents from 30% in early types to over 50% in recent years. Sunflower cultivars with a range of fatty acid profiles have been developed including high oleic acid (75–91%) with reduced levels of linoleic acid (2–17%) and high levels of alpha tocopherol (vitamin E), providing the market with very stable monounsaturated oil for cooking purposes. Midoleic cultivars are also available, such as NuSun<sup>TM</sup>, with saturated fatty acid levels of 8% and only 20-30% linoleic acid. The meal has high protein content and is used in animal feed for livestock and poultry. A small percentage of the crop is used for nonoilseed production for confectionary purposes.

#### **Sesame Seed**

Sesame seeds are used intact or as oil and meal. The seeds generally have high oil content of  $\sim 50\%$  and  $\sim 25\%$  protein, although the oil content can vary between 40-60%. The fatty acid composition also

shows a large range (Table 2). A high oleic (40%) and linoleic acid (45%) content makes the oil nutritionally beneficial. The unsaponifiable fraction of the oil contains sesamine and sesamoline that during the refining process form sesamol and sesaminol. These are strong antioxidants that give the oil exceptional resistance to oxidation and rancidity. The oil-free meal is high in protein (34–50%), depending on the variety, and has a favorable amino acid profile with high methionine and low lysine content. Today the seed is used for human consumption on bread rolls, health food and confectionary.

#### Linseed

Due to its high iodine value, linseed oil has been used primarily for industrial purposes, such as linoleum floor covering, with a high level of unsaturated fatty acids making the oil very reactive and resulting in a short shelf life. Low linolenic acid cultivars have introduced linseed to the edible food market. In 1994, the Flax Council of Canada developed the term "Solin" to describe linseed with less than 5% linolenic acid. The original hybridization work was carried out by the CSIRO in Australia with the release of two Linola cultivars in 1992 under the Plant Varieties Rights Scheme. Linola 947 was the first Solin cultivar registered in Canada. Solin cv. Linola<sup>TM</sup> 989 has been reported as 46% oil (dry basis) and 34% protein. Linseed meal has a high crude protein value but low lysine levels. It also has a high level of soluble fiber, called mucilage that is indigestible to nonruminants and reduces the energy value of the meal. Linseed is traded at 40% oil although the oil can range significantly depending on growing conditions.

#### **Safflower**

Safflower was originally domesticated for its flowers: red carthamin dye was extracted for coloring food and cloth. Early cultivars were unsuitable for commercial development due to low oil content of  $\sim 30\%$ . Breeding programs have since increased oil contents and altered the fatty acid profiles to take it from an industrial product to the edible oil market with modern cultivars containing 34% or more oil. Traditional high linoleic types contain  $\sim 68-83\%$  linoleic acid and 8-22% oleic acid (Table 2). Through plant breeding, high oleic levels (70-84%) have been achieved. Due to the wide range of environmental conditions from countries as diverse as Australia, India, and China, fatty acids may vary considerably. The meal from safflower seed is also valued as a stock feed. The protein content of the meal remains relatively low in relation to other oilseeds at only 23%.

#### **Mustard Seed**

Typically mustard oil is high in erucic acid (20-50%) and the meal is high in glucosinolate compounds  $(50-150\,\mu\mathrm{M\,g^{-1}})$ , providing pungency to mustard for condiments but reducing the palatability of the meal for stock feed. With plant breeding, very low levels of erucic acid have been achieved and current aims are to develop cultivars that are more closely related to low erucic rapeseed cultivars, with increased oleic acid content.

#### **Production and Trade**

For the purpose of this article, fruit oils will not be discussed although it is significant. Palm oil fruit (from which comes the name palmitic acid) production in 2002 exceeded 136 Mt, second only to soybean. Oilseed yields from 1982 to 2002 are shown in Figure 1 and the area grown and yields of the various crops in 2001 and 2002 in Table 2.

#### Soybean

The global oilseed market is dominated by soybean with a total of  $\sim$ 180 Mt produced in 2002, making up almost 50% of the total oilseed production (Table 3). The United States of America produced 40%, with Brazil (23%), Argentina (17%), and China (9%) also producing significant amounts. Not unexpectedly, the United States is the largest exporter followed by Brazil and Argentina.

#### Cotton

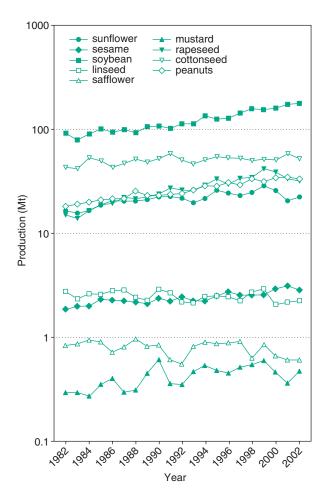
Production of cotton in the last 20 years has gradually increased (Figure 1) with the result that cottonseed is the second largest oilseed crop despite its major purpose for textile use. China is the largest producer, with ~15 Mt per annum, together with the United States, India, and Pakistan. Australia is the leading exporter of cottonseed while Mexico, Spain, Japan, Italy, Korea, and United States are large importers.

#### **Peanuts**

A global peanut production of 35 Mt is led by China (14 Mt) with India (19%), Nigeria (8%), the United States (5%), and Indonesia (3%) also being significant producers.

### Rapeseed

Rapeseed is currently rated in fifth position in world production of oilseeds with 33 Mt produced in 2002. There has been a continual decline from 1999 to 2002 (Figure 1) due partly to environmental conditions in leading producer countries. China is the largest producer of rapeseed with nearly one-third of the world



**Figure 1** Global production of major oilseed crops (log<sub>10</sub>) from 1982 to 2002 in million tons (Mt). (Adapted from Food and Agricultural Organization of the United Nations Statistics.)

**Table 3** World production of the major oilseed crops for 2001 and 2002

	Area harvested (Mha)		Production (Mt)	
	2001	2002	2001	2002
Soybeans	76	79	177	180
Cottonseed	34	32	61	54
Groundnuts	25	26	36	35
Rapeseed	22	23	34	33
Sunflower seed	18	20	21	23
Sesame seed	8	7	3	3
Linseed	3	3	2	2
Safflower seed	0.9	0.8	0.6	0.6
Mustard seed	0.5	0.6	0.4	0.5

Adapted from Food and Agricultural Organization of the United Nations Statistics.

production with India, Germany, France, and Canada also being major producers. Canada is the major exporter of rapeseed (canola) with exports of nearly 4 Mt in 2001, while France and Australia exported

nearly 1.5 Mt each. Japan is the biggest importer of rapeseed (canola) importing over 2 Mt in 2001, with China (1.7 Mt), and Mexico (0.9 Mt) also being major importers.

#### **Sunflower**

Sunflower production has increased dramatically in the period between 1982 and 2002, from 16 Mt toward 25 Mt (Figure 1). Argentina has been the major producer of sunflower seed in recent years with 17% of the total global production. Ukraine was the major exporter in 2002 with 584 000 T. Other exporters included France, Russia, Hungary, USA, and Romania. Major importers include Netherlands, Spain, Germany, Turkey, Italy, and Portugal.

#### Sesame

World production of sesame seed is increasing, from 2–3 Mt between 1982 to 3.17 Mt in 2001. All but a small part of total world production is from developing countries including India, Sudan, Myanmar, and China. India and Sudan are the largest exporters of sesame. In 2001, Japan imported 148 000 t. Other major importers were Korea, Egypt, USA, and China.

#### Linseed

World production of linseed has shown a gradual decline since the 1980s despite a peak in 1999 of almost 3 Mt (Figure 1). The total production of 2.21 Mt in 2001 was the lowest level of production in the previous forty years. The major producers of linseed are Canada, China, USA, and India. Canada is the major exporter with over 600 000 T exported in 2001.

# **Safflower**

Production of safflower has also fallen in the 1980s from ~900 000 t in 1985 to 600 000 t in 2002 (Figure 1). This may reflect the increased demand for monounsaturated oil in place of polyunsaturated oils. The major safflower producing countries are India, USA, and Mexico. The USA (21812t) and Australia (13660t) accounted for 72% of the world exports. Japan is a major importer.

#### Mustard

There has been a gradual increase in world production of mustard since the 1980s (Figure 1) from ~360 000 t in 2001 to 500 000 t in 2002. Major producers include Nepal, Canada, and Russia. In 2001, Canada exported 152 000 t of mustard while the Czech Republic, Germany, Russia, and Hungary were also significant exporters. Bangladesh was the major importer in 2001.

# **Future Developments**

Oilseeds play an increasingly important role in society, both as an edible food product and for industrial uses. The benefits include the valuable oil component and the secondary but also useful meal, which provides a high energy and nutritionally important food. Breeders have been able to optimize the characteristics of many of these crops to achieve higher levels of production and to alter the products to suit specific requirements. These changes include improvements in nutritional value, particularly with changes in fatty acid profiles and the reduction in antinutritional components. Undoubtedly the biggest changes will come in the future from genetic modification in which long term breeding programs can be dramatically reduced to relatively short periods of time. Traditional, breeding techniques rely on natural variability in plant populations to achieve change. Genetic engineering can create new products previously out of the reach of established breeding techniques. Engineering plants for specific aims or more suitable to environmental conditions can increase production. Nutritionally, fatty acid profiles can be dramatically altered to reduce saturated and trans fatty acids and simultaneously improve oxidative stability. Vegetable oils are already being substituted, in many instances, for less environmentally friendly petroleum products. The major competition to oilseed crops in the future will only be from the ever-growing range of additional oilseed types.

See also: Canola: Agronomy. Cottonseed. Peanuts. Soybean: Agronomy. Sunflower.

# **Further Reading**

Appelqvist L-A and Ohlson R (eds.) (1972) Rapeseed: Cultivation, Composition Processing and Utilization. Amsterdam: Elsevier.

Benedict JH, Treacy MF, and Kinard DH (eds.) (1994) Vegetable Oils and Agrichemicals (ISBN: 0-939809-04-4). Memphis, TN: Cotton Foundation and National Cottonseed Products Association.

Bennett M (1998) Sesame seed. In: A Handbook for Farmers and Investors. Rural Industries Research and Development Corporation of Australia.

FAO (2003) Food and Agriculture Organization of the United Nations, FAO Statistical Database 2003.

Hui YH (ed.) (1996) Baileys Industrial Oil and Fat Products, 5th edn., Series 3 (ISBN: 047159427X). Wiley.
 Karleskind A (1996) Oil and Fats Manual. France: Lavoisier Publishing.

Kimber DS and McGregor DI (eds.) (1995) *Brassica Oilseeds*, *Production and Utilisation*. UK: CAB International.

Röbbelen G, Downey RK, and Ashri A (1989) Oil Crops of the World. USA: McGraw-Hill.

Woodroof JG (1983) Peanut Production, Processing, Products, 3rd edn. Connecticut: AVI.

Weiss E (2000) Oilseed Crops, 2nd edn. Malden, MA: Iowa State Press.

## **Relevant Websites**

http://www.aocs.org – American Oil Chemists' Society.

http://www.amsoy.org - American Soybean Association.

http://www.australianoilseeds.com – Australian Oilseed Federation.

http://www.grainscanada.gc.ca - Canadian Grain Commission.

http://www.canola-council.org - Canola Council of Canada.

http://www.codexalimentarius.net - Codex Alimentarius Commission.

http://www.flaxcouncil.ca - Flax Council of Canada. http://apps.fao.org - Food and Agriculture Organization.

http://www.cottonseed.com - National Cottonseed Products Association.

www.sunflowernsa.com – National Sunflower Association.

www.regional.org.au - 10th International Rapeseed Congress.

www.rirdc.gov.au — Australian Rural Industries and Research Foundation.

# **ORGANIC GROWING OF GRAINS**

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#### Introduction

Organically grown foods are also called ecologically or biologically grown foods. Organic agriculture has become more popular over the years, being practiced in  $\sim 1-5\%$  of farmed land of most countries. Organic agriculture is an alternative to the striking increase of synthetic fertilizers and pesticide, which has become necessary to maintain high-productivity crops even if it leads to environment impacts (i.e., water degradation). This alternative agricultural method represents a viable solution especially well suited for constrained and low-input conditions (i.e., climate, regulation). Nonetheless, it cannot be assured that organically grown foods are safer, healthier, or more nutritive than conventionally grown produce. However, organically grown cereal crops might contribute to human health, for example, because it does not spread toxic pesticides in the environment. This article describes organic growing of grains and its regulations. Potential advantages and limits of organic growing of grains are also presented.

# **Definition**

Contrary to conventional agricultural practice, the concept of organically grown foods is an ethical or holistic concept based on naturalness, which involves:

- 1. the absence of synthetic inorganic chemicals (laboratory-made) for growing and processing crops, like fertilizers, pesticides or insecticides;
- 2. an agro-ecological, broad, and long-term approach throughout the food chain, including care for soil fertility, recycling of agricultural materials from in-site or community, energy preservation, stimulating the self-regulating capacity of the agro-ecosystem and care for water, soil, and air quality preservation; concepts such as genetically-modified organisms (for seeds and others) and irradiation are not acceptable, and
- 3. the integrity of living nature as a whole, not strictly focused on crop production but also on animal welfare, environment, and social impact; for example, avoidance of any process or practice that might endanger the health of agricultural workers and consumers, including minimal food processing.

A key issue in the overall sustainability of the organic farming systems, compared to nonorganic, is the financial viability. On arable systems, the financial situation is critical during conversion while specific

machinery is required, yields fall and output prices are maintained without premium. Such system requires high selling prices and strong processing and marketing network. By definition, organic agriculture would suit better for locally-grown foods, considering the potentially negative impact of transportation on the environment.

In organic agriculture, the fertility and biological activity of the soil should be maintained or increased by (1) the cultivation of legumes, green manures, or deep-rooting plants in a multi-annual rotation program and/or (2) the incorporation into the soil of organic material, composted or not, from material produced under the regulations. Furthermore, organic fertilizers may only be applied if adequate nutrition of the rotating crop or soil conditioning cannot be ensured by other methods.

Weeds are often mentioned as the most significant problem in organic agriculture. Because no herbicides are allowed, the emphasis is on prevention and control technology. Preventive measures include: reduction of the supply of weed seeds and the multiplication of weeds and destruction of weeds before the crop is sown. Measures can be taken: (1) at the farm level through crop rotation and intercropping systems, (2) at the crop level by mechanical control, and (3) at the variety level by selecting genotype for competitiveness through plant architecture, rapid juvenile growth, deep rooting, and allelopathic exudates.

Pest and disease control is not performed with synthetic pesticides. Most soil-borne pests and diseases can be controlled by stimulating biodiversity in and above the soil, by good soil management, and by choosing site-specific crops through balanced rotation and selection of resistant crop varieties.

# Regulations

Organic foods cannot be distinguished from nonorganic foods, even if analyzed for pesticides or nutritional content. Especially because premium price is paid for organic crops and foods, control is necessary to avoid fraud and determine which food is organic and which is not. Organically produced foods must meet international standards. For legally registered labeling, accredited certification agencies inspect farms and processing plants. Food production and process must be certified as organic, not the food itself. Principles are close to Hazard Analysis and Critical Control Point (HACCP), including regulations (on farmers' practices and food processing) and traceability. This means that detailed records of production techniques and inputs are subjected to audits from certification agencies.

Long before HACCP became a popular acronym, artisans of organic agriculture devised internal rules to ascertain that organic really meant organic. Within countries, legislations have been set up over the years, based on guidelines provided by Codex Alimentarius Commission created in 1962 to establish international norms for food on behalf of FAO (Food and Agriculture Organization) and WHO (World Health Organization). These norms are designed to favor food trade and protect human health, setting a minimum quality level for safety and trade. In 1999, this organization published international rules for production, processing, labeling, and commercialization of food produced according to organic agriculture. Production, processing, and commercialization (including labeling) of organic produces have been defined within the regulation. Depending on country or certifying group, the standards must be applied in full including withdrawal of all synthetic fertilizers and pesticides, after a transition period of 2 to 5 years before certifying organic products.

Worldwide, ISO Standard 65 has become the most accepted standard for organic certifiers. Within a country, control is made by representative organizations, which are, in some countries, recognized by government. Worldwide, the international market requires assurance of equivalency because definition of organic may slightly differ according to certification agency. In 1992, the International Federation of Organic Agriculture Movements (IFOAM) established the IFOAM Accreditation Program (IAP) to provide international equivalency of organic quality claims. The IFOAM is recognized as the leading group for organic agriculture organizations. A major inspiration for Codex standards, the IFOAM Basic Standards provide a framework for certification bodies and standard-setting organizations worldwide to develop their own certification standards (but cannot be used for certification on their own). Certification standards should take into account specific local conditions and provide more specific requirements than the IFOAM Basic Standards. Table 1 presents examples of approved inputs (fertilizers, soil conditioners, crop protectants, and growth regulators) for producing organic crops, based on IFOAM Basic Standards. This reflects the complexity of regulating the production of organically grown crops, a major task, which has been made through the years due to the commitment of organizations like IFOAM.

# **Food Processing Issues**

Although not ideal, the same processing facility may generally be used to process organic and nonorganic grain. First batch in the morning should be reserved to

Pest and disease control

**Table 1** Examples of approved inputs for organic crops production<sup>a</sup>

Substance Condition for use Acids (natural) (e.g., vinegar) As crop protectant or growth regulator Algal preparations (seaweeds and seaweeds preparations) As crop protectant or growth regulator Animal by-products (blood meal, meat meal, bone, bone As fertilizer or soil conditioner meal, hoof and horn meal, feather meal, wool, fur, hair) Animal preparations and oils As crop protectant or growth regulator Bacterial preparations (e.g., Bacillus thurigiensis) As crop protectant or growth regulator As fertilizer or soil conditioner Basic slag As crop protectant or growth regulator Beeswax Biodynamic preparations As fertilizer, soil conditioner, crop protectant or growth regulator By-products (biodegradable) from brewery, distillery, food, As fertilizer or soil conditioner feed, oilseed or textile processing Calcareous and magnesium amendments As fertilizer or soil conditioner Calcium chloride As fertilizer or soil conditioner Calcium hydroxide As crop protectant or growth regulator Carbon dioxide As crop protectant or growth regulator Chalk As fertilizer or soil conditioner Chitin nematicide (natural origin) As crop protectant or growth regulator Chloride of lime As crop protectant or growth regulator Clay (e.g., bentonite, perlite, vermiculite, zeolite) As fertilizer, soil conditioner, crop protectant, or growth regulator As fertilizer or soil conditioner Compost from acceptable inputs (incl. spent mushroom waste, humus from worms and insects, urban composts from separated sources which are monitored for contamination) Coffee grounds As crop protectant or growth regulator Copper salts (e.g., sulfate, hydroxide, oxychloride, octanoate) As crop protectant or growth regulator; maximum 8 kg ha<sup>-1</sup> per year (on a rolling average basis) Corn gluten meal For weed control Crop and vegetable residues, including mulch, green manure, As fertilizer or soil conditioner and straw Dairy products (e.g., milk, casein) As fertilizer, soil conditioner, crop protectant, or growth regulator Diatomaceous earth As crop protectant or growth regulator; also as pest and disease control Epson salt (magnesium sulfate) As fertilizer or soil conditioner Ethanol (ethyl alcohol) As crop protectant or growth regulator Fish and fish products As fertilizer or soil conditioner Fumigants (ethylene oxide, methyl bromide, aluminum Forbidden phosphide or other) Fungal preparations As crop protectant or growth regulator Gelatin(e) As crop protectant or growth regulator GMO (genetically engineered organisms) Forbidden Guano (excrement of seabirds) As fertilizer or soil conditioner Gypsum (calcium sulfate) As fertilizer or soil conditioner Homeopathic and Ayurvedic preparations As crop protectant or growth regulator Insects (sterilized) As crop protectant or growth regulator Kieserite As fertilizer or soil conditioner Lecithin As crop protectant or growth regulator Limestone As fertilizer or soil conditioner Lime sulfur (calcium polysulfide) As crop protectant or growth regulator Magnesium rock, magnesium sulfate As fertilizer or soil conditioner Marl (maerl) As fertilizer or soil conditioner As fertilizer or soil conditioner; human excrements must not be Manure (incl. farmyard slurry and urine; guano) applied on edible parts; no urea allowed As fertilizer or soil conditioner Microbiological preparations based on naturally occurring organisms Mineral oil (light such as paraffin) As crop protectant or growth regulator Neem (Azadirachta indica) As crop protectant or growth regulator Parasites, predators and sterilized insects As crop protectant or growth regulator As fertilizer or soil conditioner; prohibited for soil conditioning, permitted for potting mixes

> Only physical barriers, sound, ultra-sound, UV-light, traps (including pheromone traps and static bait traps), temperature control, controlled atmosphere, and diatomaceous earth

Table 1 Continued

Substance	Condition for use
Pheromones	As crop protectant or growth regulator; in traps and dispensers only
Plant preparations and extracts	As fertilizer, soil conditioner, crop protectant or growth regulator
Plant oils	As crop protectant or growth regulator
Plant based repellents	As crop protectant or growth regulator
Phosphates (natural)	As fertilizer or soil conditioner
Potassium (mineral, including sulfate of potash, muriate of potash, kainite, sylvanite, patentkali)	As fertilizer or soil conditioner; only those obtained by physical procedures, not enriched by chemical processes
Potassium bicarbonate	As crop protectant or growth regulator
Potassium permanganate	As crop protectant or growth regulator
Propolis	As crop protectant or growth regulator
Pyrethrum (Chrysanthemum cinerariaefolium)	As crop protectant or growth regulator; piperonyl butoxide is prohibited
Quassia (Quassia amara)	As crop protectant or growth regulator
Quicklime	As crop protectant or growth regulator
Rock (pulverized) and stone meal	As fertilizer or soil conditioner
Rotenone ( <i>Derris elliptica, Lonchocarpus</i> spp., <i>Thephrosia</i> spp.)	As crop protectant or growth regulator
Ryania (Ryania spaciosa)	As crop protectant or growth regulator
Sabadilla	As crop protectant or growth regulator
Salt (sea) and salty water	As crop protectant or growth regulator
Seeds (organic, if available)	Only if nontreated with forbidden pesticides
Sewage sludge (municipal)	Forbidden
Silicates (e.g., sodium silicate, quartz)	As crop protectant or growth regulator
Soap (soft)	As crop protectant or growth regulator
Sodium bicarbonate (soda)	As crop protectant, growth regulator, crop protectant or growth regulator
Sugar beet lime	As fertilizer or soil conditioner
Sulfur	As fertilizer, soil conditioner, crop protectant or growth regulator
Sulfur dioxide	As crop protectant or growth regulator
Tobacco tea	As crop protectant or growth regulator; pure nicotin is forbidden
Trace elements (boron, copper, iron, manganese, molybdenum, zinc)	As fertilizer or soil conditioner
Vermicastings	As fertilizer or soil conditioner
Viral preparations (e.g., granulosis virus)	As crop protectant or growth regulator
Wood and wood by-products (ash, bark, charcoal, sawdust, shavings)	As fertilizer or soil conditioner

<sup>&</sup>lt;sup>a</sup>Based on IFOAM Basic Standards for Organic Production and Processing (2002).

organic grain. Thorough cleaning must be used between transitions, leaving a transition period. In general, certified organic foods must be made with a minimum of 95% of organic ingredients (by weight), without considering water and salt. So-called organic foods may then be prepared with 5% of nonorganic ingredients, based on total weight of ingredients except water and salt. A major issue among organic certification groups is the list of approved ingredients and processing aids of nonorganic or synthetic origin. Table 2 presents a list of nonorganic ingredients and processing aids, permitted in organic foods.

# **Potential Advantages**

1. Alternative agriculture is adapted to low-input and constrained conditions. Organic agriculture tends

- to limit soil erosion, compaction, or degradation by using crop diversity. It also limits the use of inputs like mineral fertilizers and synthetic pesticides.
- 2. Small is beautiful. Organic agriculture is a way to say that more modern and bigger is not necessarily better. For developing countries such as Cuba, organic agriculture is a way to seek independence over imported pesticides and mass monoculture of export crops requested by rich countries. Otherwise, the economy of developed countries is too often severely dependent upon international markets. For example, organic agriculture is adapted to family farming by increasing profitability per unit. In brief, organic crops cost less to produce (no expensive chemical fertilizers and pesticides, substituted by manure), have lower yields (25–35% less for grains), but get higher price (30–100% compared to nonorganic crops, sometimes more).

Table 2	Nonorganic ingredients	(I), additives	(A), and processing aids (P) approved in organic foods <sup>e</sup>	1
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Ingre	dient (I), additive (A), or processing aid (P) <sup>b</sup>	Restriction <sup>c</sup>
I	Acorns (Quercus spp.)	Only if unprocessed and not produced in sufficient quantity in EU (only in EU Regulation)
Р	Activated carbon (charcoal)	Not in US Regulation (status pending, only as filtering aid)
A I	Agar (agar-agar) (generally used as gelling agent) Algae, including seaweeds	Only if unprocessed and not produced in sufficient quantity in EU (only in EU Regulation)
Α	Alginic acid	
Α	Amino acids and nitrogen compounds	When required by legislation (only in EU Regulation)
A	Ammonium carbonate (bicarbonate)	Only as a leavening agent
A	Ammonium phosphates (incl. monoammonium phosphate, diammonium phosphate, phosphate dibasic or monobasic)	Only in wine (max. $0.3 \text{ mg I}^{-1}$ ) (only in IFOAM)
Α	Ammonium sulfate	Only in wine (max. 0.3 mg l <sup>-l</sup> ) (only in IFOAM)
I	Aquatic organisms	Only if not from aquaculture and if not produced in sufficient quantity in EU (only in EU Regulation)
IA	Arabic gum (gum arabic) (generally used as gelling agent)	Only for milk products, fat products, confectionary, sweets, eggs (IFOAM and Codex); only if nonsynthetic and water extracted (US)
Α	Argon	Not in US Regulation
Α	Ascorbic acid (generally used as dough oxidation agent)	Synthetic form acceptable only if not available in natural form (Codex)
AP	Bacterial starters (incl. dairy cultures)	Only if not from GMO (product of rDNA technology)
P	Bark, preparation of	Only for sugar (IFOAM)
P P	Beeswax Bentonite	Not in US Regulation (pending); as releasing agent (Codex) Only for fruits and vegetable products (IFOAM); only if nonsynthetic (US)
AP	Calcium carbonate (generally used as buffer in yeast foods)	Except in coloring (EU); only if nonsynthetic (US)
AP	Calcium chloride	Only in milk products, fat products, fruits and vegetables, and soybean products (Codex); only as coagulation agent (Codex and EU); only if nonsynthetic (US)
Α	Calcium citrate	
AP	Calcium hydroxide	Only for maize tortilla flour and as processing aid for sugar (IFOAM)
A	Calcium phosphate (monobasic, dibasic, tribasic)	Uses discussed include baking powder, fortification, for yeast growth, and as a firming agent for yogurt (US); only monocalcium phosphate specified for IFOAM, Codex, and EU
AP	Calcium sulfate (generally used as dough firming agent)	Only for soybean products, cakes and biscuits, confectionery and bakers' yeast (IFOAM and Codex); only as carrier or coagulation agent (Codex and EU); only if nonsynthetic (US);
AP	Carbon dioxide (generally used as preservative agent in controlled atmosphere)	
P	Carnauba wax	Only as releasing agent (Codex)
IA •	Carob bean gum (locust bean gum) (generally used as stabilizing or gelling agent)	Only if nonsynthetic and water extracted (US)
A	Carrageenan (carrageen) (generally used as stabilizing or gelling agent)	Only if nonsynthetic (US)
P D	Casein	Not in US Regulation; only for wine (IFOAM)
P	Chlorine; calcium hypochlorite; chlorine dioxide; sodium hypochlorite	Only in US Regulation, for disinfecting and sanitizing food contact surfaces
A D	Cellulose	Only in US Regulation for use in regenerative casings as an anticaking agent (nonchlorine bleached) and filtering acid
ΑP	Citric acid	Only if produced by microbial fermentation of carbohydrate substances (US); only for fruits and vegetable products, and pH adjustment (Codex); only for oil production and hydrolysis of starch (EU)
I	Cola nuts (Cola acuminate)	Only if unprocessed and not produced in sufficient quantity in EU (only in EU Regulation)
Α	Coloring agent (color)	Only if not a synthetic substance primarily judged as being unnatural or as a "new construction" of food compounds; only if not produced by genetic engineering

Table 2 Continued

Ingred	dient (I), additive (A), or processing aid (P) <sup>b</sup>	Restriction <sup>c</sup>
I	Cornstarch (native)	Only in US Regulation (only if nonsynthetic)
AP	Cultures, dairy	Considered as bacterial starters; only if not from GMO (product o rDNA technology)
Α	L-cysteine (generally used as dough conditioner; reducing agent)	Prohibited
Р	Diatomaceous earth	Only for sweeteners and wine (IFOAM); only if nonsynthetic and as food filtering aid (US)
Р	Egg white albumen (albumin)	Not in US Regulation; only for wine (IFOAM)
AP	Enzymes	Only if derived from edible, nontoxic plants, nonpathogenic fungi or nonpathogenic bacteria; only if not from GMO; only if nonsynthetic (US); US Regulation also includes the following animal-derived enzymes: rennet-animals derived, catalase- bovine liver, animal lipase, pancreatin, pepsin, and trypsin (only in US Regulation)
Р	Ethanol	Only as solvent (Codex and EU); not in US Regulation
Р	Ethylene	Only for postharvest ripening of tropical fruit (US only)
Р	Extraction	Only with water, ethanol, plant and animal oils, vinegar, carbon dioxide, or nitrogen (IFOAM)
I	Fats and oils (other than cocoa, coconut, olive, sunflower, palm, rape, safflower, sesame, or soya)	Only if not produced in sufficient quantity in EU (only in EU Regulation)
Α	Ferrous sulfate (iron)	Only in US Regulation, for food fortification; see minerals
Р	Filtration	Only if not with asbestos or with technique that chemically react or modify food on a molecular basis (IFOAM)
Α	Flavoring agent or extract (essential or volatile oil)	Only if not a synthetic or unnatural substance; only if not produced by genetic engineering; only if not produced by nonsynthetic solvents, carriers or preservatives such as oil, water, ethanol, carbon dioxide and mechanical and physical processes (US)
I	Fructose (from cereals and tubers)	Only if not produced in sufficient quantity in EU (only in EU Regulation)
Р	Gas (incl. carbon dioxide, oxygen, and nitrogen)	Permitted for controlled atmosphere (IFOAM)
Р	Gelatin(e)	Only for wine, fruit, and vegetables (IFOAM); not in US Regulation (pending)
Α	Glucono delta-lactone	Only if nonsynthetic and not produced by the oxidation of p-glucose with bromine water (only in US Regulation)
Α	Glycerol, glycerine	Only from plant extract (EU); only if produced by hydrolysis of fats and oils (US)
AIP	GMO (from genetically modified ingredients)	Forbidden
I	Gooseberries (Ribes uva-crispa)	Only if unprocessed and not produced in sufficient quantity in EU (only in EU Regulation)
IA	Guar gum (generally used as gelling or stabilizing agent)	Only if nonsynthetic and water extracted (US)
Р	Hazelnut shells	Not in IFOAM or US Regulation
I	Horseradish seeds (Armoracia rusticana)	Only if unprocessed and not produced in sufficient quantity in EU (only in EU Regulation)
Р	Hydrogen peroxide	Only in US Regulation
Р	Irradiation (ionizing radiation)	Prohibited
Р	Isinglass	Only for wine (IFOAM); not in US Regulation
Р	Isopropanol (propan-2-ol)	Only in sugar crystallization, until 31.12. 2006 (only in EU Regulation)
Р	Kaolin	Only if nonsynthetic (US)
Α	Karaya gum (generally used as gelling or stabilizing agent)	Not in IFOAM or US Regulation
Α	Kelp	Only if nonsynthetic and for use as a thickener and dietary supplement (only in US Regulation)
Α	Kirsch (from fruits and natural flavorings)	Only if not produced in sufficient quantity in EU (only in EU Regulation)
AP	Lactic acid	Only in fermented vegetable products (Codex); only if nonsynthetic and not derived from microorganisms that are products of recombinant DNA technology (US)

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Ingre	dient (I), additive (A), or processing aid (P) <sup>b</sup>	Restriction <sup>c</sup>
IAP	Lecithin	Only if unbleached and obtained without organic solvents (Codex); bleached and unbleached forms accepted in US Regulation
I	Lesser galanga (Alpinia officinarum)	Only if unprocessed and not produced in sufficient quantity in EU (only in EU Regulation)
IA	Locust bean gum (carob bean gum) (generally used as stabilizing or gelling agent)	Only if nonsynthetic and water extracted (US)
IA	Magnesium carbonate (generally used as drying and anticaking agent)	Only if nonsynthetic (US); only in agricultural products labeled "made with organic (specified ingredients or food groups)," prohibited if labeled "organic" (US)
AP	Magnesium chloride (or nigari)	Only for soybean products (IFOAM and Codex); only as coagulation agent (EU and Codex); only if derived from seawater (US)
A	Magnesium silicate (generally used as anticaking agent for salt)	Forbidden
Α	Magnesium stearate	Only in US Regulation, only in agricultural products labeled "made with organic (specified ingredients or food groups)," prohibited if labeled "organic" (US)
A A	Magnesium sulfate Malic acid	Only in US Regulation; only if nonsynthetic (US) Only in Codex
A	Malted barley	Only from an organic source
Α	Minerals and vitamins (nutrients)	When required by legislation
Α	Mono-calcium phosphate	Only for "raising flour" (IFOAM); raising agent for self raising flour (EU); for US, see calcium phosphate
P A	Mono- and diglycerides Nisin	Only in US Regulation (only for drum drying of food) Prohibited
٩P	Nitrogen	Only nonsynthetic and oil-free grades (US)
٩P	Oxygen	Only nonsynthetic and oil-free grades (US)
Р	Ozone	Only in US Regulation
l	Passion fruit or maracujas (Passiflora edulis)	Only if unprocessed and not produced in sufficient quantity in EU (only in EU Regulation)
	Pea protein (Pisum spp.)	Only if not produced in sufficient quantity in EU (only in EU Regulation)
IA	Pectin (unmodified)	Nonsynthetic (high-methoxy) and synthetic (low-methoxy) sources allowed (US)
	Pepper (Peruvian) (Schinus molle L.)	Only if unprocessed and not produced in sufficient quantity in EU (only in EU Regulation)
P P	Perlite Phosphoric acid	Only if nonsynthetic and for use as filter aid in food processing (US Only in US Regulation; only for cleaning food-contact surfaces and
A	Potassium alginate	equipment (US) Not in EU Regulation
AP	Potassium carbonate (bicarbonate) (generally used in baking powder)	Only in cereals, cakes, biscuits, and confectionary (IFOAM); only for drying of grape raisins and sugar production (Codex and EU)
A	Potassium chloride	Equivalent to sodium chloride and for food processing (EU); only ir canned or frozen fruits and vegetables, vegetable sauces; for ketchup and mustard (Codex); only if nonsynthetic (US)
Ą	Potassium citrate	Not in EU Regulation
P	Potassium hydroxide	Prohibited for lye peeling of fruits and vegetables except for peeling peaches during the individually quick frozen (IQF) production (US) only for pH adjustment for sugar processing (Codex); not in
A	Potassium iodide (generally used as dietary source of iodine for salt)	IFOAM and EU Regulation  Only in US Regulation, both synthetic and nonsynthetic allowed; if a synthetic source is used, only in agricultural products labeled "made with organic (specified ingredients or food groups)," prohibited if labeled "organic"
Α	Potassium metabisulfite	Only for wine (only in IFOAM)
A	Potassium phosphate (monobasic potassium phosphate; potassium biphosphate) (generally used in baking powder)	Only in US Regulation, only in agricultural products labeled "made with organic (specified ingredients or food groups)," forbidden if labeled "organic" (US)

Table 2 Continued

Ingre	dient (I), additive (A), or processing aid (P) <sup>b</sup>	Restriction <sup>c</sup>
AP	Potassium tartrate; potassium acid tartrate (also called cream of tartar) (generally used in baking powder)	Only if made from tartaric acid (US); only for cereals, cakes, and confectionery (Codex)
I	Rasberries (Rubus ideaeus)	Only if dried and unprocessed, and not produced in sufficient quantity in EU (only in EU Regulation)
I	Red currants (Ribes rudrum)	Only if dried and unprocessed and not produced in sufficient quantity in EU (only in EU Regulation)
Р	Rice meal	Not in IFOAM or US Regulation
I	Rice paper	Only if not produced in sufficient quantity in EU (only in EU Regulation)
I	Rum	Only from cane sugar juice and if not produced in sufficient quantity in EU (only in EU Regulation)
I	Safflower flowers (Carthamus tinctorius)	Only if unprocessed and not produced in sufficient quantity in EU (only in EU Regulation)
I	Salt (sodium chloride)	Not included in the percentage calculation of organic ingredients; equivalent to potassium chloride (EU)
P	Silicon dioxide (amorphous)	Only as anticaking agent for herbs and spices (EU); only for wine, fruit, and vegetable processing (IFOAM); only as gel or colloidal solution (Codex)
A	Sodium alginate	0.1.
AP	Sodium carbonate (or bicarbonate) (generally used in baking powder)	Only in sugar production (EU and Codex); only if nonsynthetic (US)
Α	Sodium citrate	Not in EU Regulation
AP	Sodium hydroxide	Only for the surface treatment of traditional bakery products (IFOAM and EU); only for cereal products (Codex); only for sugar production and oil production from rape seed ( <i>Brassica</i> spp.) (EU); prohibited for lye peeling of fruits and vegetables (US)
Α	Sodium phosphates	Only in dairy foods (only in US Regulation)
AP	Sodium tartrate (generally used as stabilizing or gelling agent)	Not in US Regulation; only for cakes and confectionery (Codex)
Α	Sorbic acid (generally used as preservative agent)	Prohibited
I	Starch (from rice and waxy maize)	Only if not chemically modified and not produced in sufficient quantity in EU (only in EU Regulation)
Α	Sulfur dioxide	Only for wine (IFOAM; Codex); only in wine labeled "made with organic grapes," provided that total sulfite concentration does not exceed 100 ppm (US); not in EU Regulation
Р	Sulfuric acid	Only for pH adjustment of extraction water in sugar production (Codex); only for sugar production (EU); not in US Regulation
P	Talc	Not in US Regulation
P	Tannic acid, tannin	Only as filtration aid for wine; not in US Regulation
AP A	Tartaric acid Tocopherols (mixed natural concentrates)	Only for wine (IFOAM) Allowed if derived from vegetable oil when rosemary extracts are not a suitable alternative (US); only as antioxidant in fats and oils (EU)
Α	Tragacanth gum	Not in US Regulation
I	Unleavened bread paper	Only if not produced in sufficient quantity in EU (only in EU Regulation)
Р	Vegetable oil	Only as greasing, releasing, or antifoaming agent
A IP	Vitamins and minerals (nutrients) Water (drinking)	When required by legislation  Not included in the percentage calculation of organic
I	Watercress herb (Nasturtium officinale)	ingredients  Only if unprocessed and not produced in sufficient quantity in EU (only in EU Regulation)
Р	Waxes, plant derived (beeswax, Carnauba wax)	Only as releasing agents (Codex and EU); beeswax not in US Regulation (pending)
I	Whey powder (herasuola)	Only if not produced in sufficient quantity in EU (only in EU Regulation)
Р	Wood resin	Only in US Regulation

Table 2 Continued

Ingre	dient (I), additive (A), or processing aid (P) <sup>b</sup>	Restriction <sup>c</sup>			
Α	Xanthan gum (generally used as stabilizing or gelling agent)	Only for fat, fruit and vegetable products, cakes and biscuits (IFOAM; Codex); only for salads (Codex)			
AP	Yeast (autolysate, bakers, brewers, nutritional, smoked)	Only if nonsynthetic and not grown on petrochemical substance and sulfite waste liquor; only if not from genetic engineering; nonsynthetic smoke flavoring process must be documented (US)			

<sup>&</sup>lt;sup>a</sup> Where the substances listed in this annex can be found in nature, natural sources are preferred. Substances of certified organic origin are also preferred. <sup>b</sup> Products with bold character are most likely to be found in grain-based foods. According to IFOAM (2002), *processing aids* are any substance or material, not including apparatus or utensils, and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods or its ingredients, to fulfill a certain technical purpose during treatment or processing and which may result in the non-intentional, but unavoidable presence of residues or derivatives in the final product; some specific processes have also been mentioned as processing aids. According to Codex (2001), *ingredient* means any substance, including a food additive, used in the manufacture or preparation of a food and present in the final product although possibly in a modified form. For the sake of clarity, ingredients normally used at low concentrations have been classed as *additives*.

<sup>c</sup>Codex: Based on Guidelines for the production, processing, marketing, and labeling of organically produced foods. Codex Alimentarius Commission and the FAO/WHO Food Standards Programme (2001; <a href="https://www.codexalimentarius.net">www.codexalimentarius.net</a>). EU: Based on Council Regulation (EEC) No. 2092/91 of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs (<a href="http://www.ifoam.org">http://www.ifoam.org</a>). US: Based on Regulation of the United States Department of Agriculture (The National Organic Program) (as of 3 November 2003), according to <a href="http://www.ifoam.org">www.ams.usda.gov</a>.

Large milling and baking plants are usually absent from the organic market because they are highly automated and require significant volumes to be cost-effective.

3. Road to alternative cereals. A niche market, organic cereal foods offer interesting possibilities to develop or introduce new cereal-based foods such as those made from nontraditional or specialty grains such as ancient wheat (spelt or khorasan). Niche markets are then available through organic grains, which give access to such specialty cereals containing much fiber and often having special flavors. Some people also switch to nontraditional cereals because they are sensitive (but not necessarily allergic) to standard cereal foods from wheat.

Organic agriculture is a way of life for some persons. For others, it is a way to diversify their foods. Several people are also looking for something else; this might be translated into an opposition to the leveling of the taste and appearance of foods. This is contrary to the idea of food quality as seen by food industry: nonuniformity. For several people, the popularity of organic cereals is closely linked to nostalgia of the past, especially taste. A question of lifestyle, some want to produce these foods themselves; others are willing to pay more to get foods with specific qualities they want or believe it has. And these people are willing to pay more for this insurance on food.

4. A question of confidence. Confidence of customers in food has been shaken by food crisis as Mad Cow disease. Justified or not, lack of confidence in food is one of the reasons for choosing organic foods. Internationalization of decisions on food production may also be seen as a threat, enticing some

people to come back to alternative agricultural and food consumption practices. Some references on organic foods state that they have a higher nutritive value than nonorganic foods. Others conclude that organic foods are potentially riskier considering that some of the most powerful poisons are of natural origin (mycotoxins). Also, organic foods could be related to a wish to avoid foods from genetically-engineered organisms, due to their potentially negative impact on environment.

Are organic foods much better both for health and earth? There is no guarantee that organic foods are safer, tastier, and more nutritive than nonorganic foods. In general, it is recognized that organic foods are likely to contain less residues of agricultural chemicals than nonorganic foods. However, this source of contamination is considered very low in any food. For the moment, health risks might be more important for growers themselves (plus their relatives and neighbors) who are in contact with concentrated pesticides than the public. Toxicity of agricultural practices remains a critical issue. Organic grain and foods growers banish the use of wellknown toxicants such as concentrated chemical pesticides in the environment, with potentially questionable or not-so-sure long-term impact on human health.

# **Potential Limits**

Most of the nontraditional cereals are more expensive than typical cereals partly because they are still marginal crops. This may limit accessibility of specific cereals, due to bad environmental conditions

especially if produced by few growers. More and more of these products are now available in North American and European supermarkets. In general, higher prices must compensate for lower yields so any possibility to improve yields will lower price and popularity of organic crops. Because yields of organic crops are generally lower than nonorganic, organic agriculture is unlikely to feed the world, especially developing countries. According to most scientific studies, foods from organic-grown crops (including grains) are not more nutritive than nonorganic foods.

# **Future Prospects**

It is expected that organic agriculture will remain popular for grain growers. For organic agriculture, specific grain varieties might be developed, especially those with long straw length, likely to limit growth of weeds through shading. Better understanding of organic grain growing will benefit from research as more scientists will become interested to this sector. On the long term, expansion of the organic grain market will be faced with identity problems. In essence, it is an ecological alternative to conventional agriculture (using synthetic inputs) but is perceived as a safer and more nutritive grain, without ecological significance for citizens. The question of the quality of organic grain-based foods also remains to be addressed, considering the minimum input from ingredients used in conventional foods. Because environment certainly has a major impact on human health, the organic way to grow grains is a nice alternative to conventional agriculture.

As mentioned, it is not expected that organic growing of grain will replace nonorganic agriculture. Up to now, this alternative agriculture is mainly a phenomenon associated with industrialized countries, especially Europe and America. Within less than 50 years, land surface devoted to organic agriculture has grown from literally nothing to a few percents.

At the turn of year 2000, large food manufacturers and distributors have now embarked the organic train. This is symptomatic of the attractiveness for safe, nutritive, flavorful, and guilty-free foods for which some consumers are ready to pay an "insurance premium." Will the popularity of organic foods only grow in the hands of large food consortiums? This is difficult to determine because the organic food market is still in transition. One sure bet is that the availability of organic foods in large supermarkets has given much credibility to organic agriculture in general. We may continue to see two markets for organic foods: one from mass and probably industrialized food sectors with limited potential for addressing rural priorities,

and another for small-land or "terroir" tailored foods. Overall, the quality of these two types of organic foods might be different but they may well replace a significant part of the traditional but non-organic healthy foods. This popularity should slowly reduce prices and make these foods even more attractive to low-budget consumers. Nowadays, in Canada for example, it is very easy to get bread made from organic grain, with a special and appealing flavor and aroma due to the use of whole wheat or specialty grain. This appeal for new bread flavors and appearance is following the lasting interest for beer from microbreweries since the 1980s. Around this time, many speculated that such special beer market would not survive long. Like specialty beer, organic growing of food and grain has probably given us another lesson: it is here to stay.

See also: Chemicals for Grain Production and Protection. Consumer Trends in Consumption. Labeling of Grain-Based Foods.

# **Further Reading**

Bourn D and Prescott J (2002) A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. *Critical Reviews in Food Science and Nutrition* 42: 1–34.

Gooding MJ and Davies WP (1997) Wheat Production and Utilization – Systems, Quality and the Environment. Oxon: CAB International.

Hall DC and Moffitt LJ (eds.) (2002) Economics of Pesticides, Sustainable Food Production, and Organic Food Markets. Oxon: Elsevier.

IFST (1999) Organic food. Food Science and Technology Today 13(2): 108-113.

Knorr D (ed.) (1983) *Sustainable Food Systems*. Westport, CT: AVI Publishing.

Rizza RA, Go VLW, McMahon MM, and Harrison GG (eds.) (2002) Encyclopedia of Foods. A Guide to Healthy Nutrition. San Diego, CA: Academic Press.

Rosset P and Benjamin M (eds.) (1994) The Greening of the Revolution. Cuba's Experiment with Organic Agriculture. Melbourne: Ocean Press.

Verhoog H, Matze M, Lammerts van Bueren E, and Baars T (2003) The role of the concept of the natural (naturalness) in organic farming. *Journal of Agricultural and Environmental Ethics* 16: 29–49.

Vincent C, Hallman G, Panneton B, and Fleurat-Lessard F (2003) Management of agricultural insects with physical control methods. *Annual Review of Entomology* 48: 261–281.

Wallace J (2001) Organic Field Crop Handbook, 2nd edn. Ottawa: Canadian Organic Growers.

Woese K, Lange D, Boess C, and Bögl KW (1997) A comparison of organically and conventionally grown

Wright S and McCrea D (eds.) (2000) Handbook of Organic Food Processing and Production, 2nd edn. London: Blackwell Science.

#### **Relevant Websites**

http://www.codexalimentarius.net — This website presents regulation of the Codex Alimentarius. Guidelines on organic agriculture are described on ftp://ftp.fao.org/codex/standard/en/CXG\_032e.pdf http://www.ifoam.org — This is the website of the International Federation of Organic Agriculture Movements. This organization is recognized as the leading group and its main function is to coordinate

the international organic movement, offering, for example, standardized rules for certifying organizations from different countries.

http://www.organicstandard.com - This website updates international regulation of organic foods production.

http://europa.eu.int — This site presents regulation for European countries.

http://www.ams.usda.gov — This site shows regulation on organic foods by the US Department of Agriculture's Agricultural Marketing Service which administers the National Organic Program.

http://www.fibl.ch and http://www.darcof.dk — These sites refer to the Research Institute of Organic Agriculture (Switzerland) and the Danish Research Centre for Organic Farming, two major research organizations dedicated to organic argiculture.

# **OVEN TECHNOLOGIES**

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#### Introduction

Baking ovens were used far back into antiquity, well before recorded history. No one knows exactly when and where baking, and hence the oven, was developed. Most likely, it was a gradual process with baking tools and skills developed through a slow combination of serendipity and ingenuity (see Bakeries. Milling and Baking, History). The oven is the single most important, most expensive, and most complex piece of equipment used in bakeries. It limits the throughput, the variety, and the quality that the bakery can produce. It is also the least understood, most misadjusted, and most poorly maintained piece. It consumes the most energy and creates the most pollution, takes the most space, and generates the most controversy among its users. An oven cannot correct prior processing mistakes, but it can certainly limit everything done before. The success or failure of a baking venture is often determined by the oven, but the oven is sometimes the last item to be considered when a new bakery is being designed and equipped. Even though a well-designed and maintained oven will last for decades, its specifications often are reduced to "cost is the first consideration," rather than product suitability, lifetime utility, and maintenance costs.

# **Oven Classifications**

Ovens today may be classified by several methods, as detailed below.

# Scale (Includes Cost, Size, and Capacity)

The local "corner hot bread shop" or "in-store bake-off" will use small batch ovens. These may only operate a few hours per day, perhaps baking only a hundred loaves, and will almost be totally automatically controlled. They require very little skill to operate properly. The "baker" only needs to put the loaves (which may be frozen dough or even parbaked) into the oven and removes them when the buzzer sounds. Temperature, air circulation, and other settings are all carefully controlled by the manufacturer. Larger-scale institutional or small wholesale bakers are probably using rack or reel ovens, still manually batch loading them. They may bake a few hundred to a few thousand loaves per day. More judgment and skill is required on the part of the baker not only

because of the oven, but because the entire operation is probably a "scratch" bakery, preparing doughs and batters from individual ingredients, not from mixes or frozen items. Some of these bakers may be operating automated, thermal oil heated, deep hearth (setter) ovens. Finally, the large-scale high-speed plant bakeries baking 100 loaves per minute on each of several parallel lines will have highly automated continuous ovens in which molded dough pieces are automatically deposited into pans, proofed, loaded into a continuous oven, baked, de-panned, cooled, and sliced without human contact.

# **Products Being Baked**

Except in very small shops, breads are baked in ovens that differ from sweet dough foods such as chemical-or yeast-leavened pastries. Cookies and crackers will be baked in a different design yet, and cakes and pies require still another design. While smaller bakers may use a "one-for-all and all-for-one" oven, by far the best results will be obtained using an oven designed for the specific baked food type.

# **Physical Arrangement**

Different physical arrangements for ovens began with the descendents of the primitive beehive ovens as hearth or deck ovens, rotary hearth, rotary rack, reel, traveling tray ovens with single or multiple laps, and finally the long, continuous tunnel ovens used for crackers and cookies. Some of these designs will be described in more detail later in this article.

#### **Heat Sources**

Except in small primitive bakeries and by a few "boutique" bakers, wood is seldom used in ovens today. The energy source is usually refined petroleum, gas, or electricity. A few attempts have been made to use solar energy, but this is not yet a significant contributor except in a few isolated locations used by individual bakers living in very hot and sunny countries with a shortage of fossil fuels. Likewise, coal is seldom used because it is so dirty.

The fuel may be burnt directly in the baking chamber, though there is an increasingly popular trend toward using indirectly fired ovens in which the combustion chamber is kept separate. A relatively recent modification is to heat special high-temperature oil at some distance from the oven and then pump it to large horizontal heat exchanger plates in the baking chamber.

Within the oven, heat must travel in some fashion from the source to the raw dough. There are several mechanisms that accomplish this. Radiation, both thermal and electronic, is used, as is direct conduction between the hot oven surface and the dough. Likewise, convection (natural or forced) uses hot air to transport the heat. There are some other lesser known methods, such as direct ohmic resistance in which an electric current passes directly through the baking product, heating it throughout. Of course, there are ovens that use two or more of these forms simultaneously. When thermal sources are combined with electronic sources (microwave or radiofrequency (RF)), they are often called "hybrid" ovens. The following section presents a simplified description of the principal heat transfer modes.

# **Heat Transfer Mechanisms**

"Heat" is energy in motion. A hot object transmits its energy to another when some of the thermal vibrations are transferred. The mechanisms for heat transfer are usually classified in three basic types, radiation, conduction, and convection, with some additional variations and combinations. These are described next.

#### **Thermal Radiation**

Radiant energy does not need a medium to carry it, as it falls in the electro-magnetic spectrum. Thermal radiation is energy that is transferred when the baking product can "see" the heat source, which is often incandescent although the most effective heating wavelengths are usually in the infrared (IR) region, invisible to the human eye. For example, electrical elements may glow "red hot," or gas flames may heat ceramic blocks to incandescence. By controlling the temperature and the nature of the radiator, it is possible to determine the wavelength at which the radiant energy is emitted.

Energy transfer by radiation can be expressed by a relatively simple equation. Note the importance of the temperature term, raised to the fourth power. If the absolute temperature in degrees kelvin ( $^{\circ}$ C + 273.16) is doubled, the heat transfer rate will be 16 times as fast:

$$Q_{\rm R} = e\sigma A \left(T_{\rm h}^4 - T_{\rm c}^4\right) \Delta\Theta \tag{1}$$

where  $Q_R$  is the net radiant energy transferred, e the emissivity of the radiating body, varying between 0 and 1,  $\sigma$  the Stefan-Boltzmann constant for radiant energy, A the area to which heat is being transferred,  $T_h$  the absolute temperature of the hot, or emitting, body,  $T_c$  the absolute temperature of the cold, or absorbing, body, and  $\Delta\Theta$  the time increment over which the radiation occurs.

Radiant energy is very effective for browning surfaces, but it does not penetrate very far below

the surface. Some large ovens have a "browning" section immediately before the discharge end with IR elements that can be used to control the final surface color. IR heaters are familiar as the deep red lights above restaurant serving counters, keeping food warm while it waits for delivery to the customer. Small "toaster" and "pizza" ovens sometimes use this principle. They are relatively cheap to construct and work well on relatively thin products such as biscuits. The principle is not satisfactory as the sole energy source for thicker objects such as pan bread loaves, because the surface heats very rapidly, browning quickly before the heat can penetrate to the interior and complete the baking process. Irregular surfaces are also difficult to bake with IR, because any exposed points burn before the rest is hot. Thermal radiation is a component in nearly all ovens, but is usually accompanied by conduction and convection.

#### Conduction

The higher the temperature of a substance is, the more rapidly its molecules vibrate, and the vibration may be passed by contact from one molecule to another. If a cold substance is in contact with a hot object, the molecular vibrations are passed from the hot to the cold substance and the "heat" is transferred. The more rapidly (the hotter) the source molecules vibrate and the better the contact between hot and cold bodies, the more efficiently the energy will be transferred. Metals are good thermal conductors and still air is a very poor conductor. Ceramics are intermediate. The ability of a material to transmit heat energy through it is called its thermal conductivity.

Heat transfer by conduction may also be described by a relatively simple equation involving the temperature difference between the donating and receiving objects. In this case though, unlike radiation, the temperatures are only raised to the first power, not the fourth, so the driving force from temperature is not as dramatic:

$$Q_{c} = \kappa A (T_{h} - T_{c}) \Delta \Theta / d$$
 [2]

where  $Q_c$  is the net heat transferred,  $\kappa$  the thermal conductivity coefficient, A the area across which heat is transferred,  $T_h$  the temperature of the hot or donating body,  $T_c$  the temperature of the cold or receiving body,  $\Delta\Theta$  the time increment over which the heat transfer occurs, and d the distance, or thickness, through which the heat must migrate.

Most early baking used conduction to get the heat inside the food. Hot stones sitting in coals transferred their heat directly and rather rapidly to the dough placed on them. Even today, hearth breads are often placed directly upon the hot oven floor, perhaps separated slightly by a thin layer of bran or maize meal to keep loaves from sticking. The energy transfer is rapid and efficient so long as the bread makes good contact. Heat transfer between the dough and a pan can be improved by putting a thin layer of oil in the bottom before panning a pizza crust, for example. As a result the pizza may have almost a "fried" instead of a "baked" crust character.

Many baked foods such as biscuits (cookies and crackers) are baked upon continuous bands inside tunnel ovens. The direct gas flame heats the band and the small, thin biscuits are baked quickly, often in less than 3 min, because heat transfer is rapid. By preheating the oven band to an extra high temperature, the bottom of the dough piece is heated extremely hot, while the top is still relatively cool. The heat transfers so rapidly into the dough that small interior bubbles are generated by the rapid release of leavening and water as steam. The resulting dried product is very crisp yet very tender. Similarly, "flat breads" such as pita and tortillas are baked at even higher temperatures, up to 400°C for less than 1 min.

Conduction inside dough is usually slower than the rate at which heat can reach the surface. Calculating the heat transfer rate inside a baking food is very difficult because the heat transfer coefficient,  $\kappa$ , varies not only with the chemical nature of any substance (protein, starch, water, etc.) but also because as the product bakes, its structure and chemical composition change. The " $\kappa$ " value will even be different in various parts of the food at the same time. The crust may be dry and hard with large air-filled pores. The resulting thermal conductivity is very low. On the other hand, raised dough with greater moisture will conduct heat more rapidly. This becomes more complicated as we consider convection and the "steam pipe" effect in the following section.

#### Convection

Convection heat transfer requires a moving fluid. Convection may be "natural," caused by air and steam circulation resulting from density differences. Hot gases expand, become less dense, and rise to the top, whereas the cooler gases settle to the bottom where they are reheated, resulting in vertical circulation inside the oven. Hot gases heat the baking dough by transferring energy across a boundary layer by conduction. The boundary layer is the relatively cool, moist, and dense layer surrounding the dough piece. Natural convection is present in most ovens, though it is not very efficient and heats rather unevenly. Fan-forced convection ovens operate more efficiently by circulating the hot gases more rapidly

and the hot gases are not limited to a vertical motion. A special form of forced convection, "impingement" directs jets of hot gases perpendicularly onto the baking food at high velocity, sweeping aside the boundary layer as well as bringing the hot gases there more rapidly (Figure 1).

The equation for heat transfer by conduction resembles the heat equations for the other two principal forms. Again, the difference in temperature between the heat source (circulating fluid in this case) and the baking product is the driving force. The rate is also controlled by the convective heat transfer coefficient. Remember that, like the radiation example, this equation only describes how the heat reaches the surface, not how fast it moves to the interior of the baking product:

$$Q_{\rm v} = hA(T_{\rm h} - T_{\rm c})\Delta\Theta$$
 [3]

where  $Q_v$  is the net heat transferred, h the convective heat transfer coefficient, A the the area across which heat is transferred,  $T_h$  the temperature of the hot or donating body,  $T_c$  the temperature of the cold or receiving body, and  $\Delta\Theta$  the time increment over which the heat transfer occurs.

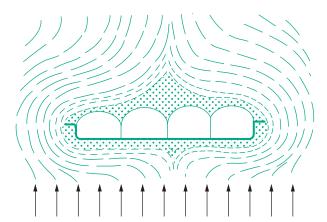
The convective heat transfer coefficient, *h*, is very difficult to predict based upon theoretical considerations, though many attempts have been made to do so. It is principally affected by the air velocity and flow pattern, but also by the temperature, pressure, and moisture content, all of which affect air density and viscosity, and hence control the properties of the stagnant boundary air layer that surrounds an object. Surface geometry also affects the convective heat transfer coefficient, especially for natural convection. For engineering scale-up purposes, it is more often satisfactory to determine the apparent convective

heat transfer coefficient,  $h_a$ , experimentally. This can be done by measuring the rate of temperature rise of a standardized metal plate, usually aluminum, placed inside the oven.

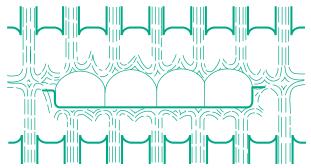
Nearly all ovens will have some convection and it has been estimated that convection and radiation account for roughly equal contributions to the net heat transfer in conventional natural convection ovens. Many large commercial ovens have forced circulation by one means or another, not only increasing baking speed, but more importantly, improving baking uniformity. Likewise, institutional food service-type convection ovens are relatively common. They have a fan that circulates the air at rather high velocities and bake in less time with more uniformity.

The most dramatic use for forced convection is in the many tens of thousands of small units built for pizza restaurants since the mid-1970s. They force the circulating air through many small nozzles  $(\sim 1.0-1.5 \text{ cm} \text{ in diameter})$  at air velocities  $\sim 150-$ 200 m s<sup>-1</sup> (meters per second). Higher velocities are possible, but they sometimes levitate the ingredient toppings. By impinging the air jets perpendicularly at the surface, these ovens sweep away the dense, viscous boundary layer and bring heat closer and more rapidly to the baking pizzas. With jets both above and below, pizzas now go from totally raw to fully baked in 5-8 min as compared with the 20-30 min required when they were baked in deck ovens. The tremendous expansion in pizza outlets was only possible after impingement ovens were developed (Figure 2).

Similar principles have been incorporated into tube-impinged breakfast cereal toasters. They increase throughput, reduce breakage, and improve quality for ready-to-eat breakfast cereals (*see* Cereals: Breakfast Cereals for more information).



**Figure 1** Flow patterns in natural convection, resulting in uneven heat transfer. (Reproduced with permission from Varilek P and Walker CE (1984) Baking and ovens: history of heat technology IV. *Bakers Digest* 58(2): 13.)



**Figure 2** High-efficiency air impingement directs small jets from both above and below, at right angles to the product surface. (Reproduced with permission from Varilek P and Walker CE (1984) Baking and ovens: history of heat technology IV. *Bakers Digest* 58(2): 14.)

# **The Steam Pipe Effect**

When engineers first began to study heat transfer in the new rapid-bake impingement ovens, they could not explain why the net heat transfer rate appeared to be higher than would be expected. Efficient convection would heat the surface rapidly, but the cold, moist interior (like in raw pizza dough) was thought to be limiting because it had a lower thermal conductivity. They eventually discovered that the improved heat transfer inside the products being baked with very high apparent convective heat transfer coefficients could be explained by an interesting mechanism. This works best on porous products with relatively high moisture contents.

Heat applied to a surface by the impingement process moves by conduction relatively rapidly across the thin solid wall of the "bubble" or cell. Moisture evaporates from the hot side and migrates as vapor across the cell and condenses onto the inner, cooler cell wall. The latent heat of vaporization then migrates across another thin cell wall and evaporates more moisture on the other side. The process continues until it eventually reaches the center where it meets a similar phenomenon approaching from the other side (Figure 3).

Not only does the heat move in, but there is also a net moisture migration to the center. The result is that in a loaf of bread fresh out of the oven, even though it has lost  $\sim 10\%$  by weight during baking, and the outside may be quite dry and crisp, the loaf center actually has higher moisture content than the

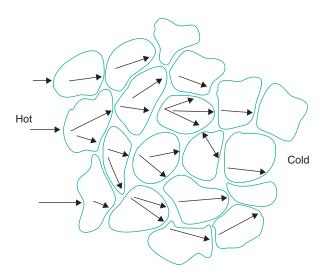
starting dough. The moisture redistributes rather quickly, and the starch and protein have been changed by the  $95-98^{\circ}$ C temperatures in the center, so the inside is cooked, not raw or dough like.

# Oven Designs and Commercial Examples

# **Traveling Tray Bread Ovens**

By around the year 1900, commercial baking had reached the point where the peel-hearth or deck and reel design ovens popular at the time could not keep up with demand. Tunnel ovens were designed in which pans entered at one end, traveled slowly through the entire length, and exited the other end. Limited automation was then practical, but the ovens were expensive and took a huge amount of floor space.

The next innovation was combining the tunnel oven design with an extension to the concept. In a reel oven, two large wheels rotate vertically inside the baking chamber, much like on an amusement park Ferris Wheel, with trays (instead of passenger seats) suspended between. Pans were placed on the trays and they revolved until the products were done. It was an important innovation, and reel ovens are still used in many smaller bakeries, because they are versatile and products requiring different baking times can be baked together (Figure 4). The traveling tray oven extended the oven from front to back by adding



**Figure 3** The "Steam Pipe Effect" can explain why heat transfer across a porous substance is more rapid than otherwise predicted, and how there is a net moisture migration toward the center. As heat moves through the cells, moisture simultaneously evaporates and recondenses, moving from the hot to the cold portions in the dough.



**Figure 4** The reel oven was once very popular in wholesale bakeries and is still used in some retail and food service operations. (Courtesy of the Reed Oven Co., Kansas City, MO, USA.)

a second set of wheels and connecting them with heavy chains, to which trays were attached. Bread was loaded and unloaded through the same door. As their size grew, they eventually reached a length such that one pass took the entire baking cycle, so the batch oven evolved into a continuous oven, with products entering and leaving continually, never crossing paths with each other (Figure 5).

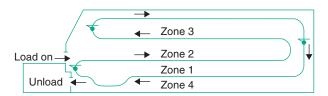
The floor space for these ovens was more efficient than the tunnel oven, because a complete lap involved two passes through the oven, front to back and back to front. Only limited baking zone control was possible though, as compared with the true tunnel oven in which product entered one end and exited the other. Some manufacturers added a second pass through the same baking chamber, creating the double-lap oven. In the USA in 1923, 93% of the industrial bread ovens were the manually peel-loaded, batch type. By 1929, as the tunnel and traveling tray ovens were introduced, the portion of peel ovens fell to  $\sim 50\%$  (Figure 6).

#### **Cracker Ovens in a Tunnel**

By 1931, the single pass tunnel oven design was no longer used for bread but the traveling trays were replaced by a solid or very heavy mesh continuous band and adopted by biscuit and cracker bakers. Typical cracker tunnel ovens are now more than 1 m wide and 100 m or more in length, for a 3 min bake. This provides for tremendous capacities, e.g., 2000—3000 kg of product per hour. Their multiple zones provide the operator with great control over product quality. For example, they are often direct-gas fired



**Figure 5** The single-lap traveling tray oven carries trays loaded with several pans of products through the oven in one pass. (Reproduced with permission from Pyler EJ (1988) *Baking Science and Technology*, 3rd edn., vol. II, p. 1188.)



**Figure 6** The double-lap traveling tray oven carries its trays through two passes of the heating chamber. (Reproduced with permission from Pyler EJ (1988) *Baking Science and Technology*, 3rd edn., vol. II, p. 1189.)

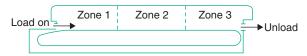
with burners both below and above the band. The burners above the band, especially, may have IR radiating ceramic elements (Figure 7).

Few basic design innovations have been made in industrial-scale bakery ovens since the mid-twentieth century, although automation, energy conservation, and increasingly sophisticated controls have evolved substantially. At the smaller wholesale, retail, and restaurant/food service scale, however, many innovations have appeared, nearly totally replacing the earlier deck and, to a lesser extent, the reel designs in many markets.

## **Rotary Rack Ovens**

Rotary hearth ovens provided advantages over the deck and peel designs, because each item was easier to access and could be put in and removed at a different time from the others. Their heavy, slow-moving, hearths were very stable and ideal for cakes and pies. In order to increase floor space efficiency, multiple decks (up to about five) were stacked. This design is currently in use in some retail and small wholesale bakeries. However, they are still slow and laborious to load and unload and require many manual transfers between forming, proofing, baking, and cooling.

An improved concept now used in many large retail and small wholesale bakeries was developed by rolling the entire rack of pans inside a large vertical cabinet oven. Products could be panned on plain or perforated trays, the rack rolled into the proofing cabinet, and when ready for baking, the entire load rolled onto the oven. Humidity could be introduced by injecting steam or by dripping water onto large metal balls stored in the wall, in the air path. Air is blown at high velocity from one side of the oven across the trays. In order to bake more uniformly, the air direction could reverse automatically, and, in the more popular current versions, the entire rack rotates during the total baking cycle. The rotary rack oven is probably the choice for most new institutional food service and retail bakeries, and sometimes they are installed in multiples for small wholesale operations, especially those that need to process a variety of products ranging from pastries and pies to bagels, as well as breads and rolls. The



**Figure 7** Band ovens with thin solid metal or heavy mesh baking bands are used principally for baking cookies and crackers. (Reproduced with permission from Pyler EJ (1988) *Baking Science and Technology*, 3rd edn., vol. II, p. 1190.)

ovens occupy a size and versatility niche where they are difficult to beat.

# **Impingement Ovens**

The ancestors of the present-day ubiquitous impingement ovens owe their existence to attempts to cool radar tubes during the Second World War. It was discovered that if air was blown on the tubes through small nozzles, the heat transfer was more efficient and the tubes had longer life. In the early 1970s, a university food service operator asked the inventor of that cooling technology how to cook pizzas, rapidly expanding in popularity with college students, faster and with shorter lead time than possible in their deck ovens. The result was a series of small ovens that blew high velocity hot air through nozzles perpendicularly onto the pizza. Baking times dropped to well below 10 min, so the large pizza restaurant chains began to adopt the ovens. The original patented design used "fingers" extending from the air plenum on the side of the oven. The fingers were perforated with drawn nozzles below collimating plates and directed the air at the baking foods. The ovens were conveyorized with an open mesh and nozzles were placed both below and above the product. Belt speed determined bake time, reducing operator error.

Forced convection air impingement ovens today use small nozzle holes, short tubes, perforated plates, or narrow slits to blow air at high velocity perpendicularly onto the baking food. The air jets or curtains "sweep" aside the relatively cool, moist, viscous, dense boundary layer close to the product surface. The result is that the heat can reach the product more efficiently. The product can be baked in a shorter time, at a lower temperature, or both. This principle is especially effective with thin foods. Many consumers will be familiar with these ovens behind the counter in their local pizza restaurant (Figure 8).

Other manufacturers produced competing equipment using tubes or slots, both of which had been used earlier in commercial equipment for other markets, to deliver the air at high velocity. Their uses expanded from the original pizza shops to encompass restaurants and food service on a larger scale. Casseroles, steaks, small bread loaves, cakes, and pies joined each other in the same oven. Even though the high air velocities result in rapid moisture evaporation, the residence time in the oven is reduced so much that products lose less moisture and generally have superior texture and increased shelf life.

Forced convection tends to provide very uniform heat, so the principle was applied to a large commer-



**Figure 8** High-speed air impingement ovens commonly bake pizzas in  $\sim$ 7 min. These rapidly replaced the slower deck oven design previously used. (Reproduced with permission from Middleby-Marshall Corp. "Press Kit" CD. ©2001.)

cial scale pie oven. Highly automated, it could change fan speed and nozzle height "on the fly," permitting different products to be baked in the same oven at the same temperature, leaving only a short break between formula changes. By the mid-1990s, several large-scale tunnel ovens using the principle of high-velocity air impingement from slots or nozzles were being used to bake baking powder biscuits (resemble English scones), flat breads, and many other products on a large scale to meet the growing needs of demanding customers like fast-food chains.

Any savings in baking times, lower temperatures, and reduction in energy use vary with the nature of the product (thickness, water content, etc.) and with the nozzle design and locations, but most importantly with the air velocity. As a general rule, the product of "time" and "driving temperature" can be reduced to about one-half, where "driving temperature" = "oven temperature" - "product starting temperature." Although it is theoretically possible to reduce the times even more by using higher temperatures and higher air velocities, the baker will soon encounter a zone of diminishing returns because the product's interior thermal conductivity becomes the limiting factor, not how fast the heat can be brought to its surface. The time and temperature reductions apply to many products. Even "Pullman" bread loaves (square sandwich bread baked in lidded pans) can be baked with about two-thirds the "time by driving temperature product." The accelerated heat transfer applies to metal as well, not just to exposed moist dough surfaces.

Baked foods' characteristics begin to change as baking times are adjusted by altering the temperature and heat transfer rate, either up or down. Baking is a complex series of reactions, many of which have different  $Q_{10}$  (relative change in rate per each  $10^{\circ}$ C change in temperature) values. As a result, the rates for leavening release, fat melting, sugar dissolution, protein denaturation, and starch gelatinization will all differ at different temperatures, and the product will exhibit different properties when the baking conditions are changed, even if the internal temperature and moisture contents are held constant. Reformulation can compensate for these changes to only a limited extent.

# **Compact Footprint Designs**

In many modern, large-scale wholesale bakeries, the oven is no longer a "stand alone" piece of equipment that must be "interfaced" at each end with the rest of the production line, but has become part of a totally unattended, integrated baking system. Product moves in a continuous, uninterrupted process, from mixing and depositing or filling at the beginning, with any proofing or relaxation required, through the oven, directly into coolers and freezers, without leaving the main conveyors, smoothly and quietly transferring trays where necessary. Manufacturing innovations include complete assembling of the entire production line and operating it to bake real product before it leaves the factory. Broken into smaller units for shipment, it can then be reassembled and put into operation relatively rapidly with a minimum of disruption to production flow in an existing bakery.

Totally automated and integrated systems like this are ideal for parbaking breads. Parbaking involves partially baking the loaves, so that they are firm and can be handled but they do not have full color development. The loaves are often shipped frozen to distribution points all across the country where they are finish baked in small rack ovens and sold to the consumer as fresh baked.

Horizontal folded path Like many other large-scale oven developments, the serpentine concept had its origins in small-scale ovens designed for retail bakers. Initially used as a small multipass cookie oven, the serpentine design borrowed concepts from the commercial traveling tray oven and produced a highly compact oven capable of baking large quantities in a small space. Trays are suspended on an extremely precise chain and guide system. They usually enter the oven near the bottom and progress from bottom to the top, typically making 7-11 closely spaced passes. In each pass, the trays are separated from those below or above by hot-oil-filled plate radiators served by a heat exchanger that can be located quite remotely from the production area, improving safety and sanitation. Most products baked in this system to date have

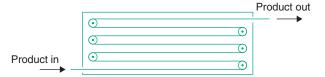


Figure 9 The horizontal path serpentine oven design conserves floor space and bakes very uniformly. It is often part of a totally integrated system that is preassembled and tested in the factory before it is installed in the customer's bakery. Thin thermal oil heat exchanger plates can be located between the horizontal runs of the conveyor and can provide much more uniform baking than side-fed recirculating air ovens, especially if the conveyors are more than 1 m wide. With a smaller external surface area to volume ratios and closer-spaced heat sources, the ovens are much more efficient than traditional designs.

been relatively thin ones, like cakes and cookies, but designs are available for full-size pan breads as well (Figure 9).

The small cavity design retains humidity, and the large radiator plate surfaces close to the product result in unusually uniform product characteristics, energy efficiency, and low baking losses in addition to the obvious space savings. Instruments are now available to monitor oven humidity in real time and control it by operating dampers or even by direct steam injection.

Spiral circular path Spiral coolers and blast freezers are relatively common, providing very long paths and hence long residence times in a relatively compact floor space. The concept has also been applied to continuous ovens. Product enters the oven supported on large baking trays sometimes attached to the conveyor by magnets. The conveyor is a long, endless, spiral, usually entering near the floor level and winding its way upward through the large hot chamber. In some designs, the chain returns in a smaller spiral down inside the outer spiral, to exit the oven below the first spiral, where the product is removed and the conveyor is automatically and continuously reloaded with raw product. Heat is often supplied by direct combustion gas burners in the center of the oven, and fans may be used to blow the hot air at the products, maintaining a uniform temperature in the oven. Though very efficient in terms of baking surface per square foot of floor space, it is very difficult to incorporate temperature, humidity, or heat transfer "zones" into this design.

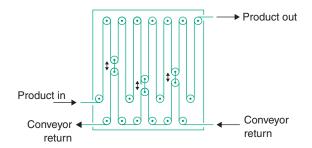
Vertical folded path Another approach to reducing floor space requirements is to operate the transport chains vertically, moving up and down through the oven as it progresses through the baking chamber.

Baking products are carried on swinging trays which remain horizontal as the transport chains move them through the oven chamber. Thermal oil heating plates may be arranged vertically between ascending and descending tracks or the ovens may be heated with forced circulation hot air. This design concept may have been inspired by the surge accumulator sections used in some automated packaging lines. This design can effectively change the path length through the oven, changing residence time "on the fly" for different products, without having to change belt speed. This is a large design advantage, though in most cases the radiant plates cannot be placed as close to or as effectively over the products as they can in the horizontal fixed-path design such as in the serpentine ovens. However, it is possible to change residence time in each zone essentially independently (Figure 10).

As the moveable double geared-pulley sets move up, the "forward" path shortens while the "empty return" path lengthens. This reduces the residence time in the oven but keeps the total chain length the same. Although a temporary regional speed change occurs while the geared pulleys are moving vertically, either the in-feed or discharge conveyor can be kept moving at a constant rate so they will not lose synchronization with either the filling or de-panning equipment. Baking times can thus be changed "on the fly" when products are changed. Since there is a great deal of thermal inertia in large oven systems, this can be a useful control for the baker because it will have a much more rapid effect than trying to change the temperature. Baking time and temperature can be stored in a computer as part of product manufacturing specifications. When the operator tells the computer that at a certain time a product change will occur, the oven can be programmed to adjust itself automatically.

Since the baking time can be changed without altering the chain speed, and hence production line speed, product is produced at a constant rate. In effect, the baking "tunnel" is lengthened for longer baking times or shortened for shorter times, all while the oven and the rest of the line continues in normal constant speed operation. This is not possible in most oven designs, especially in single-pass tunnel ovens. The same concept could also be applied to the horizontal folded path oven.

Steam baking provides a high heat transfer rate Steam is often added to conventional ovens, sometimes to control the crust texture or color. It is also used as the principal energy source for Chinesestyle steamed bread. In steamed breads, the object is to produce a relatively dense loaf with a chewy texture (varies with the style: "Northern," "Southern," or



**Figure 10** The vertical path serpentine oven design is also efficient in floor space (sometimes described as "small footprint" style) and can also be incorporated into an integrated baking system. Small trays remain suspended in a horizontal position while their conveyor moves through the oven zones. Thermal oil plates or infrared sources can be placed between the vertical conveyor paths. As the movable conveyor gears travel up, the product-containing section is shortened, matched by an increase in the conveyor return path shortens so the total conveyor length remains the same, but the baking time is reduced without changing conveyor speed.

"Guang Dong") and a smooth, white, leathery surface, rather than a conventional browned crust.

Chinese steamed bread is made similar to conventional bread rolls in the early process stages, but after it has proofed and is ready to be baked it is placed on open or perforated racks in a steamer. Traditional designs use bamboo baskets, although metal trays are often used in larger or more modern bakeries. The bread is "baked" in saturated steam until it is heated through, the starch is gelatinized, the protein is denatured, and the loaves have a shiny but leathery surface.

# **Less Conventional Designs**

Microwave baking Microwaves are familiar tools for reheating foods in the home or office and have been tried for large-scale baking. Microwaves have the advantage that the RF radiation passes easily through a dry crust and is absorbed largely by the moist inside portion. Provided the product is no more than  $\sim 10$  cm in diameter, it heats relatively uniformly throughout. However, microwaves do not produce a brown crust and in the case of bread, a toughening often occurs. Domestic microwaves operate at 2450 MHz and industrial microwaves at 915 MHz in the United States. Some countries allocate different frequencies for these uses. It is very important to isolate these high-energy frequencies from other applications, so they do not interfere with communications, for example. The lowerfrequency, longer-wavelength radiation penetrates most products much better.

Although there were numerous attempts to accelerate baking with microwaves as early as the 1960s, they were never commercially successful for

large-scale baking. In addition to the quality problems resulting from nonuniform field heating, they were very expensive to operate and difficult to control. Microwave generators (Magnetron tubes) are only ~50% efficient, converting about half the electrical input into heat. Their power level is also difficult to control. The most effective method has been "duty cycle," turning the tube on at full power for a short time, then turning it completely off. Although satisfactory for thawing foods or reheating them in domestic batch oven use, such a procedure does not work well in continuous conveyorized ovens. Also, trapping the radiation inside an operating oven, open at both ends for products on a nonmetallic belt to pass through, creates severe engineering challenges.

**RF** Ovens RF is used as an energy source in some ovens. RF is similar to microwaves but operates at a much lower frequency, hence generating longer wavelengths which are easier to contain. The energy penetrates deeper into the product and has fewer "hot spots" than with conventional microwaves. The frequencies allocated for this use in the United States are approximately 6, 13, 27, and 40 MHz. RF is used widely in industrial applications for curing plastics and providing controlled, localized, heating. In baking, however, it is principally limited to "postbaking" in the biscuit and cracker industry, where it has become very common since its successful commercialization in the UK in the 1960s. Thin products such as crackers are difficult to bake uniformly at high speed, because there are small variations in the dough composition, mixing and forming steps, and in the oven. As a result, the individual pieces may vary in moisture content and color as they leave the oven. Even if the product averages the target value (typically  $\sim 3\%$ ), when the moisture is too high in the center part of the cracker, it will cause checking or cracking as it equilibrates later.

By sending the still-hot crackers through a RF post-baking oven, the excess moisture is removed or equilibrated in a few seconds, resulting in more uniformity and less breakage. High-energy radio waves at these frequencies will preferentially couple with water molecules, drawing power to the wet portions but not to the dry ones. The small additional energy concentrated where the product needs it is sufficient to drive the extra moisture out, or to redistribute it, before the cracker has a chance to stress crack as it cools. This results in less dissatisfaction for the consumer and a greater profit margin for the baker (Figure 11).

Baking with visible light "Visible + IR" light is another relatively recent concept for rapidly baking foods, especially at the retail fast food and food



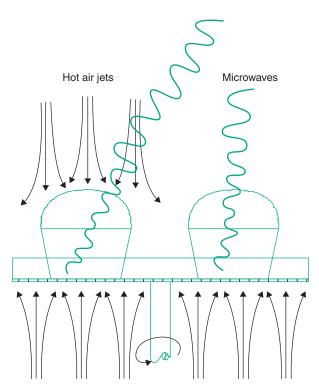
**Figure 11** RF postbaking section often follows a biscuit tunnel oven to reduce uneven moisture distribution which can cause broken product. (Courtesy of Radyne-Strayfield Corp., Milwaukee, WI 53207, USA.)

service level. A small microwave oven-size cavity contains tungsten—halogen tubes that emit a mixture of IR and visible wavelengths at extremely high intensity. The IR light does not penetrate very far beneath the surface, providing browning and flavor development as well as providing much of the total energy to the product. The intense visible light, shorter in wavelength, does penetrate somewhat into the interior. Though not as efficient in transmitting thermal energy, some is absorbed deeper into the products interior, thus accelerating the baking times considerably as compared with a conventional oven. It does not compete in speed with a microwave oven having a similar power rating, however.

Direct resistance ovens Ohmic heating is used in some specialized applications. To produce certain styles of bread crumbs, especially "Japanese style," for breading and battering foods such as fish sticks and deep fried vegetables, a simple bread dough is first mixed then the "loaves" placed between two electrodes and an electric current passed directly through the loaf. Careful control of dough moisture and salt content is necessary. The internal resistance of the moist dough generates considerable heat "internally" so in effect the bread bakes in its own steam. The moisture is driven off and the internal structure is set, but no crust browning occurs. After the loaf is removed and cooled, it is ground to the appropriate particle size and dried for use as a breading or batter thickener.

#### **Hybrid Oven Designs**

Hybrid ovens contain a combination of two or more energy sources. Technically, nearly all ovens might be



**Figure 12** When baking in a hybrid oven, the food receives air impingement on all surfaces and also absorbs microwaves throughout. Keeping the product in motion results in a more uniform bake by distributing both forms of energy more evenly. (Reproduced with permission from Walker CE and Li A (1993) Impingement oven technology. Part III: Combining impingement with microwave (hybrid oven). *Technical Bulletin of the American Institute of Baking* XV(9): 1–6.)

considered "hybrid" because they usually use conduction, convection, and thermal radiation, all three to a certain extent. The term "hybrid oven" though is usually reserved for ovens that combine "nonconventional" energy sources, sometimes in a conventional oven, sometimes totally independently (Figure 12).

Microwave oven manufacturers have long offered (largely unsuccessfully) hybrid ovens to domestic customers in an attempt to combine the speed advantages of microwave with browning and flavor development. One variation includes adjustable radiant elements in the top of the cavity and another offered a bottom hearth plate, but neither were ever large sellers. One domestic range added microwaves inside the regular size oven, but the baking was rather uneven. The most popular were the so-called convection microwave ovens. A conventionally designed microwave oven contained a small fan to circulate air heated by a separate electric element. One major problem was that US domestic electrical circuits could not provide enough power to operate the microwave and the convection heaters at the same time, but had to cycle between them. A gas-fired hybrid microwave oven was developed in Europe, but sold only limited quantities. One major problem was in preventing the microwaves from extinguishing the gas flame.

Convenience stores and vending machines offer another potential market for microwave hybrid ovens, combined in this case with air impingement. They can be installed in commercial environments where they have access to sufficient electric power. The units are basically convection microwave ovens with oversized blowers and heaters that can operate at the same time as the microwave generator. Intended for food point-of-purchase consumption, the convenience store customer would buy a refrigerated or frozen dinner from the display cabinets, remove the wrapper, and cook it in less than 3 min.

An innovative extension to this idea was developed for the vending machine market. A large cabinet resembling a soft drink vending machine contained a freezer in the base where foods such as pizzas, spaghetti, and French-fries were kept inside ingenious design boxes. The top part contains a combination microwave and hot-air impingement oven. When the customer inserts coins, an elevator automatically selects the desired food from the frozen storage magazine below, lifts it to the top, and pushes the inner noncovered container out of the over wrap box into the heating chamber. The machine is preprogrammed for the amount of microwave and thermal energy needed to thaw, reheat, and brown the customer's choice. The outer carton is then replaced and the hot snack delivered to the customer in 1 min. The concept has been used successfully for many years in remote military posts and in hospital food service operations, but has not yet become widely familiar to the general public.

#### **Summary**

Ovens have evolved slowly over thousands of years. Until recently though, the same principle — heating a food sitting on a hot shelf inside a closed chamber — described them all. Automation was not a major concept until a century ago, and innovative ways of generating and applying the energy to the product did not begin to make serious inroads in the home or commercial bakeries until the 1960s.

See also: Breads. Cakes, Chemistry of Manufacture.

# **Further Reading**

Ovadia DZ and Walker CE (1997) Opportunities for impingement technology in the baking and allied industries: Part IV. *Technical Bulletin of the American Institute of Baking* XIX(5): 1–8.

Pyler EJ (1988) "Oven Equipment" in Baking Science and Technology, 3rd edn., vol. II, chap 28, pp. 1184-1217. Kansas City, MO, USA: Sosland Publishing Co.

Varilek P and Walker CE (1984) Baking and ovens: history of heat technology IV. Bakers Digest 58(2): 13, 14.

Walker CE (1987) Impingement oven technology: Part I. Principles. Technical Bulletin of the American Institute of Baking 9(11): 1-7.

Walker CE (1991) Air impingement drying and toasting of ready-to-eat cereals. Cereal Foods World 36(10): 871 - 877.

Walker CE and Li AL (1993) Impingement oven technology: Part III. Combining impingement with microwave (Hybrid oven). Technical Bulletin of the American Institute of Baking 15(9): 1-6.

Walker CE and Sparman AB (1989) Impingement oven technology: Part II. Applications and the future. Technical Bulletin of the American Institute of Baking 11(11): 1-11.

#### **Relevant Websites**

http://www.aibonline.org http://www.asbc.org http://www.bakeryandsnacks.com http://www.bakery-net.com http://www.bri.com.au http://www.sosland.com



# **PASTA**

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# Introduction

The word "pasta" is Italian for "dough" and is generally used to describe products fitting the "Italian" style of extruded foods such as spaghetti or lasagne. It is distinguished from the "oriental" style of sheeted and cut foods called noodles. There are over 600 pasta shapes, the most popular being spaghetti, elbow, macaroni, lasagne, and shells. Pasta can be sold fresh (as made in the home or restaurant) or refrigerated but most pasta is dried (with or without eggs), canned, or frozen. There are composite products, such as ravioli, cannelloni, lasagna, etc., in which pasta is combined with meat and vegetables in a tomato-based sauce, but the ingredients for making pasta are principally durum wheat semolina and water.

The wheat preferred is durum *Triticum turgidum* L. subsp. turgidum conv. durum (Desf.) MacKey (see Wheat: Genetics and Breeding). Durum wheat, in contrast to common wheat T. aestivum L. which is used to make bread and oriental style noodles, is the hardest wheat, and durum milling produces a coarse particle called semolina, ideal for making pasta and couscous. The key features of durum wheat include its hardness, intense yellow color, and nutty taste. After conversion to pasta, this wheat produces products with good cooking quality and stability to endure overcooking, with unmatchable eating quality. Several countries (Italy, France, and Greece) have decreed that pasta be produced exclusively from durum wheat and that the use of other cereals not mentioned is considered a fraud. In Italy, fresh pasta can be produced from common wheat flour or blends with semolina. Other countries such as Spain, United States, Canada, and Australia traditionally consume, by choice, pasta made from only durum wheat, but do not have a specific law on the matter.

This article provides a brief overview largely directed at dried pasta. It covers a brief history,

the raw material requirements, and the manufacture of pasta from wheat milling to pasta drying. The assessment of pasta quality, its nutritional value, and the use of nonwheat sources to manufacture pasta is also discussed. The reader is encouraged to read the Further Reading section for much more detail on the subject.

# **Origin of Pasta**

Indications are that pasta originated from China, although there is evidence of pasta use in Italy during the Etruscan civilization (several centuries BC). The earliest written record referring to pasta was in 1279 where pasta was included among the items in a will, referred to as a "bariscella plena da macaroni" (basket full of macaroni). By the sixteenth century, pasta makers in Italy were organized into trade associations and this quickly spread to France and other parts of Europe. Originally the production of pasta involved a batch process of manual kneading, cutting of the dough, and extrusion by hand press, followed by sun drying. In about 1800 the first mechanical devices appeared in Italy, and by the late 1890s equipment comprising mixers, kneaders, hydraulic presses, and drying cabinets became available. It was not until 1934 that the first continuous press system (where semolina and water are converted into wet pasta in a fully automated system) was developed replacing the batch method of pasta preparation and today all presses are of the continuous type.

# World Production and Consumption of Pasta

The Mediterranean region is the world's largest producer of durum wheat (55–60% of world production) followed by North America (30%). Most of the durum wheat traded on the world market comes from North America. The annual durum wheat production globally over a 10 year period (1990–99) in selected countries was estimated to be 21.2–31.0 million tons (Mt). This was produced

Table 1 Durum wheat production in the major producing countries between 1992 and 2001 (thousand tons)

Year	European Union	Canada	Turkey	United States	Syria	Kazakhstan	India	Morocco	Algeria	Tunisia	Others	Total
1992	9042	3138	4000	2719	1400	2000	1500	682	1300	1323	1526	28 630
1993	6907	3358	4200	1919	1600	2000	1500	587	1100	1133	1871	26 175
1994	7977	4635	4000	2632	3064	2200	1700	2350	650	436	1917	31 561
1995	7088	4648	3500	2784	3447	2300	1900	439	1250	472	1825	29 653
1996	8741	4627	3800	3160	3028	2500	1800	2270	1600	1623	3170	36 319
1997	7199	4352	4000	2390	2000	2500	1850	882	455	700	3838	30 166
1998	9241	6042	4000	3758	2600	1000	1700	1544	1500	1143	4196	36 724
1999	7384	4341	3800	2702	2000	3000	2000	799	900	702	4511	32 139
2000	9145	5708	3000	2988	2100	2200	2000	427	490	700	4718	33 476
2001 <sup>a</sup>	6922	2987	2900	2275	3000	2500	1800	1039	1232	1100	5289	31 044
10 year average	7965	4384	3720	2733	2424	2220	1775	1102	1048	933	3286	31 589

<sup>&</sup>lt;sup>a</sup> Preliminary: subject to revision.

Data from http://www.cwb.ca/en/publications/students\_researchers/pdf/stats\_english\_2001-02.pdf.

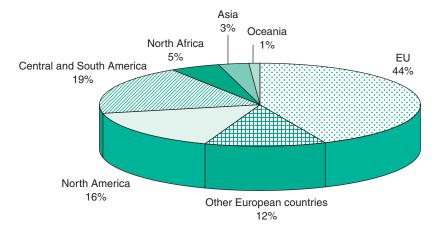


Figure 1 Approximate pasta production by region (% of world total). (Adapted from www.unipi-pasta.it/index\_economia.html.)

on a harvested area of 14-16 Mha, but this represents only  $\sim 6-8\%$  of the total global wheat production (Table 1). Annual pasta production from more than 30 countries amounts to  $\sim 10.3$  Mt (Figure 1). Italy is traditionally the leading producer with the highest per capita consumption (Table 2). Demand in other regions such as North America, Oceania, and Asia has been increasing steadily.

# Raw Material Quality Requirements for Pasta Products

Consumers of pasta are becoming more discerning in their quality requirements and less accepting of product variability. To achieve consistent quality, pasta makers must use raw materials that have the desired characteristics for processing into pasta. Currently, there are no standard measures of quality, and even for accepted parameters (as shown below) there is often disagreement over the methodology employed. Each parameter should not be viewed in isolation but used as part of a "picture of the quality" of the semolina (*see* Cereals: Grain-Quality Attributes). The important factors are given as follows.

#### **For Wheat Grain**

Appearance. Visual examination is very important to a buyer of wheat as it indicates how well the wheat will process. Most wheat-producing countries market wheat on the basis of its physical attributes.

Test weight. This is the quantity of grain that packs into a fixed volume. High values (>80 kg hl<sup>-1</sup>) indicate plump kernels undamaged by disease or environmental stress and this is correlated to the amount of semolina that can be produced from the wheat. Closely allied with test weight is the weight of 1000 kernels, which is also correlated with semolina yield, since large, plump kernels have more endosperm and hence, when milled, produce more semolina.

Physical defects. Grains with black coloration on the tips or in the crease of the grain will create

Table 2 Estimate of pasta consumption among selected countries (kg per head)

Country	Consumption			
Italy	28.0			
Venezuela	12.7			
Tunisia	11.7			
Switzerland	10.1			
United States	9.0			
Greece	8.8			
Peru	8.3			
Chile	8.2			
France	7.3			
Argentina	6.8			
Portugal	6.8			
Hungary	6.5			
Canada	6.3			
Brazil	6.1			
Russia	6.0			
Belgium-Luxembourg	5.7			
Germany-Sweden	5.5			
Spain	4.6			
Turkey	4.5			
The Netherlands	4.4			
Austria	4.2			
Australia	4.2			
Costa Rica	3.5			
Finland	3.2			
Poland	3.0			
Mexico	2.6			
United Kingdom	2.5			
Denmark-Libya	2.0			
Japan	1.7			
Romania	1.3			
Egypt	1.2			
Ireland	1.0			

<sup>a</sup>Source: http://www.ilovepasta.org/sitemap.html.

noticeable black specks in the semolina. These specks will appear in the pasta and are especially noticeable in sheeted products. The most common surface discolorations are black point, smudge (fungal infection into the crease), and mildew. Fusarium, another fungal infection, reduces semolina yield due to kernel shrivelling and makes pasta redder and duller. Fusarium is also a safety concern because of associated mycotoxins such as deoxynivalenol (vomitoxin). Other fungi include ergot (*Claviceps purpurea*), *Alternaria alternata*, and *Dreschlera tritici-repentis* which produce dark specks in semolina.

Vitreousness. Durum wheat kernels should have a translucent, vitreous appearance to ensure good semolina yield. Nonvitreous or starchy kernels tend to produce more flour than semolina upon milling because they are softer. The percentage of vitreous kernels in a sample of wheat is an important grading factor used in trading in some countries. Low vitreous percentages are often associated with a low protein content.

Moisture content. The moisture content of the wheat will vary from 8% (arid areas) to 14% (temperate areas). The miller needs to know the grain moisture content in order to determine the conditioning profile before milling the wheat to produce semolina with a moisture content of 14–15%. High moisture content leads to greater microbiological growth.

Weather damage. Frost exposure of wheat kernels during grain maturation prevents them from completely filling out, thus reducing semolina yield. Grains that begin to germinate in the head before harvest due to damp, low-temperature conditions, have high levels of the enzyme called  $\alpha$ -amylase. This enzyme degrades endosperm starch to produce sugars and can cause extrusion problems resulting in sticky pasta. The extent of sprouting can be measured by assaying for  $\alpha$ -amylase or falling number. The latter estimates the thickness of a hot wheatwater slurry by measuring the time (in seconds) for a plunger to free-fall through the paste, where a short time is indicative of weather damage.

Protein quantity. The grain protein percentage forms a part of the wheat payments to farmers in some countries (e.g., Australia and Canada). Highprotein semolina from durum wheats of good physical condition will generally yield semolina of uniform particle size with a minimum number of starchy semolina particles, and will hydrate evenly during mixing to produce pasta that is strong and elastic. When cooked, the pasta swells, leaving minimal residue in the cooking water, and remains firm to the point of serving. Semolina with low protein will produce pasta products deficient in some or all of these characteristics. A minimum protein content for pasta manufacture is  $\sim 12-15\%$  (dry matter basis). Either the Kjeldahl (acid hydrolysis) or Dumas (combustion) methods are used to determine the nitrogen content in the grain (where % protein =  $N \times 5.7$ ).

#### For Semolina

Ash. This is a measure of the mineral content (largely derived from the bran layers) remaining when all the organic content has been removed by combustion at very high temperatures. High extraction rates produce semolina with higher ash (0.9–1.1% dry weight basis) due to contamination by the bran and this reduces semolina's brightness and yellowness.

Protein quality. The protein type present in the grain affects processing properties. Gluten strength is a term used to describe the ability of the proteins to form a satisfactory network that promotes good cooking quality. The continuity and strength of the protein matrix formed during dough mixing and

extrusion is important in determining the textural characteristics of the pasta. Compared to weak gluten of the same protein level, strong gluten wheats exhibit less sticky dough with better extrusion properties and superior cooked textural characteristics. Experienced operators can judge the quality of this gluten by its feel in the hands; others need to use various physical tests. These tests include the sodium dodecyl sulfate sedimentation test, gluten index, farinograph, mixograph, and alveograph (see Wheat: Dough Rheology). Strength is particularly important for instant pastas since these have thinner walls and need more strength during processing. In contrast, popular fresh pastas require a more extensible dough and weaker gluten to improve sheeting properties. Thus, durum wheat or semolina specifications for gluten strength will vary depending on the type of final product being processed.

Color. A bright, yellow color in semolina ensures a good color in the pasta which consumers prefer. The main pigments in durum wheat responsible for the yellow color are xanthophylls and lutein. The yellow pigments can be partly degraded by the enzyme lipoxygenase during pasta processing. However, the high temperatures during drying denature the enzyme to make it ineffective. Color is typically measured using CIE (Commission Internationale de l'Eclairage) tri-stimulus values  $L^*$  (brightness),  $a^*$  (redness), and  $b^*$  (yellowness) with a chromameter or spectrophotometer. In addition, a brownish pigment caused by a copper protein complex causes a browning effect on the semolina which leads to a duller appearance in the pasta.

Speck count. Specks in semolina are caused by any material with a color that contrasts with the durum endosperm particles (brown or black). The black specks are easily detected in semolina. They also stand out clearly in the finished pasta. Brown specks arise from bran contamination and high levels indicate poor milling and/or high extraction rates. These are harder to see and their effect on pasta is to dull the appearance giving a gray effect to a normally bright product. It is impossible to completely eliminate these; therefore, an acceptable level is agreed upon between the miller and the pasta manufacturer. Measurement is normally done by visual inspection where the number of specks per unit area is counted.

Particle-size distribution. This is important since it affects the amount and uniformity of water absorption during mixing. Coarse particles hydrate slower than fine ones, and if there are too many fine particles in the mix, this will leave the coarse particles starved of water. These manifest as white inclusions in the pasta (white specks), which is unattractive to the consumer. For this reason the particle-size range should

**Table 3** Quality data of the three grades of Canada Western amber durum wheat for 2000–01

	No. 1	No. 2	No. 3
Wheat			
Wheats of other classes (%)	0.69	0.93	1.31
Test weight (kg hl <sup>-1</sup> )	82.0	82.1	80.5
Vitreousness (%)	83	68	56
1000 grain weight (g)	41.7	39.5	38.3
Falling number (s)	410	390	280
$\alpha$ -Amylase activity (units g <sup>-1</sup> )	5.5	10.5	45.5
Protein content (% at 13.5%) moisture	12.9	12.1	12.0
SDS sedimentation (ml)	42	34	31
Semolina yield (%)	67.4	66.3	65.6
Semolina			
Ash content (%)	0.69	0.67	0.69
Protein content (%)	12.0	11.2	11.3
Wet gluten content (%)	30.8	28.5	28.3
Dry gluten content (%)	10.3	9.7	9.5
Yellow pigment (ppm)	7.2	6.9	7.0
L* (brightness)	87.8	87.6	87.4
a* (redness)	-2.9	-3.0	-2.8
b* (yellowness)	30.6	29.3	28.7
Speck count per 50 cm <sup>2</sup>	29	31	42
Spaghetti dried at 70°C			
L*	77.1	76.9	75.6
<b>a</b> *	2.6	2.5	3.3
<i>b</i> *	61.8	61.3	56.1
Cooking quality parameter	47	37	41

Adapted from website www.cgc.com.ca.

not be too broad since mixing time is limited in continuous flow mixers. The typical particle-size distribution has been decreasing since the early 1980s (in the past  $630{-}125\,\mu m)$  with common ranges now being  $350{-}130\,\mu m$  resulting in quicker and better water absorption and consequently, shorter mixing times.

Nondurum contamination. An acceptable tolerance is typically 3%. New techniques have been developed to accurately determine the levels of adulteration of durum wheat semolina or pasta with common wheat.

Typical figures for quality of durum wheat and semolina are best seen in the data for the three grades of Canadian durum wheat (Table 3). The data clearly illustrate what happens to the grade as some of the characteristics deteriorate.

# Water

The water used should not have off-flavors or smells and needs to be monitored to ensure that it is safe and free from microbiological and chemical contaminants. It should not be too hard, have a low content of sodium, magnesium, and calcium ions as these would give an unpleasant flavor and color.

#### Other Raw Materials

Spinach has been used for many years and is usually added as a powder during mixing ( $\sim 2\%$  of the final product) to impart color. Tomato has also been used in much the same way. Egg imparts a change in color, texture, and increases protein content. It may be added in a liquid or dry state (four eggs - per kg of pasta). Vitamins are commonly used to fortify pasta in USA. These are added as a dry mixture (1 kg t<sup>-1</sup> semolina) and typically contain vitamin B<sub>1</sub>, B<sub>2</sub>, iron, and folic acid. Other additives include: acidifiers to give a sour taste (citric and tartaric acids); acidity correctors to modify pH of a food product (citric and lactic acids); emulsifiers to produce a homogeneous mixture of one or more immiscible phases in a food product (lecithins, fatty acids); preservatives to prolong the validity period of food products, protecting them from deterioration caused by microorganisms (sorbic acid); and flavor enhancers (monosodium glutamate).

#### The Manufacture of Pasta

An overview of the pasta making process is presented, but the reader is referred to more detailed accounts contained in the reference material.

#### Milling

The first step toward the manufacture of pasta is the milling of durum wheat to obtain semolina (see Wheat: Dry Milling). The wheat must be cleaned (by specific gravity) to remove impurities such as rocks, iron residues, straw, dust, insect fragments, and eggs. The wheat is then tempered to a moisture of  $\sim 15-16\%$  for  $\sim 4$  h before milling to toughen the bran. This ensures that the grain does not shatter excessively, as this would make it more difficult to separate bran from the endosperm in the purifier. Corrugated rolls are used to maximize semolina yield. Rolls with flutes aligned sharp-to-sharp further minimize flour production and maximize shearing during grinding. A long break system is used to allow the gradual release of coarse endosperm particles with a minimum yield of lower priced flour (Figure 2). Many purification passages are required to separate endosperm particles by their size and density. Passage through the rolling mills is alternated with sifting phases carried out by the plansifters and the purifiers. The plansifters, which are made up of superposed oscillating sieves of decreasing mesh size, have the task of separating the ground material according to particle size. The product that comes out of the plansifters is then conveyed,

by pneumatic transport devices, to the next phases of the milling process shown in the diagram.

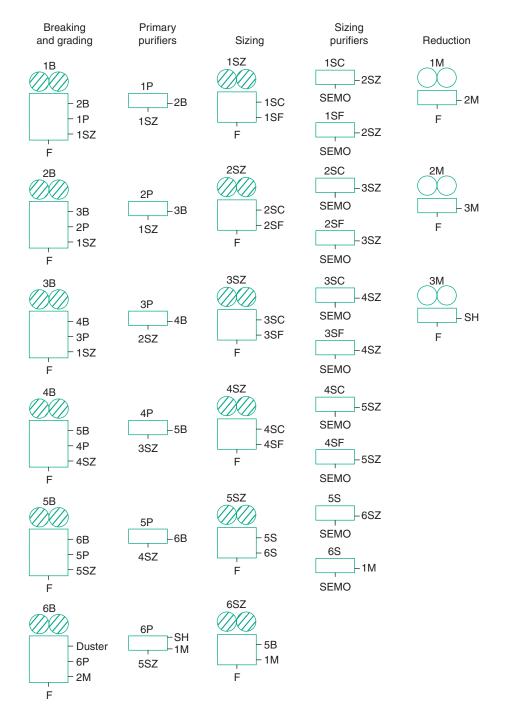
As the purifier sieve frame oscillates, an upward air current causes the material to stratify with the lighter bran-rich particles rising to the surface to float over the end of the sieve frame. The heavier endosperm particles fall through the sieve. After some initial purification, the stock is sent to sizing rolls to lightly grind the coarse particles releasing adhering bran and further reducing particle size. This is again purified and any material meeting the size settings is recovered as semolina and the rest goes onto the next sizing roll. A short reduction system is used to recover flour from the stock that is either too fine or too bran rich to be included in semolina. The reduction rolls have a smooth surface and are set closely together to release flour. Good purification is essential since bran or other dark particles are readily visible in the semolina. Commercial mills convert 65–75% of the wheat to semolina with 8-12% flour with the remainder being bran and embryo.

#### **Mixing**

This step involves combining semolina and water in a premixer. Water in a proportion of  $\sim 18-25\%$  of the dry raw materials is added at  $35-40^{\circ}$ C, to achieve a freshly formed dough containing an average of  $\sim 30-32\%$  moisture. After mixing (10-20 min), the mixture passes to the vacuum extruder (130 mm Hg) (Figure 3).

#### **Extrusion**

Dough is forced through a die in vacuum and at high pressure (80-120 kg cm<sup>-2</sup>) and this gives the pasta its desired shape by developing the dough. The vacuum helps to minimize the oxidation of pigments, reduce enzymatic and oxidative decomposition reactions, and to decrease the likelihood of bubbles being incorporated into the dough, which can cause unsightly appearances in the final pasta. Dough temperature is kept below 50°C to avoid deterioration of the gluten matrix. Rectangular dies are used for long goods (spaghetti, etc.) and circular dies for short goods (shells, macaroni, etc.) (Figure 4). The feed holes of the die inserts are usually teflon coated to produce pasta with a smooth surface while bronze inserts are used to achieve a rougher pasta surface, which helps sauces to stick better to the cooked pasta. Teflon-coated dies achieve much higher throughput rates. After extrusion the pasta is immediately subject to a blast of hot air to minimize strands sticking together. The strands then enter the predryer.



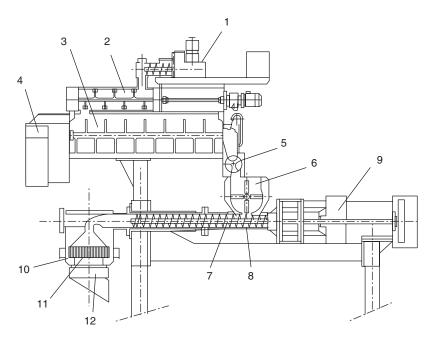
**Figure 2** Simplified durum wheat semolina mill flow: B = break roll; P = purifier; SZ = sizing roll; F = flour; S = sizing purifier; SC = coarse sizing purifier; SF = fine sizing purifier; M = middling roll; SEMO = semolina; SH = shorts. (Reproduced with permission from Kruger JE, Matsuo RB, and Dick JW (eds.) (1996) *Pasta and Noodle Technology*, p. 98. St. Paul, MN: American Association of Cereal Chemists.)

Sheeting by extrusion and rolls There are a number of different sheeting die formats. The extruder with a 300 mm head rated at 350 kg h<sup>-1</sup> gives a flat sheet of  $\sim$ 850 mm width. The sheet may be shredded to produce shortcut noodles, folded or presented as pasta Bologna. Such a machine can cut a wide range of different shapes that are stamped out while leaving

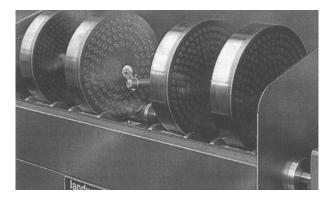
an open lattice of reworkable material which is delivered to the feed stock of pasta dough.

# **Drying**

The final step in the pasta-making process is drying. The purpose of drying is to produce a strong, stable



**Figure 3** Layout of a modern continuous press: (1) doser; (2) premixer; (3) mixer; (4) control gear box for mixers; (5) capsulism device; (6) vacuum mixer; (7) extrusion worm; (8) cylinder; (9) worm control gear box; (10) extrusion head; (11) die; and (12) cutter. (Reproduced with permission from Kruger JE, Matsuo RB, and Dick JW (eds.) (1996) *Pasta and Noodle Technology*, p. 15. St. Paul, MN: American Association of Cereal Chemists.)



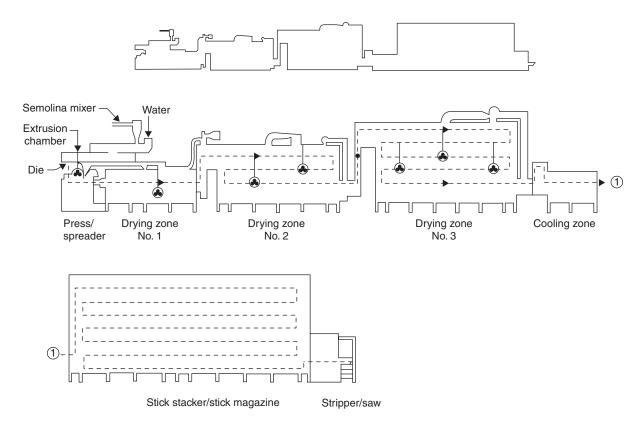
**Figure 4** Die for short goods (macaroni, elbows etc.). (Reproduced with permission from Kill RC and Turnbull K (eds.) (2001) *Pasta and Semolina Technology*, p. 155. London: Blackwell.)

product with a final moisture content of ~12.5% with low water activity to ensure a long shelf life. Moisture is removed from the surface of the pasta by a stream of hot air creating a moisture gradient within the pasta. During the drying of pasta, it is essential that the outside surface of the pasta does not dry too quickly, otherwise a large moisture difference between the inside and outside of the pasta will occur. This causes "checking" or fracture lines to develop that will lead to breakage during packaging and storage. In severe cases, the strands will fall apart during cooking, destroying its appearance and therefore consumer appeal. The drier heat creates a permanent protein

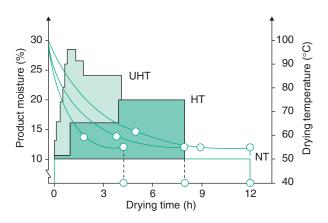
network around the starch granules, enhancing the strength and integrity of the pasta. This will prevent the starch granules from leaching into the cooking water and this will improve the firmness and bite of the cooked pasta. Excessive temperature during drying is undesirable, as it will result in brown discoloration of the pasta due to extreme nonenzymatic browning (Maillard reactions).

The pasta moves through a drying chamber and moisture falls from 30% to 12.5% and is then stabilized to ensure that any remaining moisture is evenly distributed. The product temperature is cooled down to that of the surrounding environment. Long goods which are draped over metal sticks move through the drier and the stick stacker. The pasta is then cut with high-speed stripper saws (Figure 5) to remove the bends (the portion of the strand which curves over the stick), trimming the product to the desired length. There are three different types of drying temperature used: normal temperature drying at  $50^{\circ}$ C for  $\sim 18$  h; high temperature drying at  $60-75^{\circ}$ C for  $\sim 8$  h; and ultrahigh temperature drying at 85-105°C for  $\sim 4-5 \,\mathrm{h}$  (Figure 6). Ultrahigh temperature drying has become common with benefits to quality such as increased pasta firmness and higher yellowness and higher capacity.

The final product is then packaged into cellophane or polyethylene bags or cardboard boxes. Packaging is designed to keep the product free from contamination,



**Figure 5** A simple schematic of a long goods pasta line. (Reproduced with permission from Marchylo BA and Dexter JE (2001) Pasta production. In: Owens G (ed.) *Cereals Processing Technology*, p. 113. Cambridge, England: Woodhead Publishing.)



**Figure 6** Drying diagrams at ultrahigh temperature (UHT), high temperature (HT), and normal temperature (NT) for the drying of long goods. Lines show change in product moisture and hatched areas are the temperature profiles used. (Reproduced with permission from Marchylo BA and Dexter JE (2001) Pasta production. In: Owens G (ed.) *Cereals Processing Technology*, p. 116. Cambridge, England: Woodhead Publishing.)

protect it from damage during shipment and storage, and display the product favorably while differentiating products. The typical yield for the complete process from milling to pasta is as follows: 140 kg of

durum wheat produces 95–105 kg of semolina; 100 kg of semolina produces 98.5 kg of pasta.

### **Assessment of Pasta Quality**

In traditional pasta-consuming countries the consumer is concerned about the aroma, color, appearance, texture, flavor, and nutritional value of the pasta. The pasta after cooking should maintain its texture and not become a thick, sticky mass (see Cereals: Grain-Quality Attributes). Mechanical texture is typically described by a range of terms (firmness, elasticity, stickiness, chewiness, and bulkiness) and can be measured by a sensory panel or by objective tests. Sensory evaluation is regarded as the ultimate test of pasta cooking quality and is the reference for which other methods are compared. However, some difficulties occur related to the different background and experience of the testers. To avoid subjectivity, various testing instruments have been developed to evaluate texture and all involve a means of deforming a sample and recording the force, time, and compression rate. Another test involves measuring by chemical methods, the total amount of organic matter released from the cooked pasta after immersion in water for a fixed time. This test is highly correlated with sensory evaluation.

# Pasta Products from Nonconventional Raw Materials

Although durum wheat semolina is considered the best raw material for pasta making, in some countries other ingredients are used to make pasta. Flours from other cereals such as oat, barley, millet, rye, maize, rice, sorghum, hulled wheat (spelt, emmer, and einkorn), triticale, khorasan, buckwheat, amaranth, or quinoa may be used to make pasta. Alternatively, raw materials from noncereals such as legume flour/ starches (of chickpea, bean, pea, soybean, cowpea, and lentil), protein concentrates, and various animal proteins are used. Pasta has been produced from some of these raw materials for centuries in some Eastern countries. In the absence of durum wheat it is necessary to change the processing conditions, because unconventional raw materials have proteins of inferior quality and are not able to form a gluten network. Methods that modify starch structure help to produce a starch-protein matrix (e.g., gelatinization/ retrogradation, pregelatinization of starch, and extrusion cooking) (see Starch: Chemistry and Modification). Other techniques include high-temperature drying to promote a protein network, the addition of selected additives, and fortifying raw materials with proteins. Yellow color can be enhanced by adding riboflavin, sunset yellow,  $\beta$ -carotene, and vitamin E.

#### **Nutritional Value of Pasta**

Pasta is considered a healthy food being relatively low in fat, high in carbohydrate, and having good protein content. Nutritional improvement of pasta mainly involves increasing protein and dietary fiber content and the fortification with vitamins and minerals (Table 4). High-protein flours (soybean, pea, lupin, and chickpea) can be added to increase the protein content of pasta to greater than 15% and improve the content of limiting amino acids, particularly lysine. To increase the content of minerals, vitamins, and dietary fiber, incorporation of buckwheat, whole wheat, artichoke, and amaranth pastas all claim health benefits.

Several studies have suggested that pasta reduces the increase in blood glucose in humans following a meal compared to an equivalent load of other carbohydrates, such as white bread. This is considered beneficial in reducing the risk of developing type II diabetes. Flavored pastas allow diet conscious consumers to have flavor (basil, garlic, parsley, and red

**Table 4** Nutrition values for different types of pasta

	Plain	Vitamin enriched	Egg pasta
Calories (kcal)	342	370	380
Protein (g)	12	12.8	14
Fat (g)	1.8	1.6	4.2
Carbohydrates (g)	74	74	75
Dietary fiber (g)	2.9	4.2	4.7
Minerals			
Calcium (mg)	25	17.5	29
Iron (mg)	2.1	3.8	4.5
Phosphorus (mg)	190	149	214
Potassium (mg)	250	161	223
Sodium (mg)	3	7	21
Vitamins			
Ascorbic acid (mg)	0	0	0
Thiamin (mg)	0.22	1	1
Riboflavin (mg)	0.31	0.44	0.5
Niacin (mg)	3.1	7.5	8
Vitamin B <sub>6</sub> (µg)	0.17	0.1	0.1
Folacin (µg)	34	17.5	30
Vitamin B <sub>12</sub> (µg)	0	0	0.4
Vitamin A (iu)	0	0	61
Cholesterol (mg)	0	0	94

All information per 100 g product.

Data from Kill RC and Turnbull K (eds.) (2001) Pasta and Semolina Technology. London: Blackwell.

pepper) without the addition of the high caloric sauces.

Pasta as a food represents an inexpensive means of improving diet quality in developed countries and helps to reduce hunger problems in developing countries. The unique combination of properties of cheapness, ease of preparation, versatility, nutritive value, and long shelf life will ensure that pasta will continue to play a role of importance as world demand for cereals increases.

See also: Cereals: Overview. Consumer Trends in Consumption. Grain Crops, Overview. Wheat: Agronomy; Grading and Segregation; Marketing; Dry Milling.

# **Further Reading**

Antognelli C (1980) The manufacture and applications of pasta as a food and as a food ingredient (Review). *Journal of Food Technology* 15: 125–155.

Cole ME (1991) Prediction and measurement of pasta quality (Review). *International Journal of Food Science and Technology* 26: 133–151.

Dick JW and Matsuo RR (1988) Durum wheat and pasta products. In: Pomeranz Y (ed.) Wheat Chemistry and Technology, 3rd edn., vol. 2, pp. 507–547. St. Paul, MN: American Association of Cereal Chemists.

Donnelly B (1991) Pasta: raw materials and processing. In: Lorenz KJ and Kulp K (eds.) *Handbook of Cereal Science and Technology*, pp. 763–792. New York: Marcel Dekker.

Fabriani G and Lintas C (eds.) (1988) *Durum Chemistry* and *Technology*. St. Paul, MN: American Association of Cereal Chemists.

Kill RC and Turnbull K (eds.) (2001) *Pasta and Semolina Technology*. London: Blackwell (major reference work used for "Pasta" article).

Kruger JE, Matsuo RB, and Dick JW (eds.) (1996) Pasta and Noodle Technology. St. Paul, MN: American Association of Cereal Chemists.

Marchylo BA and Dexter JE (2001) Pasta production. In: Owens G (ed.) *Cereals Processing Technology*, pp. 109–130. Cambridge, England: Woodhead Publishing.

Mercier Ch and Cantarelli C (eds.) (1986) Pasta and Extrusion Cooked Foods: Some Technological and Nutritional Aspects. New York: Elsevier Applied Science.

Troccoli A, Borrelli GM, De Vita P, Fares C, and Di Fonzo N (2000) Durum wheat quality: a multidisciplinary concept. *Journal of Cereal Science* 32: 99–113.

#### **Relevant Websites**

http://www.fas.usda.gov — This website provides information about production, imports, exports for various crops across the world. The site is monitored by the US Department of Agriculture.

http://www.cgc.ca — This website provides general information about the Canadian Grain Commission and quality data on Canada wheats.

http://www.professionalpasta.it — This is the website for the Professional Pasta Journal and contains significant amounts of information about pasta processing, history and quality. There are many free downloadable articles available.

# **PEA**

Contents

Overview Agronomy

# **Overview**

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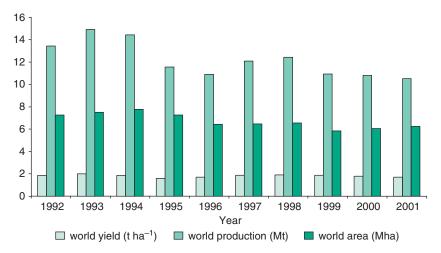
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#### Introduction

Pea (*P. sativum* L.) is one of the most ancient crop plants domesticated at around the same time as the major cereals. It is a cool-season plant requiring moderate temperatures in the range of 12–18°C with a relatively humid climate for optimum growth. Hot, dry weather is detrimental to seed set. However, it is also one of the most adaptable cool-season legumes and can give economic yields in areas that may

not conform to the optimum requirement. A symbiotic relationship with Rhizobium enables pea to fix atmospheric nitrogen and make it available for the following crop. Thus, peas are of considerable significance in cropping rotations and overall economic value in the cropping system. The field pea plant has many uses. Green peas are one of the most popular vegetable items throughout the world. Some variants with edible pods are also used as whole pod vegetable, e.g., sugar podded peas, snow peas, etc. In some parts of Africa and Burma, leaves are used as pod herbs. It is also a forage plant that makes excellent hay and silage and can be utilized as a green manure crop. Dry peas are used as a whole in confectionery and snacks, milled to produce split peas for making soups, "dhal" (a curried soup-like preparation widely used in the Indian subcontinent), flour, and canned products such as mushy peas. Dry peas are used in the feed industry, particularly in the diet of pigs and



**Figure 1** World field pea area, yield, and production (1992–2001). (Source: FAOSTAT DATABASE (1998) Food and Agriculture Organization of the United Nations. http://apps.fao.org (Dec 2002).)

Table 1 Area, production, and yield figures for the five highest producers as compared to the world total

Year	(	Canada			China			India			Russian ederatio			France			World	
	$A^a$	$P^b$	Y <sup>c</sup>	A	Р	Υ	A	Р	Υ	A	Р	Υ	A	Р	Υ	A	Р	Υ
1992	2.6	5.0	1.9	4.1	5.5	1.3	5.8	5.5	0.9	19.1	26	1.4	7.0	33.2	4.7	72.4	13.4	1.9
1993	4.7	9.7	2.1	6.3	10.0	1.6	6.1	5.5	0.9	17.1	25.3	1.5	7.3	36.6	5.0	74.9	14.9	2.0
1994	6.8	14.4	2.1	8.1	12.8	1.6	6.9	6.4	0.9	16.7	22.9	1.4	6.6	33.8	5.1	77.6	14.4	1.9
1995	7.9	14.5	1.8	6.6	10.3	1.6	7.6	6.7	0.9	14.5	12.1	8.4	5.8	27.0	4.7	72.5	11.5	1.6
1996	5.2	11.7	2.3	7.3	11.8	1.6	7.6	6.4	8.0	10.9	13.2	1.2	5.3	25.6	4.8	64.2	10.9	1.7
1997	8.5	17.6	2.1	6.7	10.0	1.5	7.3	7.2	1.0	8.6	11.9	1.4	6.1	30.5	5.0	64.6	12.1	1.9
1998	10.8	23.4	2.2	7.5	12.1	1.6	7.9	7.1	0.9	5.9	6.6	1.1	6.1	32.2	5.3	65.5	12.4	1.9
1999	8.4	22.5	2.7	8.3	10.4	1.3	7.0	7.0	1.0	5.5	6.0	1.1	4.8	26.2	5.5	58.3	10.9	1.9
2000	12.2	28.6	2.4	8.4	10.2	1.2	7.0	7.0	1.0	5.9	9.0	1.5	4.3	19.4	4.5	60.5	10.8	1.8
2001	14.0	22.0	1.6	7.5	11.0	1.5	7.0	7.0	1.0	5.8	10.0	1.7	4.2	16.8	4.0	62.3	10.5	1.7

<sup>&</sup>lt;sup>a</sup>Area ha  $\times$  10<sup>5</sup>.

Data from FAOSTAT DATABASE (1998) Food and Agriculture Organization of the United Nations. http://apps.fao.org (Dec 2002).

poultry due to their amino acid balance. This overview is focused on the production of the grain crop of *P. sativum*, referred to as dry pea or field pea.

There are over 6 million hectares (Mha) of field pea grown in the world today (Figure 1). As of early 2000s, Canada has the largest area under pea cultivation, followed by China, India, the Russian Federation, and France in that order (Table 1). Canada has dramatically increased its pea cultivation area from ~0.2 Mha to over 1.3 Mha since the 1990s. Canada also leads in the total production with more than 2 million metric tons (Mt) of crop produced in 2001, followed by France with a production of over 1.6 Mt. Eighty-eight percent of the total world production comes from the 10 highest field pea producing nations. The yield per hectare varies widely between growing regions. Amongst the largest producers,

France has the highest yields per hectare of over 4t against the world average of 1.68t and the leading producer Canada's average yield is ~2t. Average yields as low as 0.186t have been reported from Croatia. In most countries where it is produced, pea seed is retained for domestic consumption. Canada, parts of Europe, the United States, and Australia are the major exporters.

#### The Plant

Field pea belongs to the family Fabaceae and although a number of *Pisum* species were designated for various cultivated pea types, they have all been assigned to *P. sativum* L. Some taxonomists, however, still insist on subdividing field pea, garden pea, and vegetable

<sup>&</sup>lt;sup>b</sup> Production t  $\times$  10<sup>5</sup>.

 $<sup>^{</sup>c}$ Yield t ha $^{-1}$ .

pea into separate subspecies, although differences are often based on a few genes rather than broad genomic differences. All subspecies cross and recombine freely. Despite archaeological evidence indicating its cultivation as early as 6000 BC in Near Eastern and Greek settlements, there is no clear consensus on its exact place of origin. The Near East, Central Asia including Afghanistan, the Mediterranean, and Ethiopia abound with primitive forms and are sources of immense genetic diversity. A distinct species Pisum fulvum Sibth and Sm, partially cross-fertile with P. sativum, has been identified as a source of useful variation for disease and insect pest resistance and drought tolerance.

The plant, a spreading or tendril-climbing herb, is typically 30–150 cm tall (Figure 2). Papilionaceous flowers may be white or purple, or various shades of purple including pink. It is predominantly selfpollinated with cross-pollination rarely exceeding 1%. The stem is typically weak, leading to lodging as the plant gains weight. The compound leaves generally have one or more leaflets modified into tendrils, although variants are known which have no tendrils. Of significance are the forms where an entire leaf modifies to form tendrils (Figure 3) as this may help the crop to stand better by intertwining with plants. Greater attention to this character will be given while discussing genetic improvement. White flowered plants give rise to white, light greenish, or translucent testa, whereas colored flowers produce variously colored testa. Cotyledon color ranges from various shades of yellow to green, although



Figure 2 P. sativum white flowered, normal leaf type showing pod and seed development.

genotypes with red cotyledon color are also known. The testa color largely determines the seed appearance, which is of commercial significance. Traders recognize five field pea seed types (Figure 4). The round white (also known as yellow), as the name suggests, is round with white to cream testa and yellow cotyledons. The dun type is variously dimpled with greenish brown (dun) testa and yellow cotyledons. The maple type is round with brown testa that is mottled with light colored spots giving it a marbling effect and yellow cotyledons. The blue type (also known as green) has a translucent testa with green cotyledons giving the seed a bluish hue. The marrowfat is typically a large seed (>280 mg) slightly dimpled and flattened with blue/green testa and green cotyledons.

The diploid chromosome number is 14. Chromosomal interchanges can be common. Considerable attention has been given to the genetics of pea following the pioneering experiments of Mendel in the nineteenth century. Several thousand mutants have been identified and those conforming to Mendelian genetics were placed on a linkage map as early as the middle of the last century. Updated maps, now including biochemical and molecular markers, have regularly appeared in *Pisum Genetics* (see below).

# Nutritional Value, Processing, and Utilization

Field pea seed is a rich source of protein, carbohydrate, and some minerals, although the nutritional content of the seed varies with the environment and genetic factors. By far the highest proportion of the nutrient value of field pea is contained in the cotyledons, with the embryo and seedcoat contributing less than 10% to the nutritional value. The protein content is typically  $\sim 22\%$  but ranges widely, depending upon the genotype and growing conditions (Tables 2a) and 2b). Although sulfur-containing amino acid is low, it compares favorably with other grain legumes. About 60% of the carbohydrate content of the seed is made up of sucrose and oligosaccharides, starch, and crude fiber. The fat content is low and the seeds are a good source of vitamins such as thiamine, riboflavin, and niacin, although considerable loss of vitamins may occur in processing.

Antinutritional factors are present in quantities lower than in other grain legumes, and field pea has been found to have the lowest trypsin inhibitor activity among grain legumes commonly used as food in India. The flatulence activity resulting from oligosaccharides of the raffinose family is also relatively low, as are phytic acid and saponins. Lipoxygenase

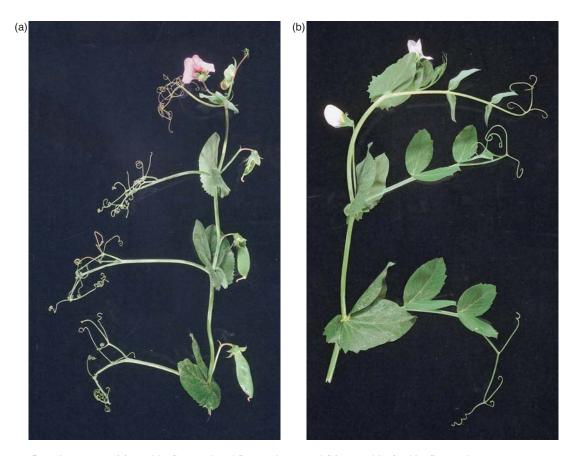


Figure 3 P. sativum types: (a) semi-leafless, colored flowered type and (b) normal leaf, white flowered type.



Figure 4 The five seed types of *P. sativum*: top left – yellow; top right – marrowfat; centre – dun; bottom left – green; and bottom right-maple.

**Table 2a** Nutritional content of field pea seed measured from a representative number of samples<sup>a</sup>

Nutritional factor	Content (g kg <sup>-1</sup>
Dry matter	889.0-918.0
Protein	193.4-273.0
Ash	21.0-33.1
Fat	7.3-23.6
Fiber	45.3-79.0
Acid detergent fiber	76.0-176.0
Neutral detergent fiber	71.0-245.0
Lignin	0.5-10.0
Calcium	0.5-1.1
Magnesium	1.0-1.5
Phosphorus	2.6-8.5
Potassium	2.0-11.0
Sodium	< 0.1 - 0.10
Sulfur	1.6-2.2

<sup>&</sup>lt;sup>a</sup> Data from Petterson DS, Sipsas S, and Mackintosh JB (1997) *The Chemical Composition and Nutritive Value of Australian Pulses.* Canberra, Australia: Grains Research and Development Corporation.

**Table 2b** Amino acid content of field pea seed measured from a representative number of samples<sup>a</sup>

Amino acid	Content (% in seed)
Alanine	0.97
Arginine	2.35
Aspartic acid	2.46
Cystine	0.34
Glutamic acid	3.85
Glycine	0.97
Histidine	0.59
Isoleucine	0.93
Leucine	1.56
Lysine	1.61
Methionine	0.19
Phenylalanine	0.99
Proline	0.97
Serine	1.03
Threonine	0.79
Tryptophan	0.18
Tyrosine	0.73
Valine	1.02
Cys + Met	0.57
Tyr + Phe	1.70

<sup>&</sup>lt;sup>a</sup>Same as in Table 2a.

activity that causes deteriorative changes during processing and storage of field peas has been found to be high, but these enzymes can be inactivated via heat treatment.

Dry pea seed is processed via soaking, germination, milling, cooking, roasting, or fermentation. In western diets the seed is mainly used in soups or in mushy pea preparations. In South Asian diets it has varied uses: as dhal (spicy soup-like preparation in the Indian subcontinent), whole pea boiled as a snack food, as

sweet and savory snacks made from pea flour, or as a supplement to wheat flour to make nutritious breads. In addition, peas can be processed into protein, starch, and fiber fractions. These products can then be used in baked goods, baking mixes, soup mixes, processed meats, health foods, pastas, and purees. There are also several industrial starch uses.

Field peas also serve as excellent stock feed. In an amino acid balanced diet, there is no limit on the inclusion rate of field peas in ration for sows, weaners, and porkers. For poultry, it can be included up to 25% in rations. It may also be fed in high doses to ruminants as a palatable energy and protein-rich feed.

# **Genetic Improvement**

The field pea yield is predominantly determined by the additive gene action, but nonadditive factors may also play a significant role. The heritability of yield may vary, depending on the environmental conditions leading to variable yield stability. The logical yield components are pods per plant, number of seeds, and seed weight. Among these pods per plant appears to be the best correlated with yield. However, selection for yield based on any one of these components is often ineffective. Further, the yield selection in early generations is not very effective as these generations are used for culling the population against easily recognized undesirable traits based on flower color, seed type, disease susceptibility, and plant type. Various breeding methods are in use and their application depends on the type and amount of genetic variation in crosses, objectives of breeding, and available resources. In Europe, the pedigree method is commonly used. Single seed descent is becoming popular to hasten the breeding and also to increase the frequency of desirable genes where recurrent selection is preferred. Bulk pedigree method has been successfully applied in Western Australian short-season environment where biomass appears to be an important asset in combating the terminal drought. Here, bulking early generation allows for natural selection to favor vigorous and tall plants.

There is considerable interest in restructuring the field pea plant since the discovery of a recessive mutant designated as afila (*af*) where leaflets are modified into tendrils. Plants with the afila characteristics are popularly known as semi-leafless, although strictly speaking they should be termed leafless. An increased number of tendrils in the semi-leafless crop helps plants to stand upright through intertwining with adjacent plants. When combined with dwarf plant stature and stiff stem characteristics, semi-leaflessness has considerably improved the standing ability of the crop. Improved standing ability has important



Figure 5 Blackspot on P. sativum caused by M. pinodes, the most widespread pea disease in the temperate zone.

implications in allowing for increased machine harvestation and enhanced aeration through the canopy that may help to reduce disease epidemics. Semi-leafless field pea varieties have become very popular in Europe, North America, New Zealand, some parts of Australia, and in countries such as India. However, in limiting environments of parts of Southern and Western Australia, conventional leaf types still outperform semi-leafless types. In such environments high biomass appears to be an important factor in determining yield, and semi-leafless types tend to have a lower biomass even at a high plant density. Breeding more vigorous and tall semi-leafless varieties presents a dilemma, as a heavier plant will tend to lodge because the stiff stem characteristic currently available is not able to support such a weight. In addition, dwarfing is counter-productive to high biomass.

Breeding against yield limitations such as diseases, insect pests, and abiotic factors has played a significant role in improving field pea yields. The impact of these factors varies with agro-ecology and crop management practices. Amongst the diseases, root rot diseases caused by Pythium spp. and Aphanomyces euteiches f. sp. pisi Pfender and Hagedorn and wilt caused by Fusarium solani (Mart.) Sacc. F. sp. pisi (Jones) Snyd. and Hans. are significant. The significant aboveground diseases are: (1) wilt caused by the bacterium Pseudomonas syringae pv. pisi Sackett, powdery mildew (Erysiphe pisi Syd.), and downy mildew (Perenespora pisi Syd.), and (2) black spot disease complex caused by Ascochyta pisi Lib., Phoma medicaginis var. pinodella (Jones) Boerema and Mycosphaerella pinodes (Berk and Blox.) (Figure 5). A number of viruses attack field peas with the most

devastating being: pea seed-borne mosaic virus (PSBMV) and pea enation mosaic virus (PEMV). Of these, PSBMV is widely distributed throughout the world, whereas distribution of PEMV is more restricted.

Resistance is available against some root rots and wilt, powdery mildew, downy mildew, and black spots caused by A. pisi and P. medicaginis var. pinodella as well as the two important viruses -PSBMV and PEMV. However, it is resistance against the black spot caused by M. pinodes, the single most important disease of field pea throughout the temperate growing zones, which has been difficult to control through breeding. Only low to moderate resistance has been identified in several screening attempts of large germplasm collections in North America, Europe, and Australasia. More recently, over 3000 accessions from the Vavilov Institute, St. Petersburg collection were screened off-shore in Ethiopia by a Western Australian based project. The more pronounced expression of partial resistance has been identified in primitive accessions originating from Afghanistan, Ethiopia, and China. However, such accessions generally carry a number of wild characteristics including extraordinarily delayed flowering. Delayed flowering is recognized to help the field pea plant to resist M. pinodes and hence it is not always clear whether the resistance shown has a true genetic basis or is an artifact of late flowering habit. More recently, it has been found that there is some variation in reaction to M. pinodes infection within the improved germplasm and that small degree of resistance scattered in a wide variety of sources can be recombined. Both wild and improved sources have been used

in recurrent selection programs across Australia and New Zealand, and some new improved lines show resistance levels that are equal to or better than the wild germplasm. Developing resistant varieties to this pathogen will be a single most significant factor in increasing field pea yield in the major temperate growing areas.

The major field pea crop pests worldwide are pea and bean weevil (Sitona lineatus L.), pea moth (Cydia nigricana F.), pea aphid (Acyrthosiphon pisum Harris), pod borers (Helicoverpa armigera Hub. and H. punctigera (Wallengren)), and pea seed weevil (Bruchus pisorum L.). The red-legged earthmite (Halotydeus destructor Tucker) is more destructive in the southern hemisphere, e.g., Australia, where pasture legumes are widely grown. There has been some work on studying variation in resistance levels, but the most promising work so far has been on pea weevil (B. pisorum) in Australia. Resistance has been identified in a wild species P. fulvum that is partially cross-fertile with the P. sativum. Resistance has been backcrossed to field pea lines that should soon be ready for use as parent in crossing. Genetic transformation has also been successful in developing resistant lines and this will be described later in detail.

Waterlogging, freezing temperatures particularly during flowering and podding, and moisture stress are the most serious abiotic constraints to field pea productivity. No true resistance to waterlogging or frost has been reported so far. There are reports regarding cold tolerance, but whether it relates to avoiding damage during frost is not known. There are two aspects of freezing tolerance. In higher latitudes, such as Europe, freezing tolerance is important during the vegetative phase in the winter-sown crop, whereas in subtropical and Mediterranean climates frost tolerance during the reproductive stage is more important. A deeper root system has been reported to increase tolerance to moisture stress. However, osmoregulation/osmotic adjustment is a more measurable character and it has shown good correlation with yield under water stress in studies done in Spain and Western Australia. Recent studies in Western Australia have shown that the most adapted field pea variety showed one of the highest levels of osmotic adjustment.

# Biotechnology and *In Vitro* Culture Techniques for Field Pea Improvement

#### **Molecular Markers and Linkage Mapping**

Molecular markers offer the potential to advance plant breeding by analysis of genetic variation, unequivocal identification of genotypes and true hybrids, purity testing of breeding lines, genome mapping, and marker-assisted selection. In field pea, classical physiological, biochemical, and hybridization-based markers such as restriction fragment length polymorphism (RFLP) have been joined by polymerase chain reaction (PCR) generated markers such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and short sequence repeats (SSRs). The polymorphic markers identified using these techniques are used to construct a linkage map of the field pea genome, indicating the position and relative genetic distances between markers along chromosomes.

When markers that are closely linked to desirable genes have been identified and validated in specific environments, plant breeders can use them to select plants on the basis of genotype rather than phenotype. The application of marker-assisted selection can reduce unreliable phenotypic screening and cultivar development time as markers enable the selection of genotypes at the seedling stage. Molecular linkage maps and trait mapping are valuable tools for characterizing the genetics of disease resistance, localizing resistance loci on linkage maps, and identifying linked polymorphic DNA sequences that might be used for marker-assisted selection. This is particularly true for traits that are inherited in a quantitative manner, for which quantitative trait loci (QTL) may be identified.

In field pea, a linkage map of the pea genome localizing QTLs for field resistance to Ascochyta blight and for plant developmental stage has been developed using classical and molecular markers. In addition, an RAPD-based genetic linkage map has been constructed, comprising seven linkage groups. Genetic relationships among Pisum accessions have also been assessed using random amplified microsatellite site (RAMS) type markers produced using SSR primers at low annealing temperatures. The largest distance observed was 22% and among P. fulvum accessions was 40%. The maximum distance between P. sativum and P. fulvum accessions was 46%. Interspecific hybridization to transfer useful traits from P. fulvum and wild-type P. sativum accessions such as ssp. P. elatius to cultivated P. sativum would also benefit from techniques such as marker-assisted selection and following QTLs. Targeted application of existing molecular techniques and the advent of new techniques such as the analysis of gene function through functional genomics will yield rapid gains in the understanding of field pea genetics.

#### **Somatic Embryogenesis**

The success of *in vitro* culture techniques such as transformation is reliant on the capacity of field

pea tissue to regenerate whole plants, usually via somatic embryogenesis. Somatic embryogenesis is the initiation and development of embryos from cells that are not products of gametic fusion. Somatic embryos are identical to normal zygotic embryos and are equipped with both the shoot and root pole and only need to be germinated to obtain complete plants. Somatic embryogenesis is useful for the rapid production of large numbers of plants from a single explant. In the absence of a system of nucleo-cytoplasmic male sterility in field pea, the cloning of the best hybrids by somatic embryogenesis and the production of artificial seeds could provide a means of utilizing hybrid vigor. In field pea, regeneration via somatic embryogenesis has been achieved from leaf-derived callus, shoot apices, immature zygotic embryos, and from protoplasts of zygotic embryo axes. Shoot apices and immature zygotic embryos are now routinely used as initial explants. Success in regeneration and especially in somatic embryogenesis is highly dependent on the donor plant genotype. In field pea, significant quantitative variation has been observed in inheritance of the ability to form somatic embryos with  $\sim 80\%$  of the observed genotypic variation due to additive gene effects. The distribution of F<sub>3</sub> family means from a cross between a line which was responsive to somatic embryogenesis and an unresponsive line indicated the presence of a few major genes in the control of somatic embryogenesis in field pea.

#### **Transformation**

Plant genetic engineering provides an opportunity to introduce traits such as pest and disease resistance, improved protein quality, and herbicide tolerance from previously unavailable sources. Since the first production of transgenic field pea plants in 1992, Agrobacterium-mediated genetic transformation has become a relatively routine procedure in a number of laboratories worldwide. The embryonic axis of immature seeds or the lateral cotyledonary meristems from germinating seed are the most responsive target explants, and regeneration is generally via organogenesis. A transgenic field pea genotype with significant pea weevil resistance has been developed in Australia. This cultivar is transformed with α-amylase inhibitors, which confer 99.5% resistance to pea weevil in glasshouse and field trials.

In addition to *Agrobacterium*-mediated transformation, several other approaches have been developed to produce transgenic field pea plants: transgenic callus has been obtained by electroporation of protoplasts, as has transient expression following particle bombardment of meristems. Microinjection, particle gun, agroinfection, or the pollen tube

pathway are at present less efficient or require expensive and sophisticated equipment. As in other species, transformation efficiency is dependent on the genotype, explant type, and other physical parameters. Community acceptance of cultivars developed via the transformation technique will determine the future of this technology.

# In Vitro Mediated Interspecific and Intergeneric Hybridization

Interspecific hybridization is the crossing of two species from the same genus. This allows the exploitation of useful genes from wild, unimproved species for the benefit of the cultivated species. The *Pisum* species are diploid self-pollinators sharing a similar karyotype. *P. fulvum* is the only separate wild species, and a useful source of disease and insect resistance. Fertile hybrids of *P. sativum*  $\times$  *P. fulvum* have been produced via conventional unidirectional crossing with *P. fulvum* as the pollen donor.

Intergeneric crossing is a further technique for improving genetic diversity within species. It is possible to regenerate plants from protoplasts in field pea via organogenesis and somatic embryogenesis. Somatic protoplast fusion, whereby genomes from different genera are combined without pollination through protoplast fusion, has been attempted for intergeneric hybridization between field pea and grass pea. A low frequency (5%) of protoplast fusion has been achieved; however, these hybrids have not been regenerated to whole plants.

#### **Double Haploid Production**

Doubled haploids (DHs) are plants derived from a single pollen grain and doubled artificially to form homozygous diploids. A DH individual has two identical homologs, so that the amount of recombination information is equivalent to a backcross. The advantage of using a DH population in molecular mapping is that all individuals are homozygous. Therefore, DHs may be transferred between different laboratories and environments for assessing the effect of the environment on gene expression. Field pea, along with many of the large seeded leguminous species, has traditionally been considered recalcitrant to this technique. Recently, success has been reported in producing embryos from isolated microspores of field pea. Research is ongoing to overcome barriers to further embryo development and plant regeneration. Successful production of doubled haploids on a routine basis would reduce cultivar development time, and provide excellent recombinant inbred lines for molecular mapping applications. The

development of doubled haploid plants also has a direct implication on breeding as it achieves homozygosity in segregating populations in a single generation as opposed to five to six generations using a conventional breeding cycle. This enables selection of stable lines to start much earlier.

# **Grain Quality and Marketing**

Market requirements for grain quality relate predominantly to shape, size, and color (testa and cotyledon). For all seed types large size is desired, and in marrowfat there may be a limit for the minimum seed size from the buyer. With blue pea, resistance to bleaching is an important criterion. Round white types must have smooth round surface with white to creamy color and bright yellow cotyledons. The dun types should ideally have a mix of greenish to brownish testa, fewer and shallower dimples with bright yellow cotyledons. Maple type that is mainly used for pigeon and bird feed must have white markings on a brown testa and yellow cotyledon. Both round white and dun types are milled, as are blue types. However, currently there are no standards for milling recovery. Seed moisture content at sale is normally between 10% and 15%. All types of field peas are generally accepted by the feed industry, but a preference is made for the noncolored type as they tend to have less antinutritional factors. The Southeast Asian market for sprouting field pea prefers dun types with large seed size and fewer hard seeds. Irrespective of the type, the following qualities are important for visual assessment in the trade:

- levels of admixture,
- levels of insect damage and presence of live or dead insects,
- product color,
- product size,
- product cleanliness, and
- product uniformity.

About one-fifth of the field pea production is traded throughout the world, which compares with temperate cereals where  $\sim 5\%$  is traded. The world field pea production has been declining in recent years, but trading across the board is showing a slow increasing trend. The prices have been generally steady since the early 1990s, but wide variations occur depending upon the season, time of the year, and proximity to the market. Canada and France are the world's leading exporters with Canada dominating the export in recent years. The Netherlands is the largest importer of field pea with Germany, Spain, Belgium, and Luxembourg emerging as rapidly growing importers. Demand for field pea from India is also increasing.

In general, the market for field pea is rather unsophisticated both in terms of product definition and in terminology used. For instance, in dun-type field peas that are normally milled, traders often use slight variation in testa color in an attempt to discount price that is irrelevant to the product utilization. There are various terminology used to describe the same product as in the case of white round type that is traded as yellow pea from North America. To address this issue some major exporters met at Winnipeg, Manitoba, Canada in 2000 for the International Pulse Quality Committee Meeting. They resolved to initiate a process to facilitate marketing of pulses through development of standardized nomenclature and methods of testing quality parameters in order to meet consumer needs. The Committee aimed at approaching International Pulse Trade and Industry Confederation (CICILS/IPTIC) and various major importing organizations to report their work and consult on future developments. It is expected that standardizing nomenclature and methods for quality evaluation will facilitate improvement of quality assurance in the international pulse trade including field peas. There has been limited progress so far, but updates on developments will be regularly posted on the CICILS website.

See also: Chemicals for Grain Production and Protection. Consumer Trends in Consumption. Cultural Differences in Processing and Consumption. Genetically Modified Grains and the Consumer. Genome Mapping. Genomics. Grain, Morphology of Internal Structure. Grain and Plants, Morphology. Grain Production and Consumption: Overview; Africa; Asia; Europe; Cereal Grains in North America; Oceania. Nitrogen Metabolism. Pea: Agronomy. Plants: Diseases and Pests. Pulses, Overview. Stored Grain: Invertebrate Pests. Taxonomic Classification of Grain Species. Variety Identification of Cereal Grains. Variety Registration and Breeders' Rights.

#### **Further Reading**

Casey R and Davies DR (eds.) (1993) Peas: Genetics, Molecular Biology and Biotechnology. Oxon, UK: CAB International.

FAOSTAT DATABASE (1998) Food and Agriculture Organization of the United Nations (http://apps.fao.org (Dec 2002)).

Hagedorn DJ (ed.) (1984) Compendium of Pea Diseases. St. Paul, MN: APS Press.

Hebblethwaite PD, Heath MC, and Dawkins TCK (eds.) (1985) *The Pea Crop: A Basis for Improvement*. London: Butterworths.

Knights R (2000) Linking research and marketing opportunities for pulses in the 21st century. *Proceedings* 

of the Third International Food Legumes Research Conference, Adelaide. Dordrecht: Kluwer Academic.

Makasheva RK (1986) The Pea. Rotterdam: A.A. Balkema.

Morton RL, Schroeder HE, Bateman KE, Chrispeels MJ, Armstrong E, and Higgins TJ (2000) Bean Alpha Amylase Inhibitor 1 in Transgenic Peas (Pisum sativum) provides Complete Protection from Pea Weevil (Bruchus pisorum) under Field Conditions. Proceedings of the National Academy of Sciences of the United States of America 97, pp. 3820-3825.

Petterson DS, Sipsas S, and Mackintosh JB (1997) The Chemical Composition and Nutritive Value of Australian Pulses. Canberra, Australia: Grains Research and Development Corporation.

Pisum Genetics, published periodically by Pisum Genetics Association, Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717, USA. (The journal can be viewed at the following website — http://hermes.bionet.nsc.ru/pg/.)

Salunkhe DK and Kadam SS (eds.) (1989) Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization, vol II, pp. 215-251. Boca Raton, FL: CRC Press.

Sutcliffe JF and Pate JS (eds.) (1977) The Physiology of the Garden Pea. London: Academic Press.

Weeden NF, Ellis THN, Timmerman-Vaughan GM, et al. (1998) A consensus linkage map for Pisum sativum. Pisum Genetics 30: 1-4.

#### **Relevant Websites**

http://cicilcsiptic.org - CICILS/IPTIC website. http://apps.fao.org - Faostat Database, Food and Agriculture Organization of the United Nations, 1998.

http://www.pea-lentil.com - USA dry pea and lentil council site with USDA policy positions and research updates.

http://interactive.usask.ca - Saskatchewan Agriculture summary of dry pea production, agronomy and uses.

http://www.ext.nodak.edu - North Dakota State University dry pea production and agronomy over-

http://www.hort.purdue.edu - Alternative field crops manual overview of dry pea history, production, agronomy and uses.

http://eesc.orst.edu - Oregon cover crops dry pea overview including abiotic and biotic factors.

http://www.smallgrains.org - University of Minnesota dry pea overview including cultural practices and economics of production.

http://www.sarep.ucdavis.edu - University of California SAREP database field pea including images.

http://www.mda.state.mn.us - Minnesota Department of Agriculture field pea overview and links to other relevant websites.

http://www.grdc.com.au - Australian Grains Research and Development Corporation field pea growing guidelines including disease information.

# Agronomy

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#### Introduction

Pea (Pisum sativum L.) is commonly grown in temperate areas of all continents. It has many uses: immature pods and seeds are used as green vegetables, either fresh or frozen; it is used as green forage for grazing animals, in situ or as hay or silage; and it is grown for its mature seed, which itself has many uses. Growing pea for its mature seed is the focus of this

Pea grown for grain is known variously as field pea, dry pea, combining pea, or protein pea (French pois protéagineux). It is grown in rotation with cereals and oilseeds in a wide range of environments, always as part of an integrated farming system. In North America and northern Europe, pea is generally sown in spring, harvested in mid-late summer, and followed by winter wheat. It is not commonly grown more than once every 4 years on the same land, and the rotation could also include winter wheat or barley, spring wheat or barley, oilseeds, or other grain legumes. In southern Europe and Australia, pea is sown in autumn or winter, harvested in early summer, and usually followed by wheat. In this case as well growing pea more than once every 4 years is not desirable, and the rest of the rotation comprises cereals and oilseeds. Pea is mostly grown under dryland conditions, but will respond to irrigation. However, limited resources for irrigation are often better used on higher valued crops, such as sugar beet and potatoes in the UK.

#### **Farming System Benefits of Pea**

The most important reason farmers grow pea is for the benefits conferred on other crops in rotations. The most obvious benefit comes from pea's ability to

Rotation	Blackgrass population 1999 (plants m <sup>-2</sup> )	Winter wheat yield 1998–99 (t ha <sup>-1</sup> )	Direct margin <sup>a</sup> 1996–99 (Euros ha <sup>-1</sup> )	
Continuous winter wheat <sup>b</sup>	13.0	6.4	407.0	
Spring barley-spring pea-winter wheat <sup>b</sup>	3.4	7.9	604.4	

<sup>&</sup>lt;sup>a</sup> Direct margin was defined as gross income minus protection costs, tillage costs, and crop seed costs, and is averaged over the whole life of the experiment.

Source: Chauvel B, et al. (2001) Crop Protection 20, 127-137.

acquire its own nitrogen (N) from the atmosphere, some of which is left in the soil where it can be used by following crops. One study in Western Australia found that wheat grown after cultivation of pea yielded 41% more grain, and averaged 1.7% more grain protein, than wheat with no added N fertilizer after wheat cultivation. Similar results have been obtained in North America. A common rule of thumb in the UK is that pea gives an extra ton per hectare of winter wheat. Pea also contributes to the farming system by breaking life cycles of diseases that affect other crops, and by allowing more flexible weed management. This is true even when weeds develop resistance to selective herbicides, which is happening increasingly in Australia, Europe, and North America. In France, pea rotations enabled successful management of herbicide-resistant populations of black grass (*Alopecurus myosuroides* Huds.). In this case, growing spring pea rather than the more profitable winter cereals allowed nonselective control of weeds germinating in late autumn and early winter. This gave better subsequent wheat yields, and a more profitable rotation overall (Table 1). In the Mediterranean, agricultural environment of southern Australia where early sowing is crucial to maximizing yields of cereals, oilseeds, and other grain legumes such as lupin and faba bean, pea increases opportunities for nonselective weed control because it is usually sown later.

Despite these benefits, farmers in many parts of the world are wary of growing pea. Pea usually yields less grain than wheat or barley, so is not generally as profitable in its own right as these crops. This is an important reason why farmers do not grow more peas in many European countries, and in Australia. Pea grain yields are also more variable from year to year than cereal yields. In Germany, it has been suggested that growing mixtures of pea with faba bean results in less season-to-season yield variability than either crop on its own. But the biggest disincentive to growing peas is harvesting. Even the most erect pea cultivars are prone to lodging at maturity, and many lodge completely. This means that, even with expensive harvester attachments to assist picking up the crop, harvesting pea is slower, and the likelihood of causing serious damage to the harvester, by putting soil or stones through it, is greater than harvesting other crops.

### **Adaptation of Pea**

#### Soils

Pea is best suited to sandy loam through to clay loam soils with neutral pH. It will, however, grow on a more diverse range of soils than other grain legumes. For example, in Australia it can be grown successfully on soils with pH ranging from 4.5 to 9.0 (measured in a 1:5 soil extract in 0.01 M CaCl<sub>2</sub>), although lupin is better adapted at the acid end, and lentil at the alkaline end, of this spectrum. Pea is not so well suited to very sandy soils, on which premature water deficits can develop as a consequence of its shallow root system. In environments with long dry periods after harvest, fragile pea stubbles on sandy soils are also at great risk from wind erosion. Neither is pea very well suited to very heavy soils with poor internal drainage because the crop is very sensitive to waterlogging.

#### Rainfall

Pea is one of the best-adapted grain legume species to dry environments. Vigorous early growth, early flowering of most cultivars, and early maturity mean a greater proportion of growth occurs under cool, humid conditions, and therefore water-use efficiency is higher than in species like lupin and chickpea. This applies both to spring-sown crops in North America and winter-sown crops in Australia (Table 2), and means in very dry seasons pea will produce at least some grain while other grain legumes may produce none at all. However, it also limits the ability of pea to respond to better growing conditions, so pea yield is often more stable across seasons than the yield of other grain legumes (Figure 1).

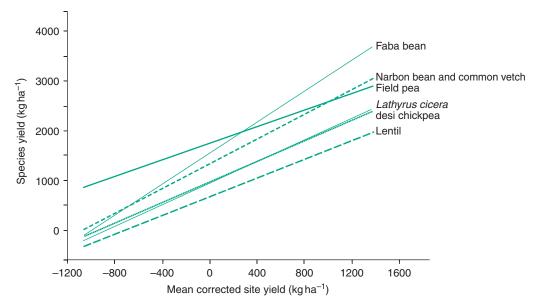
<sup>&</sup>lt;sup>b</sup> Rotations compared over three seasons (1996–97, 1997–98, 1998–99) on a site where blackgrass had developed resistance to aryloxyphenoxypropionate herbicides after 13 winter crops in 16 years and seven applications of fenoxaprop-P-ethyl or clodinafoppropargyl in the last 6 years. Figures for the winter crop rotation are averaged over three cultural strategies differing mainly in intensity of herbicide use, and figures for the spring crop rotation are averaged over four strategies.

Table 2 Water-use efficiency (kg ha<sup>-1</sup> mm<sup>-1</sup>) of a number of grain legume species in North America and Australia

		Australia South-west Western Australia <sup>d</sup>		
	Saskatchewan <sup>a</sup>	Northern Great Plains <sup>b</sup>	North Dakota <sup>c</sup>	South-west Western Australia
Pea	11.3	8.5 (29)	9.8 (3)	10.4 (4)
Chickpea	6.6	6.2 (24)	` ,	5.7 (3)
Lentil	6.2	4.8 (30)		4.5 (3)
Soybean		3.4 (11)	3.0 (3)	. ,
Dry bean		2.9 (15)	5.1 (3)	
Faba bean		. ,	` '	10.8 (4)
Lupin (Lupinus albus)				6.7 (4)

<sup>&</sup>lt;sup>a</sup> Data from Cutforth HW, et al. (2002) Canadian Journal of Plant Science 82, 681-686.

Figures in parentheses denote the number of trials contributing to individual means.



**Figure 1** Linear regressions of mean species yield against mean corrected site yield for 7 pulse species. Data are from 36 sites in Western Australia between 1994 and 1996. (Reproduced from the *Australian Journal of Agricultural Research* 50: 375–387 (KHM Siddique *et al* 1999) with permission of CSIRO Publishing. http://www.publish.csiro.au/nid/40.htm.)

#### **Growing a Pea Crop**

# **Cultivar Choice**

There is considerable diversity among pea cultivars. Initially, the type of pea to be grown must be chosen: whether white (yellow), blue (green), marrowfat, dun or maple; and then a cultivar among these types chosen. The type of pea will depend largely on market possibilities. Most peas produced in Europe and North America are white (yellow) or blue (green), and most in Australia are dun. Taking advantage of the higher prices for blue (green) or marrowfat peas requires more attention to grain quality than for other types. In particular, in dry environments achieving

large enough seed to satisfy market requirements is difficult.

Great improvements have been made in the standing ability of pea cultivars over the past 30 years. This is due to improvements in stem strength, shorter internodes, and use of the semileafless or *afila* character. Semileafless pea has more interlocking tendrils than conventional leafed plants, and therefore forms a more rigid canopy framework than conventional pea. These canopies are better able to withstand the wind-imposed shearing forces that normally cause pea canopies to lodge. Nearly all European and North American pea cultivars are semileafless, but most cultivars grown in Australia have conventional

<sup>&</sup>lt;sup>b</sup> Data from Miller PR, et al. (2002) Agronomy Journal 94, 261–272.

<sup>&</sup>lt;sup>c</sup>Data from Anderson RL, et al. (2003) Agricultural Water Management 58, 255-266.

<sup>&</sup>lt;sup>d</sup> Data from Siddique KHM, et al. (2001) European Journal of Agronomy 15, 267–280.

leaves. Semileafless cultivars are likely to become more prominent in Australia over the next decade.

Another important criterion for cultivar choice is disease tolerance. There are cultivars resistant to powdery mildew (*Peronospora viciae*), downy mildew (*Erysiphe pisi* f. sp. *pisi*), bacterial blight (*Pseudomonas syringae* pv. *pisi* and pv. *syringae*), *Fusarium* wilt (*Fusarium oxysporum*), and several virus diseases. Breeders around the world are also devoting considerable effort to improving tolerance to *Ascochyta* and *Mycosphaerella*.

# **Sowing Time**

In the Mediterranean environments of southern Europe and southern Australia, sowing time is determined by rainfall pattern, although in Australia it is further complicated by the need to avoid *Mycosphaerella* blight. In northern Europe and the Great Plains of North America, avoiding the cold winter is also important.

In Mediterranean environments hot, dry summers are followed by cool, moist winters. Spring becomes progressively hotter and drier. Annual crops like pea are sown in late autumn or early winter, grow over winter and spring, and mature in late spring or early summer. Sowing crops as soon as the soil is moist enough to support plant growth enables it to achieve most of its growth under cool humid conditions conducive to high water-use efficiency, as well as maximizing the length of the growing season. It also allows grain-filling to occur under cooler, moister conditions because early-sown crops flower earlier than late-sown crops. In Australia, pea is usually sown later than other annual crops. Its early maturity relative to other crops means it doesn't lose yield as quickly when sowing is delayed, but the real purpose is to reduce Mycosphaerella infection. This is discussed further below.

Where soils freeze in winter, sowing must be delayed until the soil is warm enough for germination— at least  $4-5^{\circ}$ C. There are winter cultivars, whose seedlings survive a certain amount of freezing after autumn sowing. Some of these are grown in France, but spring pea is more common. Sowing as soon as the soil is warm enough maximizes yield potential for the same reasons that early sowing does in Mediterranean environments. However, sowing too early can expose young seedlings to frost damage. If the aboveground parts of the seedling are killed, new shoots regenerate from subsurface axillary buds. In Canada, an early sown, frosted but regenerated crop often has as good a yield potential as a latesown unfrosted crop. Early sowing also increases the risk of frost during early pod growth. This can devastate yields. Frost damage during pod growth also occurs in Mediterranean environments, but in

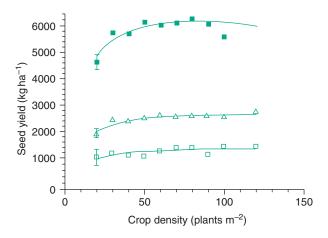
Australia spring frosts are sufficiently unpredictable to have only a minor bearing on sowing time decisions.

#### **Sowing Management**

Traditionally, pea was sown into cultivated seedbeds. This still happens, but in North America and Australia, it is increasingly being sown into undisturbed soil, often into standing cereal stubble. This reduces the risk of soil erosion from both wind and water, reduces the degradation of soil structure resulting from excessive cultivation, and avoids mixing weed seeds through the top 15 cm of soil, which can lead to more late-germinating weeds in the crop. Retention of standing stubble can improve crop water relations by trapping snow in the preceding winter, which adds to soil moisture storage in North America, and reducing evaporation from the soil surface by creating a thicker boundary layer. Standing stubble can also provide extra support to pea plants, which facilitates harvesting, but may delay spring sowing by retarding soil warming.

Pea should be sown 4–8 cm deep. Sowing at the shallow end of this range is an advantage for very early spring sowing into cold soil, but deeper sowing is preferable in environments where the soil surface dries rapidly. Crop damage from soil-applied herbicides is less with deep sowing.

Pea grain yield increases with increasing plant density when densities are low, but the rate of increase gradually declines until yield reaches a plateau, or even begins to decline (Figure 2). Simplistic analyses of this response choose the density at maximum yield



**Figure 2** Grain yield response of field pea *cv*. Swing to plant population density at seven locations in Saskachewan, Canada in 2001. Open squares — Swift Current, lowest yielding site, closed squares — Outlook, highest yielding site, triangles — average of seven sites. (Reproduced with permission from Johnston AM, *et al.* (2002) *Canadian Journal of Plant Science* 82, 639—644.)

as the target farmers should aim for, but the greater cost of seed compared to the grain produced means that the true economic optimum occurs at a lower density. In addition, grain yield is not the only criterion used to make plant density recommendations. Generally, it is better to err on the high side rather than the low side because dense crops compete better with weeds than thin crops, and have more interplant connections via tendrils, thus forming a more rigid canopy, which will better resist lodging. Dense canopies facilitate harvesting of prostrate conventional leaved cultivars too because, even though they invariably lodge, their interplant connections aid crop feeding into the harvester. Recommended target densities from various parts of the world are given in Table 3.

The necessary seed rate to establish the target density depends on seed size, percentage germination, and seedbed losses. In the UK, the following formula is recommended for calculating seed rates, and similar formulas are used elsewhere:

Seed rate (kg ha<sup>-1</sup>)
= (Thousand seed weight (g)
$$\times$$
 target population (plants m<sup>-2</sup>)  $\times$  100)/
(% germination  $\times$  (100 – seedbed losses))

Seedbed losses in Europe are greater with very early sowing when the soil is cold: UK growers expect losses as high as 15–18% for February sowing, but only

**Table 3** Recommended target plant densities in selected peagrowing regions of the world

Country	Target plant density (plants m <sup>-2</sup> )	Other specific information
United Kingdom <sup>a</sup>	65 70 95	Vigorous marrowfats Large blues and whites Early maturing small blues
Canada (Alberta) <sup>b</sup>	75–90	
Australia (Victoria) <sup>c</sup>	40-60	Tall varieties
, ,	70-80	Semidwarf varieties
Australia (Western Australia) <sup>d</sup>	45	Tall conventional leafy types
,	55	Semileafless and semidwarf types

<sup>&</sup>lt;sup>a</sup> Data from Heath MC, et al. (1991) Annals of Applied Biology 118, 671–688 and PGRO Crop Bulletin Number 1 http://www.pgro.co.uk/main/bull/bul no1.html.

7–8% for April sowing. In Australia, seedbed losses are generally less than 10% and are often ignored in seed rate calculations. Using the seed rate formula requires knowledge of the thousand seed weight and germination %, for which each seed lot must be tested. In Canada and UK, an electrolyte leakage, or electrical conductivity (EC), test is available for seed vigor. This is related to the number of microscopic cracks in the seedcoat, resulting from mechanical damage at harvest. These cracks allow substances that can stimulate pathogen growth to leak from the seed into the surrounding soil. Only the highest vigor seed (lowest EC value) should be used for early sowing into cold soil but less vigorous seed may be suitable for later sowing.

The soil surface should be left as even as possible after sowing, and free from stones, stumps, and other obstacles that could interfere with harvesting. This is especially important with conventional leaved cultivars that are prostrate at harvest, but also applies to modern, erect, cultivars that still sometimes lodge. Rolling pea crops after sowing helps by pushing rocks under the surface and crushing soil clods that might otherwise damage harvesting machinery. In some cases, heavy rubber-tired or steel rollers are towed behind sowing machinery, but pea can be rolled without significant damage until plants are  $\sim$ 15 cm high. Rolling before emergence is preferable because a rolled crop must be allowed to recover before any postemergent herbicides are applied. Rolling when the crop is emerging should be avoided because emerging seedlings are very fragile.

#### **Crop Nutrition**

Nitrogen (N) Pea requires the same plant nutrients as other crops but, as it is a legume, there is usually no need for N fertilizer if the crop is well nodulated. Pea forms root nodule symbioses with Rhizobium leguminosarum, which also forms symbioses with a number of other agriculturally important legumes, including faba bean, lentil, and vetch. It is different from the species that infect clover, medic, lupin, or chickpea roots. In Europe, R. leguminosarum is widely distributed in agricultural soils, so good nodulation can be achieved without seed inoculation. It is not so widespread in North America or Australia, where seed should be inoculated if sowing into soil that has not grown pea before. Trials in Australia show that inoculation can more than double grain yield under such circumstances. R. leguminosarum is not particularly acid tolerant, so does not survive as well in acid as in alkaline soils. In Australia, it is recommended that all pea crops be inoculated if sown on soils with surface pH  $\leq$  6, whether or not pea has

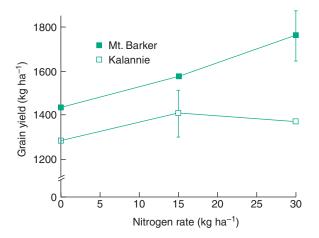
 <sup>&</sup>lt;sup>b</sup> Data from Park B and Lopetinsky K (eds.) (1999) Pulse Crops in Alberta, p.
 60. Edmonton, Alberta: Alberta Agriculture, Food and Rural Development.
 <sup>c</sup> Data from Carter JM (1999) Field Pea Growers Guide, p. 6 Melbourne, Victoria: Agriculture Victoria.

<sup>&</sup>lt;sup>d</sup>Data from Littlewood N (ed.) (2002) 2003 Crop Variety Sowing Guide for Western Australia. South Perth, Western Australia: Department of Agriculture.

been grown previously. Inoculum has traditionally been applied to seed as a slurry of peat-based culture in water or a methyl-cellulose solution (this aids adhesion to the seed) but more recently granular inocula on various carriers have been developed. These are placed in the seedbed at sowing rather than applied to the seed beforehand. The granular inocula have a longer shelf life than peat-based cultures and do not require refrigeration. Seed-applied inocula also have the disadvantage of having to be applied to the seed within a few days of sowing since the bacteria do not survive long on the seed surface. Fungicide seed dressings can be toxic to rhizobium on the seed, and large nodulation and yield reductions are possible. Recent Canadian research has shown that granular inocula are more effective on pea than inocula applied to the seed both in the presence and absence of the fungicides, metalaxyl and thiram.

While there is generally no need to use N fertilizer on pea, small amounts can be beneficial under certain circumstances. Pea seedlings depend on soil N before effective nodules form and in both Canada and Australia 5–20 kg ha<sup>-1</sup> of starter N is recommended on soils with very low mineral N. Effective nodules are slower to form in acid soils, and starter N can be helpful even when mineral N levels are relatively high (Figure 3). Higher rates of N can suppress N fixation and, in Canada, delay crop maturity and promote disease and lodging.

**Phosphorus (P)** Many soils on which pea is grown are P deficient, especially in their native state. Furthermore, much phosphate applied to soils is immobilized in insoluble forms before plants can use it. Many



**Figure 3** Effect of starter nitrogen on field pea *cv*. Dundale at two sites in Western Australia in 1997. Nitrogen was applied by top-dressing urea at sowing. Vertical bars represent the least significant difference at the 5% probability level.

European soils have been farmed for a long time so their capacity to immobilize P is close to saturated, and here P fertilization is basically replacing what is removed in grain. In North America and Australia, more P is usually necessary. The appropriate rate depends on soil P content, which is determined by soil analysis, and recommendations are based on locally developed calibrations. 20 kg ha<sup>-1</sup> P is a typical application rate, but higher rates are necessary on some soils.

Potassium (K) and sulfur (S) Pea grain contains considerable amounts of K and S (Table 4), and pea requires more of these nutrients than cereals. The need for K is also diagnosed by soil analysis, and in Europe K deficiency can be more significant than P deficiency. In Australia, many soils naturally contain sufficient K that no more needs to be added. This is especially true of the heavier-textured soils where pea is usually grown, but will change in the future as native reserves of K are depleted and higher yielding cultivars with higher K requirements are adopted. K fertilizers are very soluble in water and high rates can have adverse osmotic effects on germination if drilled with the seed. In Europe and North America, K fertilizer is banded a few centimeters below or beside the seed to avoid this. In Australia, P fertilizer is sometimes banded below the seed, but K fertilizer is often broadcast either before or after sowing. Its high solubility means it is rapidly leached into the crop's root zone by winter rains.

Much of the P fertilizer used in the past contained considerable amounts of sulfur, so S deficiency is uncommon. It is sometimes seen in North America and Europe but rarely in Australia.

**Table 4** Average amount of plant nutrients removed by 1 t of pea grain<sup>a</sup>

Nutrient	Quantity removed in 1 t grain
Nitrogen (kg)	37
Potassium (kg)	9.1
Phosphorus (kg)	4
Sulfur (kg)	2
Magnesium (kg)	1.2
Calcium (kg)	0.9
Iron (g)	59
Zinc (g)	26
Manganese (g)	20
Copper (g)	7

<sup>&</sup>lt;sup>a</sup> Compiled from Petterson DS, et al. (1997) The Chemical Composition and Nutritive Value of Australian Pulses, pp. 64. Kingston, ACT, Australia: Grains Research and Development Corporation; and Hickling D (ed.) (1997) Canadian Peas Feed Industry Guide, pp. 21. Winnipeg, MB: Canadian Special Crops Association; and Regina, SK: Western Canada Pulse Growers Association.

Micronutrients The most commonly deficient micronutrients are manganese (Mn), zinc (Zn), and molybdenum (Mo). Mn deficiency causes a disorder known as Marsh Spot, and can occur in all peagrowing regions. It is more common in Europe than elsewhere. Zn deficiency is most common in Australia. Both Mn and Zn are less available on alkaline soils, so deficiencies can be induced by excessive liming. Mo is less available on acid soils, so liming can correct a deficiency. In general, if micronutrient levels are adequate for cereals, they should also be adequate for pea. Soil tests have been developed for micronutrients, but regular tissue testing of crops is the best way to diagnose developing deficiencies.

#### **Weed Management**

Pea is not particularly competitive against weeds, and weed control in a pea crop can be troublesome. Herbicides are almost essential for producing a good crop, and the modern trend to reduce tillage has increased reliance on herbicides. Fortunately, more herbicides can be used in pea than in any other grain legume: there are currently 218 herbicide products registered for use on combining pea in the UK (many of them have the same active ingredient though: 122 of them contain glyphosate). Weed spectrum and farming system vary enormously between the world's peagrowing regions, so only general principles will be outlined here. Regional literature should be consulted for details on specific herbicides and weeds.

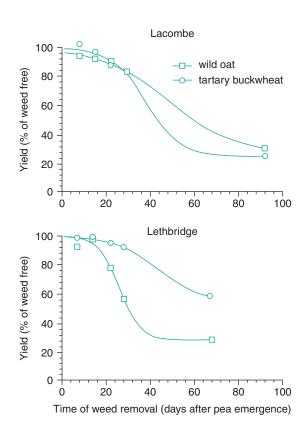
The potential weed burden should be reduced as much as possible before the crop is sown. This fits well with the need to delay sowing in Australia to reduce disease severity, as the delay gives annual weeds time to germinate and still be controlled by cultivation or nonselective herbicide before the crop is sown. The same applies to autumn and winter germinating weeds with spring sowing in North America and Europe. However, it is likely that more weeds will germinate with the crop in both systems, and preemergent herbicides are usually applied. Postemergent herbicides are also often necessary. They should be applied as early as is consistent with allowing weeds time to germinate. Early weed removal is important for maximizing grain yield (Figure 4). The weeds present and the growing environment determine how critical early removal is. Many postemergent herbicides cause crop damage if applied at the wrong growth stage of the crop, or if applied to a stressed crop. Rolling can cause enough stress for normally safe herbicides to cause unacceptable damage, and in Australia, it is recommended that no postemergent herbicides are applied within 2 weeks of rolling. In the UK, a leaf wax test is used to assess

the safety of some herbicides. It involves dipping plants in a solution of the dye crystal violet and observing how much dye adheres to the leaves. The less that adheres, the more wax is on the leaves, and the safer it is to apply herbicide.

Weeds germinating late in the life of the crop compete less strongly than early germinating weeds, and have less effect on grain yield. However, to minimize weed seed carryover into the next phase of the rotation, they should be controlled. In Australia "croptopping" is commonly practiced. This involves spraying low rates of nonselective herbicide (usually paraquat) onto the maturing crop so that it disrupts maturation of weed seeds. Pea's early maturity is well suited to this practice, because the crop suffers little damage when weeds are at the right stage for maximum effect.

#### **Disease Management**

Pea is subject to many diseases. The most important fungal and bacterial diseases are described in **Table 5**. Pea is also affected by a large number of virus



**Figure 4** Effects of time of removal of wild oat and tartary buckwheat on pea grain yield loss for two sites in Alberta, Canada. Data are means of 3 trials at each site from 1996 to 1998. (Reproduced with permission from Harker KN, *et al.* (2001) *Weed technology* 15, 277–283.)

Table 5 Some of the more important fungal and bacterial diseases affecting pea

Disease Causal organism		Type of damage	Occurrence	Management		
Fungal diseases						
Damping off	Pythium spp.	Seed and seedling rot	All pea-growing regions	Fungicide seed dressing Use high vigor seed		
Aphanomyces root rot	Aphanomyces euteiches	Root rot	North America, Europe	Avoid infested sites, rotation with oats		
Fusarium root rot	Fusarium solani f. sp. pisi	Epicotyl, hypocotyl, and taproot rot	All pea-growing regions	Rotation		
Blackspot, Ascochyta or Mycosphaerella blight	Ascochyta pisi, Mycosphaerella pinodes, Phoma medicaginis	Spotting or larger black lesions on leaves, stems and pods, footrot	All pea-growing regions	Fungicide seed dressings, foliar fungicide, rotation, delayed sowing		
Downy mildew	Peronospora viciae	Fluffy lesions on lower leaf surfaces, or with systemic infection severely stunted plants that die before flowering	All pea-growing regions	Resistant cultivars, fungicide seed dressings, rotation, destruction of infected residue from previous crops		
Powdery mildew	Erysiphe pisi (syn. E. polygoni)	White powdery lesions on upper leaf surfaces that can spread to stems and pods	All pea-growing regions	Resistant cultivars, foliar fungicide, rotation, destruction of infected residue from previous crops		
Sclerotinia white mold	Sclerotinia sclerotiorum	Plants wilt and rapidly die Affected areas have a water-soaked appearance, and white fluffy growth may be seen, especially near soil surface	All pea-growing regions	Rotation, avoid growing in rotation with canola or oilseed rape		
Fusarium wilt	Fusarium oxysporum f. sp. Pisi	Wilting and eventual death of plants	North America, Europe	Resistant cultivars, rotation		
Bacterial diseases						
Bacterial blight	Pseudomonas syringae pv. pisi and Pseudomonas syringae pv. syringae	Water-soaked lesions on leaves, pods and stems that later become brown and necrotic	All pea-growing regions	Resistant cultivars, plant uninfected seed		

Compiled from Kraft JM and Pfleger FL (eds.) (2001) Compendium of Pea Diseases and Pests, 2nd edn., 67pp. St. Paul, Minnesota, USA: APS Press; and Kaiser WJ, et al. (2000) Foliar diseases of cool season food legumes and their control. In: Knight R (ed.) Linking Research and Marketing Opportunities for Pulses in the 21st Century, Current Plant Science and Biotechnology in Agriculture, vol. 34, pp. 437–455. Dordrecht: Kluwer.

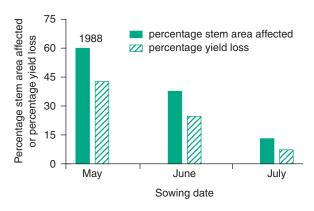
diseases, including alfalfa mosaic virus, bean leaf roll virus, bean yellow mosaic virus, cucumber mosaic virus, pea enation mosaic virus, and pea seedborne mosaic virus. Management consists of using virus-free seed (many viruses are seed borne), discouraging aphids (which spread many of these viruses within a crop) and using resistant cultivars. This article briefly describes some agronomic considerations in *Ascochyta/Mycosphaerella* blight management but for other diseases readers should refer to references provided.

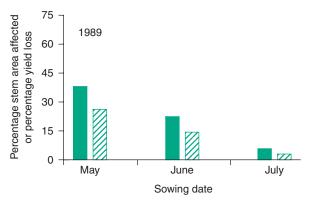
Ascochyta/Mycosphaerella blight or black spot This disease is found in all pea-producing areas of the world and can cause up to 75% yield loss. It is caused by a complex of three fungi – Ascochyta pisi,

Mycosphaerella pinodes, and Phoma medicaginis – and symptoms included leaf, stem, and pod spotting, and foot rot. M. pinodes is the most widespread of the causal organisms, and the most damaging.

The causal fungi can be spread on the seed but, in Australia at least, seed transmission is a minor source of infection compared to spores released from the residue of previous crops, and fungi persisting in the soil. These spores can be carried several kilometers by wind and, once a crop is infected, current lesions act as a secondary source of spores for further spread.

Management of the disease consists of trying to minimize the risk of infection rather than controlling an infection that has begun. Seed can be tested for infection and treated with fungicide if excessive levels are found. Different pea-growing regions have





**Figure 5** Effect of delaying sowing on percentage stem area of field pea affected by *Ascochyta* blight, and percentage grain yield loss relative to plots kept disease-free by weekly fungicide sprays at Horsham, Victoria, Australia in 1988 and 1989. Data are means for 15 pea cultivars. (Adapted from Bretag TW, *et al.* (1995) *Australian Journal of Experimental Agriculture* 35, 531–536.)

different threshold infection levels at which seed treatment becomes worthwhile. Crop rotation is an important tool in reducing infection from old crop residues. In most pea-growing regions, it is recommended that pea not be grown more often than once every 4 or 5 years. In Australia, it is also recommended that pea crops are no closer than 500 m to stubbles from any of the previous year's crops.

In Australia, delayed sowing reduces infection risk, and plays an important role in blackspot management. Spore release begins when old crop residues are wet by rain, and continued release depends on temperature. Usually, by early winter, spore release is declining and crops sown then develop little infection (Figure 5). In Western Australia, a computer model has been developed that takes all these factors into account and, when the locations of previous pea crops in an area and local weather data are provided, produces maps of blackspot risk for different sowing times.

Foliar fungicides can give effective control, but grain yield responses are not usually reliable enough to make them economically viable. Chlorothalonil is registered for *Mycosphaerella* control in Canada. There are also significant differences between cultivars in their susceptibility to *Ascochyta* and *Mycosphaerella*, but all existing cultivars suffer appreciable damage. Nevertheless, understanding of the genetics of tolerance to this disease is improving rapidly, and eventually good resistance should become available.

#### **Invertebrate Pest Management**

Pea is subject to many invertebrate pests, including insects, arthropods, and nematodes. Some of the more important insect, mollusc, and arthropod pests are listed in **Table 6**. Effective chemical control is possible for most of these pests, but agronomic management

also influences how serious a problem many of them become. The interested reader should consult more specialized references given in the Further Reading section.

#### Harvesting

Pea is harvested with the same machinery (combine harvesters) as cereals. Picking up and feeding a lodged crop into the harvester has traditionally been the biggest challenge. While erect semileafless cultivars have gone a long way to overcome this problem in Europe and North America, attachments such as crop lifters, special pea pick-up fronts, and flexible cutter bars usually make the job easier. Rolling after sowing also helps by removing obstacles such as stones and soil clods that can cause considerable machinery damage if picked up with the crop. Swathing is an alternative to direct harvesting.

To maximize grain yield and quality, pea should be harvested at the right time. In Canada and Europe, this is when seed moisture falls to 20%, in Australia the recommended moisture content for harvest is 15%. It is likely that the crop will have some green tips at this stage, and perhaps some green pods as well. Grain harvested at this stage will need drying to avoid spoilage in storage. Often this can be achieved simply by circulating dry external air through the grain in silos. Even earlier harvesting can result in an unacceptable level of immature seed in the sample, and later harvesting in more severe lodging and hence greater difficulty picking up the crop, more insect damage (especially from the pea weevil, Bruchus pisorum), more shattering losses, and greater mechanical damage to the grain. Late harvesting also increases the risk of cotyledon bleaching in blue (green) and marrowfat pea. Desiccating crops approaching physiological maturity with diquat can

Table 6 Some of the more important invertebrate pests affecting pea

Common name	Scientific name	Type of damage	Occurrence
Seedling pests			
Wireworm	Agriotes spp., Ctenicera spp., Limonius spp., Melanotus spp.	Feeding on seed, and later on roots of emerged plants	North America, Europe
Cutworm and Armyworm	Agrotis spp., Apamea spp., Euxoa spp., Peridroma spp., Pseudaletia spp., Sporodoptera spp.	Feeding on emerged seedlings	All pea-growing regions
Red-legged earthmite	Halotydeus destructor	Feeding on emerged seedlings	Australia
Lucerne flea	Sminthurus viridus	Feeding on emerged seedlings	Australia
Slugs	Deroceras reticulatum, Arion hortensis	Feeding on leaves	Europe
Vegetative and reproductive	ve pests		
Snails	Helix spp.	Feeding on leaves, contamination of grain	Europe, Australia
Pea aphid	Acyrthosiphon pisum	Feeding on stems and petioles, also flowers, pods, and leaves	All pea-growing regions
Bluegreen aphid	Acyrthosiphon kondoi	Feeding on growing point	Australia
Cowpea aphid	Aphis craccivora	Feeding on growing point	Australia
Thrips	Thrips spp. and Frankliniella spp.	Feeding on young leaves and growing points	All pea-growing regions
Pea leaf weevil (known in UK as pea weevil)	Sitona lineatus	Feeding on leaves and root nodules	North America and Europe
Reproductive pests			
Pea weevil	Bruchus pisorum	Feeding on developing seed, which may continue after harvest	Most pea-growing regions, not UK
Pea moth	Cydia nigricanus	Feeding on developing pods, and seeds	North America, Europe
Native budworm	Helicoverpa punctigera syn. Heliothis punctigera	Feeding on leaves, pods, and seeds	Australia
Lucerne seed web moth	Etiella behrii	Feeding on developing seeds	Australia

Compiled from Kraft JM and Pfleger FL (eds.) (2001) Compendium of Pea Diseases and Pests, 2nd edn., 67pp. St. Paul, Minnesota, USA: APS Press; Biddle AJ, et al. (1988) The Pea Growing Handbook, 6th edn., 264pp. Peterborough, UK: PGRO; and Carter JM (1999) Field Pea Growers Guide, 20pp. Melbourne, Australia: Agriculture Victoria.

assist achieving even maturation, and in accelerating maturation late in the season in North America and Europe. Desiccating with glyphosate is not recommended because it can reduce the viability of harvested seed.

Pea grain is fragile, especially at low moisture contents (below 14%). Luckily it threshes easily, so the drum or rotor speed should be as low as possible, and the concave closed only enough so that pods are threshed. Once it has been harvested the seed should be handled as gently, and as little, as possible to prevent further mechanical damage. Damage from too vigorous threshing and excessive handling includes split grain and reduced seed viability, but also microscopic cracks in the seedcoat, which reduce the vigor of viable seeds.

See also: Pea: Overview. Plants: Diseases and Pests. Pulses, Overview. Stored Grain: Physico-Chemical Treatment.

#### **Further Reading**

Anderson RL, et al. (2003) Agricultural Water Management 58: 255-266.

Biddle AJ, Knott CM, and Gent GP (1988) *The Pea Growing Handbook*, 6th edn., pp. 264. Thornhaugh, Peterborough: The Processors and Growers Research Organisation.

Bretag TW, Keane PJ, and Price TV (1995) Effect of ascochyta blight on the grain yield of field peas (*Pisum sativum L.*) grown in southern Australia. *Australian Journal of Experimental Agriculture* 35: 531–536.

Carter JM (1999) Field Pea Growers Guide, 20pp. Melbourne, Victoria: Agriculture Victoria.

Carter JM (1999) Field Pea Growers Guide, 6pp. Melbourne, Victoria: Agriculture Victoria.

Chauvel B, Guillemin JP, Colbach N, and Gasquez J (2001) Evaluation of cropping systems for management of herbicide-resistant populations of blackgrass (*Alopecurus myosuroides* Huds.). *Crop Protection* 20: 127–137.

- Cutforth HW, McConkey BG, Ulrich D, Miller PR, and Angadi SV (2002) Yield and water use efficiency of pulses seeded directly into standing stubble in the semiarid Canadian prairie. Canadian Journal of Plant Science 82: 681-686.
- Harker KN, Blackshaw RE, and Clayton CW (2001) Timing weed removal in field pea (Pisum sativum). Weed Technology 15: 277-283.
- Heath MC, Knott CM, Dyer CJ, and Rogers-Lewis D (1991) Optimum plant densities for three semi-leafless combining pea (Pisum sativum) cultivars under contrasting field conditions. Annals of Applied Biology 118: 671 - 688.
- Hickling D (ed.) (1997) Canadian Peas Feed Industry Guide, 21pp. Winnipeg, Manitoba: Canadian Special Crops Association Regina, Saskatchewan: Western Canada Pulse Growers Association.
- Johnston AM, et al. (2002) Field pea seeding management. Canadian Journal of Plant Science 82: 639–644.
- Kaiser WJ, Ramsey MD, Makkouk KM, et al. (2000) Foliar diseases of cool season food legumes and their control. In: Knight R (ed.) Linking Research and Marketing Opportunities for Pulses in the 21st Century, Current Plant Science and Biotechnology in Agriculture, vol. 34, pp. 437-455. Dordrecht: Kluwer.
- Knott CM (1987) A key for stages of development of the pea (Pisum sativum). Annals of Applied Biology, 111: 233-244.
- Kraft JM and Pfleger FL (eds.) (2001) Compendium of Pea Diseases and Pests, 2nd edn., 67pp. St. Paul, MN: APS Press.
- Kraft JM, Haware MP, Halila H, Sweetingham M, and Bayaa B (2000) Soilborne diseases and their control. In: Knight R (ed.) Linking Research and Marketing Opportunities for Pulses in the 21st Century, Current Plant Science and Biotechnology in Agriculture, vol. 34, pp. 457-466. Dordrecht: Kluwer.
- Littlewood N (ed.) (2002) 2003 Crop Variety Sowing Guide for Western Australia. South Perth, Western Australia: Department of Agriculture.
- Miller PR, McConkey BG, Clayton GW, et al. (2002) Pulse crop adaptation in the northern great plains. Agronomy *Journal*, 94: 261–272.
- Park B and Lopetinsky K (eds.) (1999) Pulse Crops in Alberta 149pp. Edmonton, Alberta: Alberta Agriculture, Food and Rural Development.
- Petterson DS, et al. (1997) The Chemical Composition and Nutritive Value of Australian Pulses, 64pp. Kingston, ACT, Australia: Grains Research and Development Corporation.
- Pritchard I (1993) Growing Field Peas. South Perth, Western Australia: Department of Agriculture.
- Siddique KHM, Loss SP, Regan KL, and Jettner RL (1999) Adaptation and seed yield of cool season grain legumes

- in Mediterranean environments of south-western Australia. Australian Journal of Agricultural Research 50: 375–387 (http://www.publish.csiro.au).
- Siddique KHM, et al. (2001) European Journal of Agronomy 15: 267-280.
- Snowball K and Robson AD (1991) Symptoms of Nutrient Disorders: Faba Beans, Field Peas. Perth, Western Australia: Soil Science and Plant Nutrition, University of Western Australia.
- Thompson IP, Greco N, Eastwood R, Sharma SB, and Scurrah M (2000) Integrated control of nematodes of cool season food legumes. In: Knight R (ed.) Linking Research and Marketing Opportunities for Pulses in the 21st Century, Current Plant Science and Biotechnology in Agriculture, vol. 34, pp. 491-566. Dordrecht: Kluwer.

#### **Relevant Websites**

http://www.general.uwa.edu.au - The Centre for Legumes in Mediterranean Agriculture (CLIMA) is a collaborative center bringing together expertise from the WA Department of Agriculture, CSIRO, the University of Western Australia, and Murdoch University. It describes pea research of a more strategic nature than that on the Department of Agriculture website, as well as research on a broad range of crop and pasture legumes.

http://www.grainlegumes.com - European Association for Grain Legume Research as an organization encourages research on all grain legumes, but their publications contain a lot of information relevant to pea.

http://www.sardi.sa.gov.au - South Australian Research and Development Institute's website contains the same type of information as the previous site, but with a South Australian flavor.

http://www.pgro.co.uk - The Processors and Growers Research Organisation has conducted applied research on pea and other pulse crops, and provided services to growers in the UK since the 1940s. It is necessary to become a member of the organization to access most information on the website.

http://www.agric.wa.gov.au - WA Department of Agriculture's website contains much practical information on growing pea in an Australian context as well as summaries of a great deal of applied agronomic research.

# **PEANUTS**

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#### Introduction

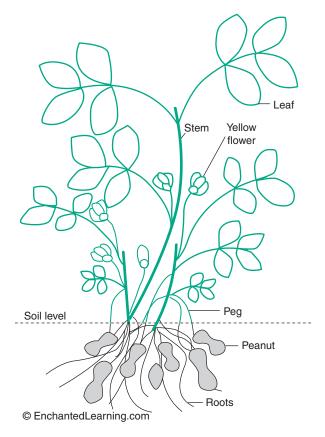
Peanut (*Arachis hypogaea* L.) is a tropical legume grown over a diverse range of environmental conditions, with the main commercial production occurring in the northern hemisphere. Peanut is used widely throughout the world, being one of the principal oilseeds as well as a high-quality food for human consumption. Over the past five years, the crop has been grown on  $\sim$ 21 million hectares (Mha), with an annual production of  $\sim$ 30 million metric tons (Mt).

This article provides a brief overview of the peanut crop and concentrates on its unique features, which lead to its reputation as a high-value, high-quality, and extremely adaptable grain legume. The article provides information on aspects including its historical background, distribution, utilization and quality issues, morphology, breeding, and recent innovations in biotechnology, which offer some exciting prospects for increased productivity and improved quality. The article purposely concentrates on aspects that are of interest, and of relevant expertise, to the author.

#### **Historical Background**

Peanut (sometimes referred to as groundnut) and the genus *Arachis*, originated in central Brazil in South America. Early archaeological evidence suggests that peanut was domesticated in northern Argentina and eastern Bolivia, and subsequently grown in Mexico, the Caribbean Basin and throughout Brazil, and the coastal regions of Peru. It is believed that the crop did not reach other parts of the world until after Columbus arrived in America, after which time it was taken from Brazil to Africa and the Far East by the Portuguese. The Spaniards are believed to have taken the crop to the western Pacific, Indonesia, and China early in the sixteenth century.

Arachis hypogaea is an annual member of the family Papilionaceae, which shows a unique feature of maturing their fruits underground (Figure 1). The plant comprises a number of subspecies – fastigiata var. fastigiata (Valencia) and var. vulgaris (Spanish), both bunch forms; and subspecies hypogaea var. hypogaea (Virginia bunch and runner). Large



**Figure 1** Diagram of a peanut plant (*Arachis hypogea*) showing its unique underground fruiting habit. (Reproduced with permission from <a href="http://www.enchantedlearning.com/subjects/plants/pages/peanutplant.shtml">http://www.enchantedlearning.com/subjects/plants/pages/peanutplant.shtml</a>.)

variations in growth habit (erect versus spreading), branching type (alternate or sequential), maturity (90–160 days), and traditional market types (Virginia, Spanish, or Valencia) exist. The present distribution of peanut covers from 40°S to 40°N in warmer growing regions of tropical, subtropical, and Mediterranean climates, where soils are light, neutral, or alkaline, and rainfall (plus irrigation) exceeds ~400 mm per season.

#### **World Peanut Production and Utilization**

#### **Production and Yields**

The world average nut-in-shell production was  $\sim$ 29 Mt during the period 1996–2000, and has been growing at an annual rate of 2.5% since 1970. While total world production has increased, there has been significant regional variation, with most of the growth occurring in Asia, followed by

Table 1 Annual peanut production and yields for the ten major world-producing countries during the periods 1972–75 and 1996–2000

Country	Production 1972–75 ('000 Mt)	Production 1996–2000 ('000 Mt)	% Change since 1972–75	Average yield 1972–75 (kg ha <sup>-1</sup> )	Average yield 1996–2000 (kg ha <sup>-1</sup> )	% Change since 1972–75
China	2 204	11 463	420	1.2	2.84	136
India	5 473	7 131	30	0.77	0.91	18
United States	1 618	1 655	2	2.68	2.91	8
Nigeria	588	1 310	123	0.55	1.11	103
Indonesia	549	979	78	1.29	1.52	18
Senegal	759	676	-26	0.80	1.02	27
Myanmar	414	563	36	0.62	1.16	87
Zaire	276	442	60	0.62	0.77	24
Argentina	367	412	12	1.06	1.47	38
Chad	64	393	517	0.82	0.98	20
Rest of the world	3 984	4 084	2.5	0.76	0.85	11
World production	16 296	29 108	77	0.91	1.37	51

Adapted from Revoredo CL and Fletcher SM (2002) World Peanut Market: An Overview of the Past 30 Years. Research Bulletin No. 437. The Georgia Agricultural Experiment Stations, College of Agricultural and Environmental Sciences. The University of Georgia.

Africa and America. Most of the growth in Asia has been in China, with a dramatic increase of 420% between 1975 and 2000, which makes China the first ranking peanut producing nation (Table 1). This increase in production has been due to both increased area planted (nearly fourfold) and increased yields (more than doubled). These increases have been associated with recent market reforms encouraging greater areas of peanut production, as well as new technologies including plastic mulching, which have substantially improved yields. In India, most of the increase in production can be explained by the increase in the area, since yields have remained stable during the last 30 years. Production in the US has remained relatively static over the same period, with only minor changes in area grown and yields per ha. Yields in Africa remained relatively stable, although levels are only half that of more developed countries. The exception to this trend is Nigeria, the main west African producer, which showed a substantial improvement in yield over the past 30 years.

#### Utilization

Peanut is grown for its kernel, oil and meal derived from crushing kernels, as well as the vegetative residue (haulms). Nearly 50% of the world production is currently crushed for cooking oil, although large variations between countries exist. For example, India and Argentina crush  $\sim$ 90% of the production compared to countries such as Indonesia, Mexico, and Ghana who directly consume over 95% of the crop for food purposes. Over the past 30 years, there has been consistent increase in peanut used as a food crop, owing to its excellent nutritious makeup, being high in oil, protein, and carbohydrate. Peanut

kernels can be consumed fresh, dry, boiled, or roasted. In developed countries such as the US, Europe, and Canada about half the production is used for peanut butter, with the remainder used for salted peanuts snacks and a range of confectionary products.

The peanut haulms are of a very high nutritional quality and are fed to cattle and other animals as a source of high protein. The shells of the mature pods can also be used as a fuel, a high-fiber filler in animal feeds, a mulch on gardens and more recently as a possible source for deactivated carbon production.

#### **Trade**

The world trade market for peanuts can be considered as a residual market, in that only a very small proportion of the world production is traded owing to most of the production being utilized domestically. While the average share of world peanut production exported over the past 30 years has remained constant at 5%, the total volume of exports has grown steadily from  $\sim$ 1.1 Mt in 1976 to 1.5 Mt in 2000. The three major exporters China, Argentina, and USA have comprised over 60% of the total world trade. The peanut trade has steadily shifted from a crush-foroil to a market-for-food purpose and this has meant that there is a requirement now for higher quality peanuts, especially with low aflatoxin contamination (see below). Strict quality requirements necessitating that aflatoxin levels meet strict regulatory limits (e.g., <4 ppb total aflatoxins in European markets) have impeded a number of Asian (China, Vietnam, India) and African (Nigeria, Sudan) countries in competing in the edible peanut market. Indeed, high levels of aflatoxin in exported product forced the European Union to temporarily ban Chinese imports during 2002, and to enforce

legislation requiring stricter testing procedures at ports of entry.

# **Morphology and Growth Physiology**

Being of indeterminate botanical type, peanut supports simultaneous growth of vegetative and reproductive structures, and so compete for nutrients and assimilate throughout most of the crop life cycle. Flowers first appear  $\sim 25-35$  days after planting, depending on the maturity of the variety and the thermal environment under which the crop is grown, and continue to be produced until final maturity. The rate of flower initiation increases to a maximum and then declines as the pod load increases and the crop approaches maturity. Bursts of flowering are common after rain, following drought periods during the vegetative phase, and can lead to more synchronous pod set and higher yields and quality given favorable growing conditions during the pod-filling period. Peanut has a self-fertilized flower that withers soon after fertilization. Rates of natural outcrossing are low and have been estimated at between 0.7% and 2.5%. After 5-7 days, the base of the fertilized ovary (gynophore or peg) begins to elongate towards the soil, penetrating the soil up to a depth of 4-5 cm. The ovary then begins to swell and turns horizontally away from the plant (Figure 1), which takes between 15 and 20 days from the initial flowering event. The pod then expands rapidly until reaching full dimensions characteristic of the variety, with seed cotyledon growth over the next few weeks. Because of the slow rate of pod addition, many pods must be added and seeds initiated to form a full pod load on the plant. Pod growth rates vary depending on temperature, water status, varietal maturity, and partitioning ability.

#### Farming Systems

In most developed countries, peanut is grown as a sole crop under rainfed or irrigated conditions, using fully mechanized cultural practices, including large-scale mechanical planting, spraying (weeds and diseases), and harvesting equipment. Friable soils, including red ferrosols and lighter textured sands, are commonly selected to allow mechanised harvesting equipment to operate efficiently and minimize pod losses which can be significant on harder setting soils. In countries such as the USA and Australia, it is not uncommon for one farming family to farm 500 or more ha per annum. Peanuts are generally grown in a rotation of one peanut crop in two or three summer crops in order to minimize soil-borne diseases, use

fertilizers more efficiently, and allow more effective weed control.

In developing countries throughout Asia and Africa, smaller scale peanut production on 1-2 ha plots is more common, under a variety of cropping systems. Peanut is renowned for its superior drought tolerance and is often grown in very marginal regions where other legume crops such as soybean and mung bean fail to produce grain. In regions such as India, peanut is often grown as "dual purpose" crop, where both kernels for a cash crop and haulms for animal fodder are produced in seasons with average to aboveaverage rainfall, while only high-quality fodder are produced in severe drought years. Peanuts are important in rice-based sequential cropping systems throughout much of Asia. The crop is either planted with irrigation before a rice crop, or grown on residual soil moisture after the rice harvest.

Peanut is also commonly grown as an intercrop with longer season annual and perennial crops, including castor, cassava, cotton, and sugar. For example, in India considerable yield per ha advantages of up to 65% have been demonstrated, when using 6–8 rows of peanut to a single row of pigeon pea. Peanut can be grown underneath perennial tree canopies such as coconut, cocoa, oil palm, and rubber, owing to its effective shade tolerance. Under these systems, as well as providing biologically fixed nitrogen, peanut can provide cash income until the plantation trees are old enough to produce their end products.

# **Biotic and Abiotic Constraints to Yield**

#### **Insect Pests**

Around 360 species of arthropod pests are known to attack peanuts before harvest in different regions of the world. In general, the foliage pests are of lesser importance than the soil-inhabiting pests, which can cause direct economic damage to pods. A large number of thrips species are however known to attack peanut and have become very important vectors of some of the debilitating peanut viruses such as tomato spotted wilt virus (TSWV). The most important soilborne pests in the developed world are the lesser corn stalk borer, Elasmopalpus lignosellus, and white grubs, the larvae of which attack pegs and pods and cause substantial economic damage. These insects also cause pod damage, which can lead to aflatoxin contamination in kernels following infection via the fungus Aspergillus flavus.

#### **Fungal Pests**

The most important fungi causing economic damage to peanut crops around the world are the leaf spots (early and late) and leaf rust. These diseases cause leaf necrosis and can lead to severe yield losses if not effectively controlled by fungicides. A number of highly efficient chemical formulations have been developed over recent years to control both diseases, hence effective control can be achieved when using a rigorous spray program aimed at optimizing chemical efficacy and inoculum load. Genetic resistance is available, however there is a known negative association between foliar disease resistance and yield potential.

A number of soil-borne diseases can cause significant economic losses in peanuts, via seedling and plant death, as well as via direct pod damage due to pod rots. The most important diseases include white mold (*Sclerotium rolfsii*), *Cylindrocladium* black rot (CBR), and *Sclerotinia* blight.

#### **Viruses**

Many viruses infect peanut and historically they have not caused widespread economic losses. This situation has changed over the past decade or so, with the arrival of the potyvirus peanut stripe virus (PStV) in Asia and the USA, and TSWV in the USA. Both these viruses have caused significant yield losses and are threatening the viability of peanut production in specific regions. A system of cultural practices has been implemented to control these diseases, including planting date and plant and row spacings to control thrip vectors such as the western flower thrip. Some genetic tolerance has been identified for TSWV in the USA, although total immunity has not been developed and there is evidence of a breakdown in this resistance. There is no known genetic resistance to PStV, although a recent collaborative project involving Australian, Indonesian, and Chinese scientists has developed resistant breeding lines using a transgenic coat protein mediated resistance approach.

#### **Water Stress**

The major effect of soil and crop water deficits on peanut productivity relates to deceases in canopy coverage, pod initiation, and conversion into viable pods and ultimately on pod yield. Peanut has a reputation of being an extremely drought tolerant crop, which is associated with a number of important traits, including its ability to withstand very low relative leaf-water contents (e.g., 29%), deep-rooting habit and indeterminate reproductive system which gives enormous plasticity in rainfed environments typified by intermittent and end-of-season patterns of drought stress. Significant genetic variation in these traits exists and breeding programs aimed at incorporating these traits in combination are currently underway to further enhance peanut drought resistance.

**Table 2** Depth of peg penetration (mm) as a function of penetrometer resistance (MPa) in the surface 1.5 cm of soil during a 21 day pegging period for peanut plants (cvs. Robut 33-1 and McCubbin) grown in pots

Penetrometer resistance in surface 1.5 cm of soil (MPa)									
Cultivar	0.5	1.0	2.0	3.0	4.0	5.0	6.0	7.0	
Robut 33-1	43	39	12	9	6	5	5	5	
McCubbin	60	50	14	9	5	4	5	5	

Peanut can be particularly susceptible to drought stress during the early reproductive stage, as soil water deficits then can influence peg and pod development through direct effects on plant water status and assimilate supply, or through effects on peg penetration and calcium uptake in dry surface soils. Peg entry and pod development are extremely sensitive to soil hardness, which often increases with decreasing soil water content. Table 2 illustrates that the depth of peg penetration (and subsequent pod growth) in two peanut cultivars declines linearly as soil strength (as measured by penetrometer resistance), increased from 0.1 to 2.0 MPa, with cessation of peg growth above 2.0 MPa.

#### **Nutritional Constraints**

As for most crops, peanut requires an adequate supply of each essential element during the growing season for maximum growth.

The specific nutritional issues of most importance for peanut production include calcium (Ca) and biologically fixed nitrogen (BFN). It has been well documented that low soil-water content in the podding zone can decrease Ca uptake and induce Ca deficiency in peanut and lead to abortion of developing seeds, leading to empty pods, referred to as "pops." In aerial fruiting plants, Ca is absorbed by roots and transported via the transpiration stream (xylem) to the developing pod, and is highly immobile in the phloem of the plant. As the pegs and pods of the peanut plant do not transpire, Ca cannot be translocated from the root to the developing pod, and hence pods rely on their Ca requirement via direct absorption through pods from the soil solution. Calcium, usually applied as gypsum, has been widely used to increase the yield of peanut under drought conditions occurring during early pod set. Large seeded Virginia types have also been shown to be more sensitive to drought-induced Ca deficiency than smaller seeded Spanish types.

Peanuts have a very effective BNF system and are promiscuously nodulated by strains of rhizobium of the cowpea miscellany group. Inoculation of seeds with effective rhizobia have led to substantial increases in peanut yield in fields, which have not previously grown peanuts. It is generally accepted that once a field has successfully grown a peanut crop, it should not require future rhizobium applications. Estimates of fixed nitrogen as large as 300 kg N ha<sup>-1</sup> have been reported for well-watered crops, with reports that BNF can supply over 80% of the plants total N uptake. Although most of the N in the crop is removed when the pods are harvested, numerous experiments have shown that significant N may be left in the soil for uptake by subsequent crops.

#### **Light and Temperature**

While growth and yield of peanut are known to be linearly related to total solar radiation receipt, recent data have shown that reproductive development can be strongly related to photoperiod (day-length) in sensitive cultivars. Peanuts were previously thought to be day-neutral, with phenology mainly driven by temperature. Controlled environment and field experiments have since determined that reproductive efficiency (i.e., cumulative flowering, peg and pod number, and allocation of dry matter to pods) after flowering can be modified considerably by varying photoperiod. The response has been characterized as quantitative short-day to day-neutral, with apparent variation among cultivars. More recent data has also discovered that significant photo-thermal interactions in this response exist in peanut, with the sensitivity of reproductive efficiency increasing at higher temperatures (e.g., mean daily temperatures exceeding 26°C).

Peanut is known to grow well under a wide range of temperatures, with daily optima for the rate of development, growth processes, and yield being  $\sim 25^{\circ}$ C to  $30^{\circ}$ C. High temperatures in excess of  $35^{\circ}$ C can reduce leaf area development, reduce the number of pods and result in lowered harvest indices and yields.

# Quality, Nutritional, and Food Safety Issues

#### **Nutritional Aspects**

Peanuts are an excellent source of nutrition for both humans and animals. Table 3 gives a proximate composition of peanut kernels, and clearly shows that they are a rich source of protein, oil, carbohydrate, and minerals. Peanuts are also a good source of niacin, folic acid, phosphorous, vitamin E, and phytosterols.

Peanuts are consumed as oil, whole as snacks, in confectionaries, and peanut butter. As a human food, they are often roasted before use, which accentuates the nutty flavor that contributes to consumer acceptance. Nearly two-thirds of the world production of peanuts are crushed and utilized as high-quality oil, making it one of the world's leading oil crops.

Table 3 Proximate composition of peanut kernels

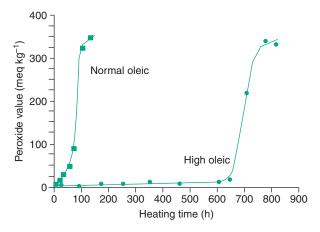
Product	% Composition
Moisture	5.0
Protein	30.0
Oil	48.0
Carbohydrate	15.5
Crude fiber	3.0
Ash	2.0

Peanut oil is mostly composed of triglycerides of eight fatty acids. Around 80% of these fatty acids are either oleic acid (monounsaturated, C 18:1) or linoleic acid (polyunsaturated, C 18:2). Generally, these two fatty acids vary inversely. Of the eight commonly detectable fatty acids in peanut oil, linoleic is the only one that is polyunsaturated. The second double bond in a linoleic acid molecule renders it far more susceptible to oxidation. This "oxidative rancidity" generates off-flavors in peanut products, and hence limits the shelf life of products in retail outlets. The monounsaturated oil type in peanuts is also known to be "heart healthy." Recent studies in the USA have shown that peanuts and peanut butter lowered blood cholesterol levels as effectively as olive oil in moderate fat diets. It was also found that diets including peanuts and peanut butter lowered cardiovascular disease risk by 21%, whereas the low-fat diet decreased the risk by only 12%.

In the late 1980s, researchers at the University of Florida discovered a (naturally occurring) mutant peanut plant with over 75% oleic acid, and linoleic acid below 5% of fatty acids, which they subsequently described as "high oleic" germplasm. This trait has recently been bred into adapted commercial varieties, which are currently being released in the USA and Australia. This characteristic will provide even greater benefits to both product shelf life and human health. The increased shelf life is best illustrated by the peroxide value (a measure of shelf life) data shown in Figure 2, which shows how high oleic peanuts maintain low PV's (and hence no rancid off-flavors) during an accelerated aging test.

# **Food Safety Issues**

Aflatoxin Aflatoxin is a human carcinogen that contaminates peanuts, particularly under end-of-season drought and makes them unsafe for human consumption. Aflatoxin production occurs in peanut kernels particularly under drought conditions consequent to infection by *Aspergillus flavus* and *A. parasiticus*. The "within-season" seed infection by the fungi is also responsible for aflatoxin production depending on



**Figure 2** Peroxide value over time as a measure of oxidation of high and low oleic peanut oils. (Reproduced with permission from O'Keefe, Wiley, and Knauft (1993) *Journal American Oil Chemists Society* 70: 489–492.)

the conditions during the postharvest-drying phase in the field, or during storage conditions.

The aflatoxin problem is a worldwide phenomenon, but is particularly severe in developing countries in Africa, South, and Southeast Asia, where food safety and security systems are not well developed to protect consumers against unsafe food products. There is a very low level of awareness about impacts of aflatoxin contamination on human/livestock, particularly in peanut-producing countries of the semiarid tropics, and hence the source of contamination remains invisible and its effects and potential impacts on human and livestock health are not well documented. The human impact, in terms of mortality and morbidity, in peanut-growing countries in the developing world is enormous, with estimates of up to 20 000 deaths per year arising from aflatoxin-related illnesses being made in some Southeast Asian countries. In more developed countries, consumers are more aware of food safety issues such as aflatoxin, and are increasingly demanding peanut products meet strict regulatory standards. Current strategies to reduce aflatoxin levels to below regulatory limits (e.g., 15 parts per billion (ppb) in retail products in the USA and Australia) involve a post-farm-gate process of selective segregation achieved through blanching and color sorting in shelling plants. This strategy, although effective, is time-consuming, wasteful, and consequently, very expensive. These costs are continually passed onto growers and hence, threaten the viability of peanut farming in rainfed-production systems. In developing countries, aflatoxin regulatory limits for peanut have been stringently applied and have recently affected export of peanut and its products (see above).

A number of potential solutions have been proposed to minimize aflatoxin at its source, the farm. Use of agronomic management methods, including pre- and postharvest strategies that minimize aflatoxin contamination by the fungus Aspergillus flavus/parasiticus have shown to be effective. A number of peanut varieties have been shown to have some aflatoxin tolerance, which seems to be associated with drought avoidance and escape mechanisms. Recent research is also investigating the potential for using a biocontrol approach, where nontoxigenic strains of the Aspergillus flavus fungus are applied to the soil, and infect kernels and hence competitively exclude toxigenic strains.

Cadmium Cadmium (Cd) is a heavy metal, which can accumulate in foods such as peanut and potentially lead to kidney damage if high levels of intake occur over prolonged periods. Recent research has shown that peanut can accumulate high Cd levels on acidic sandy soils, especially where there has been a history of phosphatic fertilizer application, which had contained Cd as a by-product. Developed countries have begun to apply strict regulatory standards, which have recently affected some peanut exporting nations. A number of management practices have been proposed to minimize Cd accumulation in peanuts and readers are referred to the website listed at the end.

Anaphylaxis Anaphylaxis is a sudden, severe, potentially fatal, systemic allergic reaction to peanuts that can involve various areas of the body (such as the skin, respiratory tract, gastrointestinal tract, and cardiovascular system). Symptoms occur within minutes to 2 h after contact with the allergy-causing substance, but in rare instances may occur up to 4 h later. Anaphylactic reactions can be mild to life-threatening, and seem to have an annual incidence of  $\sim 30$  per 100 000 persons. The best treatment currently is avoidance of peanuts and peanut products. Medical researchers are working on developing treatments, such as vaccination, to prevent or decrease the symptoms of serious food allergy to peanut consumption. Before developing such treatments, it is necessary first to identify the specific proteins that are responsible for triggering the allergic response. A number of the proteins responsible for peanut allergy have already been identified. Medical treatments to desensitize or minimize peanut and other food allergy reactions are under development, but will not be widely available for several years.

### **Breeding and Biotechnology**

The primary objectives of most peanut-breeding programs are to develop high-yield potential, resistance to local environmental, and disease stresses in conjunction with broad or specific adaptation to the appropriate cropping system. As peanuts are grown under a variety of agro-ecological systems, local breeding objectives will undoubtedly change between regions. Once general adaptation and disease resistances have been developed, breeding efforts in the more developed countries have tended to concentrate on quality issues such as improved flavor, high oleic oil, blanchability, and kernel size.

There is enormous genetic variability available for most traits of interest, including host-plant resistance to disease and insects and along with important agronomic and quality traits. This germplasm has been collected and preserved thanks to the extensive collections made in South America during the early to mid-1900s. The most significant collection is housed at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India, where some 12 000 accessions are maintained, along with more than 70 wild *Arachis* species.

The most common breeding approach used by peanut breeders is the pedigree method, which allows selection for highly heritable traits in early generations. Generally, plant and pod type are the focus of this selection, which allows progeny numbers to be rapidly culled, after which emphasis can be placed in later generations on quantitative characters such as yield and quality traits. Backcross breeding has not been used widely in peanut owing to the lack of simply inherited traits for disease and insect resistance. The technique is however being used widely in the USA and Australia to backcross the single gene high oleic trait.

The biotechnology revolution occurring in a range of important crop plants has yet to be realized in peanut. This has been largely due to the lack of polymorphism for allozymes or restriction fragment length polymorphisms (RFLPs), which has restricted the use of marker technology for improved selection of quantitative traits such as yield. Several research institutions have been able to transform peanut with exogenous DNA and generate fertile plants from transformed tissues. This technology has opened up enormous possibilities for expanding the genetic diversity of peanut. Recent applications of this technology include genetic transformation for disease resistance to PStV and TSWV.

See also: Nitrogen Metabolism. Nutrition: Effects of Food Processing. Plants: Diseases and Pests. Pulses, Overview.

#### **Further Reading**

Ashley JM (1984) Groundnut. In: Goldsworthy PR and Fisher NM (eds.) *The Physiology of Tropical Field Crops*, pp. 453–494: Wiley.

Bell MJ, Bagnall DJ, and Harch G (1991) Effects of photoperiod on reproductive development of peanut (*Arachis hypogaea* L.) in a cool subtropical environment: II. Temperature interactions. *Australian Journal of Agricultural Research*, 42: 1151–1161.

Boote KJ and Ketring DL (1990) Peanut. In: Stewart BA and Nielson OR (eds.) "Irrigation of Agricultural Crops," Agronomy Monograph 30, pp. 625–717. Madison, WI: American Society of Agronomy.

Isleib TG, Wynne JC, and Nigam SN (1994) Groundnut breeding. In: Smartt JT (ed.) *The Groundnut Crop*, pp. 552–623. UK: Chapman Hall.

Nigam SN (1992) Groundnut – A Global Perspective: Proceedings of an International Workshop, 25–29 November, 1991, 548pp. AP, India: ICRISAT Centre.

O'Keefe SF, Wiley VA, and Knauft DA (1993) Journal American Oil Chemists Society 70: 489-492.

Pattee HE and Young CT (1982) Peanut Science and Technology. Yoakum, Texas: American Peanut Research and Education Association.

Reddy PS (1988) *Groundnut*. New Delhi, India: Publications and Information Division, Indian Council of Agricultural Research.

Revoredo CL and Fletcher SM (2002) World Peanut Market: An Overview of the Past 30 Years. Research Bulletin No. 437. The Georgia Agricultural Experiment Stations, College of Agricultural and Environmental Sciences. The University of Georgia. May 2002. (www.ces.uga.edu).

Wright GC and Nageswara Rao RC (1994) Groundnut water relations. In: Smartt JT (ed.) *The Groundnut Crop*, pp. 281–335. UK: Chapman Hall.

Wright GC, Hubick KT, Farquhar GD, and Rao RCN (1993) Genetic and environmental variation in transpiration efficiency and its correlation with carbon isotope discrimination and specific leaf area in peanut. In: Ehleringer JR, Farquhar GD, and Hall AE (eds.) Stable Isotopes and Plant Carbon-Water Relations, pp. 247–268. San Diego, CA: Academic Press.

#### **Relevant Websites**

http://www.ars.usda.gov — aflatoxin biocontrol. http://www.ars.usda.gov — Agricultural Research Service, USDA.

http://www.enchantedlearning.com.

http://www.foodallergy.org — anaphalxis in peanuts. http://www.clw.csiro.au — cadmium in grain legumes. http://www.dpi.qld.gov.au — managing aflatoxin. http://sacs.cpes.peachnet.edu — National Peanut Research Lab., USA.

http://agnews.tamu.edu – Texas A&M University, USA. http://www.peanut-institute.org – The Peanut Institute.

# PET FOODS

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#### Introduction

#### What Are Pet Foods?

Pet foods have been manufactured since the 1940s in the USA and Europe, and today there are manufacturing plants in most developed countries. They originated from animal feedstuffs based on nutrition that were manufactured for livestock. The close relationship between pets and owners led to the development of certain sensory requirements to attract the consumer. The products were made with more attractive shapes and colors to satisfy the owners and with special flavorings to ensure that the animals were happy. However, the core products are nutritionally well balanced to give a complete diet for an animal so that they can live a healthy life if fed only on the pet food.

There are also some special products known as "treats" that may be more highly flavored, or have a special chewy texture. These products are aimed at providing an enjoyable experience for the animal and its owner, and are not necessarily so well balanced nutritionally.

There are many types of pets in the modern world, but cats and dogs dominate all the production in the pet food market by a large margin with sales of over five billion pounds worldwide. The next levels of pet foods are for fish, small mammals such as rabbits, guinea pigs, and ferrets, birds, and then the products begin to merge with animal feeds for farm animals and reptiles. In this article only the products made for cats and dogs are described in detail, and some of the products made for fish and small mammals are briefly mentioned.

# **Types of Foods**

The manufacture of pet has taken two main forms in the past century; these may be described as wet and dry types. Using the mature UK market as an example, the proportions of each type of product sold in 2001 are shown in Table 1. Wet pet foods were developed originally as canned products that had moisture contents of 65-70% w/w, similar to cooked meat and gravy, or raw meat. They could be used straight from the tin and were easily digested by the animals. In the

retail market, cans were sold mainly in 400 and 800 g sizes. These were satisfactory for cats and small dogs, but were a little small for large dogs. The packs of cans became very heavy and new forms were developed, such as the intermediate-moisture products with much less water (20-30% compared with 65-70% w/w for cans) that could be sold in large plastic tubes. The food could be easily sliced from the roll to provide the meals. However, they were not very successful and it was the dry products, with moisture content of  $\sim 10\%$  that were preferred as the main alternative to canned pet foods.

More recently, laminated plastic and foil pouches have been introduced with individual meals that are lighter than cans but offer the same types of wet pet foods. In the market for cats they have captured 20% of the wet sales.

Initially dry products were developed as biscuits that could be added to supplement meals prepared at home. These might have been made up of meat and bones from the butcher, or the scraps from a family meal. However, with the advent of the new technology of extrusion cooking, it became possible to prepare a dry food from a well-balanced range of ingredients and was a complete meal. This new type of dry food had considerable advantages in weight for the shopper, removing 60% of the water in canned products plus the tin. The remaining dry solids, which were sold in paper or laminated plastic sacks had a long shelf life and were liked by the pets. Therefore, they began to replace the wet foods in several markets. However, the wet foods were still preferred by many customers for other reasons that the successful introduction of the small individual meal pouches of wet foods has helped to maintain market share for this form of pet food.

For other animals such as fish and small mammals most foods are sold as dry foods, except for some intermediate moisture products for fish. The dry products may be in the form of flakes, extruded rods or

Table 1 UK market for different types of pet foods 2001

Type of pet food	Cat market (%)	Dog market (%)
Wet	76	39
Dry	21	40
Semi-moist	1.5	1
Treats	1.5	14
Mixers	0	6

pellets, and simple mixtures of different forms of grains, pellets, and flakes.

### **Dry Pet foods**

#### **Biscuit Technology**

Two manufacturing processes are used for dry pet foods, traditional biscuit-making technology or extrusion cooking. Originally, biscuit making was developed by transferring knowledge from the bakery industry. Basic equipment was available to mix, sheet and cut out the shapes, and bake dough into a dry biscuit texture. In some cases, the dough was sheeted by forming extrusions with wide fan-shaped slit dies to produce a sheet, rather than rollers. In most processes the waste dough and biscuit material was added back into the mixer to form the new dough.

The recipes for the biscuits were largely cereal-based products (Table 2) with a small addition of meat, fat, and bone meal to provide flavor rather than nutrition. Some of the doughs were also allowed to ferment overnight (e.g., cracker doughs) to develop flavor, but generally they were mixed and allowed to stand for  $\sim$ 2 h.

The dough produced by mixing the above recipes with water at a low level of 20-30% w/w was often stiff and crumbly, which could be sheeted out by extrusion and cut to form the biscuit shapes. These were usually simple geometric forms such as ovals and squares, but could also be formed into the wellknown bone shape. After the pieces were shaped, the baking process was set up to give some aeration to the dough structure by rapid vapor creation, and then to dry out the biscuit to create their hard texture and color. This was not easy to achieve for dog biscuits, because of their size and the time available in the oven. Generally, biscuits were baked with a dry outer region and a moister center that would equilibrate with dry regions over several days, to give an average moisture <10% w/w (Figure 1). At this moisture

Table 2 Recipes for dog biscuits

Ingredients	%	%	%
Wheat flour	50.7	31.3	71.5
Wheat meal	0	31.3	0
Crumb	30.8	0	20.0
Bone meal	9.1	3.1	0.0
Meat solids	5.4	1.9	5.7
Salt	0.7	0.6	0.6
Malt	0	0	1.7
Fat	2.5	0	0.0
Soda	0	0.6	0.3
Corn meal	0	31.3	0.0
Yeast	0.7	0	0.1

level, the starchy structure would have a crunchy texture and good shelf life in the packs. Occasionally, this baking method caused problems because of the stresses created in the biscuits. The products could crack along lines of weakness and the biscuits could break into two or more pieces.

The traditional dog biscuit had a limited scope for development because of the long baking process. It also had other shortcomings, in that it had a fairly slow production rate and occupied a large factory area. Therefore, the development of extrusion cooking as an alternative method for dry pet-food production seized the imagination of manufacturers worldwide.

# **Extrusion Cooking Technology**

Extrusion cookers were invented in the 1940s to manufacture snacks from maize grits, but by the 1950s they were being adapted for the production of pet foods. At first the machinery was fairly crude and not well understood, and had a relatively small throughput of a few 100 kg h<sup>-1</sup>. The recipes were still based on a high level of cereals, such as wheat and maize. Later, they were enriched with proteins from meat meals and soy meal and flour (Figure 2 and Table 3).

The potential that could be envisaged for extrusion cooking provided the driving force for its development, and the equipment manufacturers strove to meet the needs of the pet food industry. Extrusion cookers are continuous processing units that transform a dry mix feedstock into a plastic fluid with the addition of 10-20% w/w of water, within their barrels. This fluid can be shaped and expanded from the dies at the end of the machine and cut into pieces with a rotating knife.



Figure 1 Baked biscuits.

The benefits of using extruders, compared with biscuit technologies, were as follows:

- continuous processing with low manpower requirements;
- small factory space;
- range of shapes and sizes created with different dies;
- low moisture process to reduce energy in drying (baking);
- sterile products;
- short residence time for control of thermal history of materials; and
- efficient processing.

The improvements in manufacturing units and understanding of the process by operators have both been enormous since the 1960s with production rates doubling every 10 years or so, to reach an amazing 5-10 ton per hour (t h<sup>-1</sup>) on the best modern plant.

Extrusion cooking – raw materials The basic process starts with the recipe and its components. These are selected for the qualities of flavor and nutrition, their interaction in the process, and their price. In all extrusion cooking processes, the raw materials interact with the processing variables of screw design, rotational speed, barrel temperature, die dimensions,



Figure 2 Extruded dry dog food.

Table 3 Recipe for extruded dog food

44%
17%
16%
17%
1%
5%

and feed inputs to create the system variables within the machines and eventually the product characteristics of the extrudate. This complex multivariate process was difficult to study in the early machines because they were sealed systems. It was only in the 1980s that CCFRA with its split barrel APV MPF 50 twin screw machine was able to provide good information on the processing of cereals.

Most dry pet foods are based on the transformation of starch by a combination of high temperature and powerful shearing forces into a dispersed polymer fluid. Therefore, the starch content of a recipe is very important in determining the physical characteristics of the extruded products. All raw materials have some effect in the process and they may be viewed in terms of their potential in an extrusion process using Guy's classification system (Table 4).

The development of the current understanding of the role of raw material components on the transformation of starch and the extrusion and expansion phenomena at the die has led a diverse range of products and greater control of their quality.

There is one problem with dry extruded pet foods because of the starch content. Cats in particular are not suited to a starch-rich diet and they have problems with the digestion of starch. Generally, the pet foods are made in dense form with a low degree of cooking, or dispersion of granular starch.

Extrusion machinery – preconditioning The powder mix for the extrusion cooking process can be metered directly into the extruder barrel and processed without difficulty. However, the extruder manufacturers have developed a secondary unit to warm the powders and to add water and fats prior to processing in the main barrel. This additional treatment has been shown to increase processing rates and reduce energy and wear costs. Little or no gelatinization of the starch occurs in these units because they operate at  $<100^{\circ}$ C and the moisture being usually <30% w/w is too low to reach the melting temperature,  $T_{\rm m}$ , for starch.

**Table 4** Guy's classification system for ingredients by their functional effects in extrusion cooking

Number	Functional group	Typical examples
1	Structure forming	Starch, soy, and gluten
2	Dispersed phase fillers	Soy, bran, and uncooked starch
3	Plasticizer/lubricants	Water and oils
4	Soluble solids	Sugars, dextrins, salt
5	Nucleants	Calcium phosphates and carbonates, fibers
6	Coloring materials	Added and Maillard reactants
7	Flavoring materials	Added and Maillard reactants

Extrusion machinery – extrusion cookers The heart of the process is an extrusion cooker where the recipe is transformed and prepared for forming into products as it is extruded. M. Riaz describes the various types of extruders, including single and twin screw designs that are available in the market and explains the differences between various types. There have been at least 30 companies selling machines in the market worldwide, but today one or two large single screw machines and three large twin screw machines dominate the world markets.

In all these extruders the moist mix of powder and water is compressed and sheared so that it becomes hot enough (120–160°C) to melt the crystalline structures within native starch granules. Once softened, the starch granules are partly dispersed by the shearing action of the screws or paddle elements in the machine, to give an expandable cell-wall material for gas bubble expansion at the die exit. The differences in commercial machines depend on their production capacity and their ability to control the process in terms of the temperature and the degree of starch dispersion.

In pet foods, the starch dispersion is relatively low after the melting of starch has been achieved. This gives a fairly dense extrudate with an expansion of  $1.5-2\,\mathrm{ml\,g^{-1}}$  specific volume for the extrudate. The ideal extruder is one with a short residence time in the hot shearing zone and a variable speed screw, which operates independently of the throughput rate at the lowest possible moisture. This type of extruder can be set to disperse starch to the required level at the lowest temperature and fastest throughput. Most extruders offer a compromise between these ideals and running costs, but the latest machines appear to offer more benefits and efficiency than the older models.

Extrusion machinery — coating and drying Once cut, the extrudate can be coated by spraying with a gravy and then dried in a moving or fluidized bed to its final moisture content of <10% w/w. The size of the dryer is dependent on the throughput and the moisture level, which may vary from 18% to 30% at the die exit, according to the process and the extruder.

#### **Wet Pet Foods**

The basic concept for wet pet food is a plate of meaty chunks surrounded by a meaty gravy or jelly. In practice, the meaty chunks may be made from reformed meat meals or vegetable proteins, such as soy or wheat gluten, or mixtures of both. The gravy is carefully thickened with gums and starches to control its physical character, both when hot and cold. Methods for forming the chunks have changed during the 1990s

and now include the use of extrusion cooking to texturize proteins, and simpler thermal methods to gelatinization of starch within meat slurry.

#### Formation of Meat Chunks - Extrusion Cooking

The earliest method for the economic production of meat chunks was the texturization of soy flour by extrusion cooking. In this process the protein is hydrated to 35-40% moisture w/w and heated to 160°C in a high shear field to produce a fluid melt, similar to the polymer melt of starch developed in dry extrusion cooking. Soy proteins have a tendency to aggregate when denatured and disrupted by heating above their denaturation points of 75–100°C. The use of high shear fields in the extruder prevents this from occurring until they are pumped into the die channels. In long die channels, laminar flow can occur at the normal flow rates with only small shearing forces. This allows the protein molecules to align and form hydrogen and hydrophobic bonds to create layers of fibrous material not unlike meat fibers.

The extrusion through the die channels is usually at 120–140°C, and at the exit some water vapor evaporates from the matrix to open up the texture and leave an alveolar structure in the chunks that are cut at the die.

Texturized vegetable protein (TVP) can be made with a minimum of 45% soy protein, but becomes stronger and tougher with increasing protein levels up to 90%. The material made from flours or concentrates is satisfactory for pet foods and can carry other materials within its continuous phase. Generally, some starch is used to form a dispersed phase within the smooth continuum of the protein. Other proteins and meals can also be added to enrich the chunks that too will be dispersed in the main soy protein phase.

If the secondary protein becomes dominant in the recipe, it may replace the soy as the continuum. Thus, when wheat gluten is used at 40-50% of a mixture, it may become the continuous phase. This protein has been used extensively in chunks for cat food because cats prefer it to soy products.

In the development of texturized proteins, a new type of process has been developed by Clextral of Firminy in France. This process is based on the use of higher moisture (60–80%) in the melt and a long cool die in which the proteins are allowed more time to form their fibrous structure without evaporation of water vapor to disrupt them.

# Formation of Meat Chunks — Thermal Gelling of Starch

In this process, wheat flours or other cereal flours are mixed into meat slurry to form a paste. The wheat

proteins hydrate to provide sufficient viscosity to help keep the solids in suspension until the paste is heated to gelatinize the starch granule of the flour. At this point the slurry becomes a solid gel whose strength increases on cooling and can be cut into small slices or chunks as required. This simple process may be carried out with special equipment to make it more efficient. In a continuous process the slurry is pumped through a forming tube, which is heated to warm the slurry and eventually gel the starch before it is extruded and cut.

This type of product and the texturized protein chunk must be stable in a canning process where the chunk is heated in gravy to 110-120°C for a period of 15-20 min. It was shown by that soy proteins must have a well-texturized protein structure to survive. In starch systems the presence of retrograded starch and some cross-linking may help the granules to retain the structure of the chunks.

#### **Intermediate Moisture Products**

The final type of wet cat and dog food was developed as a long-life product that could be stored at ambient temperatures and packed in light plastic bags or sleeves. Normally, a wet product will decay and be spoiled by microbiological growth once it has been removed from its can or pouch. However, if the water activity is reduced to < 0.7 with a low pH of 4-5 and the product is pasteurized, it will remain in good condition for several months. It can be sold in plastic packs in normal retail outlets at ambient temperatures, alongside the dry and canned pet-food products (Table 5).

#### **Treats and Other Foods**

This section covers a set of products generally called "treats" and some other dry foods. Treats may be

Table 5 Intermediate moisture products

Ingredients	%
Poultry meal	32.0
Maize	17.8
Maize gluten	10.0
Soybean meal	8.0
Corn syrup/beet fiber	12.0
Beet fiber	4.0
Potassium sorbate	0.2
Propylene glycol	8.0
Phosphoric acid	2.0
Vitamin/mineral mix	3.0
Fats	3.0

made in many forms but are usually dry products that for dogs can be carried by the owner and dispensed to the animals during their walk. The products may take the form of chewy leathery items, or individual biscuits can be made into bone shapes or produced by co-extrusion as mini-beefsteaks or "marrow" filled bones. The extrusion cooking units are very well suited to producing interesting shapes and being able to use special recipes to manufacture tasty products. For cats the treats are given in the house or garden. Again, one relies on special shapes and colors to please the owners, and on strong flavors and crunchy textures to satisfy the pet.

For the other pets that are kept in the home, the foods are not so well defined and are made as animal feedstuffs, with less thought about shape and color and more on nutrition and wholesome character. Some foods may be shaped into pellets by a forming extruder or pelleting press and some may be flaked as grain by flaking rolls or shaped as wet flour slurry on a roller dryer. The last mentioned method is used for the thin flakes fed to pet fish.

To summarize, it can be seen that the pet-food industry is dynamic and full of invention to increase efficiency and improve its products. The market has grown at a fast rate since the 1990s and there appears to an overall increase in the pet population with a slight movement toward cats instead of dogs.

See also: Animal Feed. Cereals: Overview. Extrusion Technologies. Nutrition: Effects of Food Processing.

#### **Further Reading**

Frazier PJ, Crawshaw A, Stirrup JE, Daniels NWR, and Eggitt PWR (1980) Factors affecting texture formation during extrusion cooking. Proceedings of 2nd International Congress on Engineering and Food. London: Applied Science Publishers.

Guy RCE (1986) Extrusion cooking versus conventional baking. In: Blanshard JMV, Frazier PJ, and Galliard T (eds.) Chemistry and Physics of Baking, pp. 227-235. London: The Royal Chemical Society.

Guy RCE (2001a) Extrusion Cooking: Technologies and Applications. Cambridge: Woodhead Publishing.

Guy RCE (2001b) Raw materials for extrusion cooking. In: Guy RCE (ed.) Extrusion Cooking: Technologies and Applications, pp. 5-27. Cambridge: Woodhead Publishing.

Guy RCE (2001c) Understanding extrusion. Food Engineering and Ingredients 26: 29–31.

Guy RCE and Horne AW (1987) Extrusion cooking and coextrusion of cereals. In: Blanshard JMV and Mitchell JR (eds.) Food Structure – Its Creation and Evaluation, pp. 331–350. London: Butterworths.

Riaz MN (2001) Selecting the right extruder. In: Guy RCE (ed.) *Extrusion Cooking: Technologies and Applications*, pp. 29–49. Cambridge: Woodhead Publishing.

Rokey GJ (1994) Pet food and fish food extrusion. In: Frame ND (ed.) The Technology of Extrusion Cooking, pp. 144–89. Glasgow: Blackie Academic and Professional.

Smith WH (1972) *Biscuits, Crackers and Cookies*. London: Applied Science Publishers.

#### **Relevant Websites**

http://www.AACCnet.com – American Association of Cereal Chemists.

http://www.webofscience.com — Web of Science. http://www.campden.co.uk — Campden & Chorleywood Food research Association.

http://www.waltham.com – Waltham Nutrional Foundation.

http://www.hillspet.com - Hills Petfoods and Nutrion.

#### **PLANTS**

Contents

Diseases and Pests
Whole-Plant Utilization

#### **Diseases and Pests**

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#### Introduction

The importance of diseases and pests in terms of food crops provides a context that all can relate to. In order to recognize the symptoms caused by pathogenic fungi and bacteria, viruses, and other plant pests, it is necessary to be familiar with the way in which healthy plants appear and how they behave. It is in this vein that a reminder of general plant physiology is given, and the vulnerable stages in plant development and growth are highlighted. An element of detective work is often necessary when identifying the cause behind plant abnormalities; the results of attack by various types of pests can appear very similar to the effects of nutrient deficiencies. "Cereal," "combinable," or "grain" crops are terms used to include those that are grown to produce seed for human consumption, that can be harvested with a combine and have small, hard seeds. Barley, maize (corn), millet, oats, rice, rye, sorghum, triticale, and wheat are monocotyledonous and have a growing point at

ground level (Figure 1a). The dicotyledonous plants including amaranth, beans, buckwheat, canola (oilseed rape), chickpeas, cotton, lentils, linola/linseed, lupins, peas, peanuts, quinoa, safflower, soybeans, and sunflowers have growing points aboveground level (Figure 1b). How these two types of plants grow, and in general this varies from plants to plants, determines their susceptibility to the various pests and diseases. An introduction to both infection and infestation of plants from pathogens and pests, respectively, is provided. Approaches to disease and pest management using both natural and man-made remedies are given. Some crop species are more resistant to attack (whether from grazing animals or microscopic pathogens), whilst others can only survive under sheltered conditions.

#### **Importance of Diseases and Pests**

The term "importance" is usually associated with positive attributes, e.g., "an important crop is one that has many uses." Detrimental factors are equally important, however, as they can have a huge, negative impact. Plants are dependent upon their environment; they require available nutrients, water, and sunlight in sufficient quantities to allow them to grow. If disease or pest damage occurs, normal plant function is

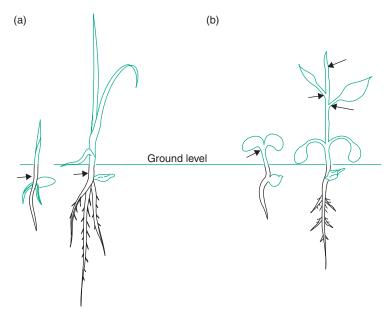


Figure 1 Location of plant growth points (arrows): (a) monocotyledons and (b) dicotyledons.

reduced, leading to injury or death unless the disease or pest is managed.

#### **Fit for Purpose**

The main effect of diseases and pests that occur in crop plants is to reduce the yield. For profitable food producers, the main concern from pests and diseases is the effect they have on the marketability of the crop. Often, if either yield or quality is reduced, at least the crop can still be sold, even if it is for a lower price than planned. If both factors are reduced, the crop will not be profitable at all. If this is the case for more than a season or two, such losses are unlikely to be sustainable. If a crop does not make money, there is no economic sense in growing it. When crops are grown to sell in niche markets, they must be fit for the purpose, as with organic produce. In this case, the way in which the crop is grown must meet certain, strict specifications throughout the growing season in order to retain its status. Part of the requirement in this example is not to use the majority of chemical pesticides (including fungicides) even when the crop is under attack. For the organic producer, not treating could mean reduced quality of produce, but treating could mean losing organic status and not being able to sell at the premium rate. Where market demand is present and there is fierce competition for a share, high standards of food quality and safety are vital.

#### **Food Safety**

The safety of food has become increasingly important over the years; besides affecting the marketability of a crop, it can have serious implications on consumer health. Pests and diseases can have a direct effect on the safety of food crops, such as the presence of mites in cereal-based products leading to the development of asthma and some molds that produce mycotoxins. There is also a safety issue with the presence of pesticides remaining in the harvested part of the crop, when high levels of diseases or pests were present during the growing season and were controlled to protect the quality of the product. Extensive testing to set maximum acceptable levels for both mycotoxins and pesticide residues is in progress. Typically, the agreed limits are based upon levels measured in food that is currently available and that has found to be safe. Whilst food safety is obviously a serious issue, the food crops currently produced are of a very high standard. In many countries it may appear that safety limits are now used as a reason for rejecting a proportion of the crops where production is high, rather than because there is a serious risk to consumers.

#### **Healthy Plants**

Once a seed has germinated and developed into a seedling, it will grow to maturity over a period of weeks, months, or years depending upon its genetic makeup and the nutrients available to it. During this time, photosynthesis is carried out within the chloroplasts of the green plant cells. Intercepted solar energy is used to convert carbon dioxide and water into carbohydrates and oxygen. The simple carbohydrates produced in this way are then processed into starch

(for efficient storage), the structural component of cell walls (cellulose), fats, oils, or proteins.

For a plant to be healthy, photosynthesis must be efficient, the required raw materials must be available, the chlorophyll within the leaves must be working correctly, and the carbohydrates produced must be transported to the growing points of the plant. Plants need various mineral nutrients (such as nitrogen, phosphorus, potassium, sulfur, calcium, magnesium, manganese, and molybdenum) for the more complex molecules that they produce during growth. When these nutrients are in short supply, symptoms of deficiency are seen in the plants and damage caused by disease may be increased. The accumulation of carbohydrates is important in maintaining the plant's species when they are sequestered either into the seeds of grain crops, or storage organs as with potatoes. Vegetative propagation via rhizomes or stolons may also give rise to new mature plants in some plant types.

#### **Vulnerable Stages in Plant Growth**

The "success" of a pathogen or pest depends upon its ability to invade a host, grow, and reproduce before causing death to the plant. Diseases and pests cause most damage to plants when they act upon critical points in the processes involved in plant maintenance, growth, and reproduction. These can be summarized as follows.

- 1. Reduction in the rate of plant photosynthesis. The reduction of green leaf area caused by pest damage or diseases such as mildew, scab, and rust will impair the interception of solar radiation.
- Reduction of the plant's ability to take in water and nutrients by preventing uptake through the roots or blocking the transport through the plant. As the components necessary for photosynthesis are restricted and the supply of water ceases,

- plants become chlorotic and wilted. Diseases falling into this category include *Fusarium* foot rot, eyespot, and take-all; pests include slugs and insect larvae that damage stem bases and roots.
- 3. Interference with seed formation leading to infertile flowers, nonviable seed, or the replacement of seed with fungal structures. Damage caused to the lower stem can lead to infertile flower heads that do not produce any seed and may be described as "blind," or if seed is produced it is severely shriveled and of poor quality. Damage caused by insect larvae feeding on developing grains (e.g., wheat orange blossom midge) may prevent normal germination of the seed and will reduce its quality. Seed production is prevented in several species of grain by smut, bunt, or ergot infection.

Plants are more vulnerable to attack at certain points in their life cycle than at others, particularly during the early stages of growth. As their biomass increases, they become more resilient to damage; this is illustrated in cereals with the damage caused by bulb-fly larvae. When the eggs of the bulb-fly hatch, each larva burrows into the base of a plant and feeds on the stem and leaf material there, this prevents the flow of nutrients and water up to the green leaves, resulting in their death. When the eggs hatch early in the season, there will only have been a single tiller produced; with this destroyed, the entire plant will die. When the eggs hatch at a later stage in the season, several tillers will have been produced and even if one or two are killed, the remainder will continue to grow and more tillers will be produced. By the time, the crop has reached maturity, there will be little effect on overall yield.

Another example is aphid infestation of canola (oil-seed rape). Because aphids feed via a proboscis that must be pushed into plant tissue before sap can be extracted (Figure 2), they attack new, tender shoots

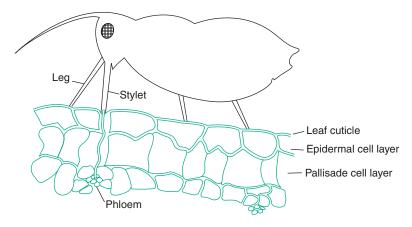


Figure 2 Aphid feeding on plant sap through its stylet.

and are unable to penetrate older stems. A crop at an early stage in development contains plants that are completely accessible to aphids and may be killed if sufficient numbers build up during this vulnerable stage. Once plants have grown and have tougher stems, only their new shoots and flower buds can be penetrated. As flower sites are particularly tender, the fertility and amount of seed set may be reduced or the yield may be adversely affected with the restricted energy reserves available for storage within the seeds.

Storage conditions are very important to keep seeds and tubers healthy and to start with a clean crop the following season. Seedlings are extremely vulnerable to attack from many types of organism and severe attacks at this stage are likely to be fatal. Once seedlings have reached a certain stage, they are equipped better to withstand attack. If the pest or disease is present in epidemic proportions, however, they are still at risk and at best, may suffer reduced yield. With the presence of inoculum and the means for its spread, diseases rapidly take hold. Attacks to flowers tend not to be fatal, as the plant is mature at the stage when they are produced but yield is still likely to be reduced.

#### **Plant Infection by Pathogens**

The causal agents of plant diseases (pathogens) may be airborne, soilborne, splash-dispersed, or carried by insect vectors. These causal organisms are mainly fungi, bacteria, or viruses, although very few bacteria are associated with cereal diseases.

#### Table 1 Examples of cereal diseases caused by fungi

Crop(s)	Disease	Pathogen	Dispersal
Barley	Net blotch	Pyrenophora teres	Seed
Cereals	American powdery mildew	Sphaerotheca morsuvae	Air
Cereals	Black stem rust	Puccinia graminis	Air/insects
Cereals	Ergot	Claviceps purpurea	Soil/air
Wheat + barley	Eyespot	Pseudocercosporella herpotrichoides	Soil
Cereals	Foot and root rot	Fusarium culmorum	Soil
Cereals	Head blight	Fusarium culmorum	Soil
Cereals	Powdery mildew	Erysiphe graminis	Air
Cereals	Rusts	Puccinia spp.	Air
Cereals	Seedling wilt	Fusarium culmorum, F. avenaceum, F. gramineaum, F. nivale, F. poae	Soil
Cereals	Sharp eyespot	Rhizoctonia cerealis	Soil
Cereals	Take-all	Gaeumannomyces graminis	Soil
Maize	Common smut	Ustilago maydis	Air
Maize	Southern leaf blight	Cochliobolus heterostrophus	Air/splash
Wheat + barley	Glume blotch	Leptosphaeria nodorum	Splash
Wheat + barley	Loose smut	Ustilago nuda	Air
Wheat	Common bunt or stinking smut	Tilletia caries	Seed/air
Wheat	Septoria	Septoria tritici	Splash

#### **Fungal Diseases**

A fungus is a multi-celled organism that lacks photosynthetic ability; therefore, it relies on other organisms (animal or vegetable) to provide the necessary energy for growth and reproduction. If a fungus does not cause much damage to its host organism, it is described as saprophytic, but if the host is greatly damaged or killed, the fungus is categorized as parasitic.

The main causal organisms of plant diseases are the true fungi (*Eumycota*); the three subdivisions into which most pathogenic fungi fit are:

- 1. the *Ascomycotina* (e.g., powdery mildew *Erysiphe graminis*);
- 2. the *Basidiomycotina* (e.g., the rust and smut fungi); and
- 3. the *Deuteromycotina* (e.g., cereal eyespot *Pseudocercosporella herpotrichoides*).

The first two groups contain fungi that have both asexual (cell division) and sexual (cell fusion then division) stages to their life cycles, the third contains fungi whose sexual stage has not been identified (if they have one). Table 1 shows examples of cereal diseases caused by fungi.

The sexual stage allows the alteration of genetic makeup over time as many generations are passed through. By this process, the fungus may gradually change in a way that enables it to infect plants that were previously resistant to its attack. When this occurs, the resistance of the plant is described as having broken down (although it is actually the pathogen's properties that have changed rather than those of the

host plant). During the course of several years, a newly developed crop cultivar will initially be resistant to infection from the strains of pathogens present in the environment and a large area will be sown with this cultivar. In this way, a heavy selection pressure is placed on the fungi, as only those that able to overcome the plants' resistance will be able to survive. Any strains of fungi that develop the ability to infect their host plants will have a great advantage and will multiply quickly. As the numbers of this "new" strain increase in proportion to the "old" strains to which the plant cultivar was resistant, the plants are more frequently attacked until the resistance loses its strength completely. This is an important consideration when producing crops, and is one of the key driving forces behind plant breeding programs, in addition to improving yield and crop quality. The process of pathogen evolution and its effect on a monoculture of a cultivar has been described as a cycle of "boom" and "bust" in terms of crop success. As the success of a new resistant cultivar encourages growers to use it, there is a boom, but when the resistance breaks down, there is a bust as the cultivar rapidly succumbs to widespread disease.

Life cycle of brown rust in wheat (*Puccinia recondita*) This fungus may infect wheat and rye, but is particularly damaging to the former host. Disease symptoms occur on the leaf blades in the form of scattered orange-brown spots (uredosori) that are often surrounded by a pale halo of leaf tissue.

Dispersal The uredosori release huge quantities of uredospores to be spread by the wind, particularly in warm weather with frequent rainfall (although hot conditions will halt its spread).

Penetration When a uredospore lands on the surface of a plant under conditions of suitable temperature  $(15-20^{\circ}\text{C optimum})$  and humidity (high), it will produce a germination tubule. As the tubule grows between the plant cells, it is recognizable as hyphae and will penetrate cells producing a haustorium, which it uses to remove nutrients from the host cell, thus enabling its own growth.

Mechanism of injury By removing nutrients from many host cells simultaneously, the growing fungus causes areas of chlorosis around each infection site. The growth of superficial mycelium on the leaves and the localized areas of chlorosis and necrosis reduce the green leaf area available for converting solar radiation into nutrients by photosynthesis.

Propagation Once established at an infection site, the fungus will produce fresh pustules (uredosori) that will release further uredospores. The infection cycle may be repeated many times during the summer growing season. The pathogen is able to survive between seasons by living on volunteer plants and straw in this way. As temperatures fall, teliospores and haploid basidiospores are also produced, which infect a noncereal host, such as meadow rue (*Thalictrum flavum*), and the cycle continues through winter and releases accidiospores that are able to infect cereals again.

Control Resistant cultivars will prevent infection from established fungal strains. Regular monitoring of the crop for early signs of disease will allow fungicide to be applied in time to prevent serious damage from new fungal strains.

#### **Bacterial Diseases**

All bacteria are single celled with no cellulose cell wall, chloroplasts, or mitochondria within them; they can only be seen as individuals under a light or electron microscope. Plant pathogenic bacteria have rod-shaped cells and rely on contact with a host organism to obtain the energy they need to grow and reproduce. They do not have a dispersal mechanism, so must rely on naturally occurring vectors (such as rain, insects, and soil-dwelling organisms) to move their cells into contact with a suitable host. Plant cells are well protected by rigid walls and a cuticle, so bacteria are only able to gain entry through wounds or natural openings (e.g., stomata). Diseases caused by bacteria are rare within cereals; they are a greater problem for broad-leaved plants and their fruit or tubers. The types of diseases caused by bacteria can be split into three main groups according to the way in which they act upon the host. Table 2 shows examples of cereal diseases caused by bacteria.

1. Parenchymal diseases are recognized easily once they have taken hold; in the affected area of the host plant, the cell walls are broken down and become incapable of holding their original shape, resulting in a soft, weak mass.

Table 2 Examples of cereal diseases caused by bacteria

Crop(s)	Disease	Pathogen
Maize Oats Wheat	Bacterial wilt Halo blight Yellow slime (Tundu disease)	Erwinia stewartii Pseudomonas coronofaciens Corynebacterium tritici

- Vascular diseases cause the host plants to wilt as the xylem and phloem pathways become blocked by thick, sticky substances produced by the bacteria, preventing transport of water around the plant.
- Meristematic diseases occur when bacteria cause excessive plant growth in a localized area by stimulating either cell division, cell enlargement, or a combination of the two.

Life cycle of bacterial wilt in maize (*Erwinia stewartii*) Symptoms range from mild to severe: long, pale green/yellowish streaks on leaves extending from the points of insect damage; early flowering and the production of very few ears; and no ears produced, or complete plant death.

Dispersal The main vector of these bacteria is the corn flea beetle (*Chaetocnema pulicaria*), but the soilborne larvae of *Diabrotica longicornis* and *Phorbia cilicrura*, that attack the plant roots, also carry them.

Penetration The vector damages the plant when it feeds, allowing bacterial cells to enter through the wounds and into the host's vascular system.

Mechanism of injury Once inside the host, the bacteria feed on it by producing enzymes to degrade the pectin of cell walls. By-products are produced in the form of sticky polysaccharides that build up to block the vascular system and preventing the normal flow of nutrients around the host.

**Propagation** Bacterial cells divide rapidly once they have gained sufficient nutrients to enable them to do so. Vectors feeding on infected plants will carry some bacterial cells to other plants and continue the infection cycle.

**Control** The main method of control is to target the flea beetle (vector) population with early season insecticides; this can be combined with the use of resistant cultivars.

#### **Viral Diseases**

An individual virus is submicroscopic (~50 nm across) and can only be seen using an electron microscope; it generally consists of a single strand of DNA or RNA encapsulated within a protein coat. Because of their simplicity, viruses require other cells (e.g., animal, plant, or bacterial) to provide the building blocks and energy required to replicate themselves. Different types of viruses are specifically adapted to infect different organisms; those that infect plants

tend to contain RNA and are coated with a simple protein layer when traveling between cells. When a virus comes into contact with a host cell, the RNA passes into the cell, leaving the protein coat on the outside. The RNA causes a template strand (that is complementary to the original) to be formed from the cell's DNA; this template is then used to replicate many strands that are identical to the original. Upon entering the host cell, the initial viral RNA strand causes the host cell to produce enzymes (RNA polymerases) that catalyze the replication of new viral RNA strands. The plant cell is also used to produce the protein coating to enclose the new RNA strands before the cell is completely destroyed and the new batch of viruses released and may remain in the intercellular spaces to infect new cells, or may enter the plants transport system and be dispersed throughout the plant. Table 3 shows examples of diseases caused by viruses.

When they are outside a host's cell, viruses are relatively fragile and easily killed. The success of viruses is due to the way they are actively spread from plant to plant via various vectors such as aphids, leafhoppers or whitefly (all of the order *Homoptera*), soil-dwelling nematodes, fungi, or within seeds. These insect and nematode vectors share a common morphology in their feeding organs; they have a protruding "beak" called a stylet that is able to penetrate the outer cuticle of plant surfaces and enter plant cells. Within this group of vectors, the two main categories into which virus transfer falls are termed "stylet-borne" and "persistent."

Stylet borne As the name suggests, viruses transmitted in this way are carried from an infected plant by a vector within or stuck on the outside of the stylet

Table 3 Examples of cereal diseases caused by viruses

Disease	Vector
Barley stripe mosaic virus	Pollen/infected seed
Barley yellow dwarf virus (BYDV)	Aphids especially Rhopalosiphum spp.
Barley yellow mosaic virus	P. graminis (soil fungus)
Maize chlorotic dwarf virus	Graminella nigrifrons (leaf hopper)
Maize dwarf mosaic virus (MDMV)	Many aphid species
Maize mosaic virus	Peregrinus maidis (plant hopper)
Maize rough dwarf virus	Laodelpax striatellus (plant hopper)
Oat blue virus	Macrosteles fascifrons (leaf hopper)
Oat mosaic virus	P. graminis (soil fungus)
Rice dwarf virus	Nephotettix cincticeps (leaf hopper)
Rice tungro virus	N. impicticeps (leaf hopper)

after a feeding session from the outer layers of a host plant. When a new feeding place is reached (on another plant or a different location of the same plant), the plant's cuticle is punctured and the viruses delivered from the stylet into close contact with internal plant cells. The "soybean mosaic virus" is transmitted in this way.

Persistent This type of transmission occurs by the movement of infected sap taken from the host plant's transport system. When the vector feeds on the sap of a plant containing virus particles, it ingests the mixture without harming the pathogens' structure. The virus particles may remain dormant within the vector for several hours allowing a greater chance of spreading the disease to a new plant, or they may retain their virulence and be able to cause infection immediately as they come into contact with a host cell. Upon moving to a new feeding position, the viruses are injected deep into the transport system of the new plant, as with the "pea enation mosaic virus."

Regardless of the manner in which a virus enters its host plant, once inside it is able to penetrate the host cells, rapidly multiply, and spread causing disruption of normal growth. By repressing production of the plant's necessary enzymes, symptoms are seen on the outside of the plant. These symptoms are the basis for the classification of the different types of virus pathogen:

- 1. Stunting and dwarfing result from a loss of ability to grow normally.
- Color change occurs when chlorophyll production is reduced, forming a mosaic, mottled pattern of yellow and green on the leaves. Variations on chlorosis, followed by necrosis, may cause interveinal discoloration or concentric rings of yellow and brown.
- Leaf and stem distortion results from abnormal cell proliferation or development as occurs in virus leaf curl diseases.

Life cycle of barley yellow dwarf virus in cereals Despite its name, barley yellow dwarf virus (BYDV) infects many grass species, including barley, oats, wheat, and rye. Symptoms are seen as bright yellowing (wheat and barley) or reddening (oats) of the leaves from the tip back towards the stem. Other symptoms may include necrotic spotting, stunting, increased tillering, and bleached, sterile ears.

Dispersal Many species of aphid carry this virus from one feeding site to the next. After an aphid feeds for 24–48 h on an infected plant, there is a short latent period (during which the virus will

not be passed on), then the virus will be transmitted from that aphid for the next few weeks.

Penetration The virus particles are injected by the vector directly into the host's vascular system from which they are able to enter large numbers of host cells throughout the plant with relative ease.

Propagation and mechanism of injury As the viruses replicate themselves, they use the host cell contents and leave empty, dysfunctional cells behind them. Due to their rapid multiplication, they cause large areas of cells to become necrotic simultaneously. When a crop is infected at the seedling stage, large areas can be wiped out completely, as the plants grow, they are able to tolerate the infection better, if they are not infected until late in the season, there will be very little damage to the crop.

Control The only effective method is by reducing the aphid (vector) population, this can be combined with the use of resistant cultivars and reducing the amount of wild grasses surrounding the crop as these are a source of inoculum.

#### **Plant Damage by Pests**

#### **Birds and Mammals**

Although some species of birds will eat insects rather than seed, there are those (sparrows and pigeons in particular) that will eat seeds at every opportunity: during storage, when planted in the field, at germination and during early plant development. If seeds are damaged, they are unlikely to remain viable and will not germinate. Wild burrowing and grazing animals such as rabbits, hares, gophers, badgers, rats, and mice (and their relatives around the world) can be serious pests once seeds have germinated. If a germinated seed is damaged, the seedling produced may be stunted or grow abnormally if at all. If young plants that are attacked survive, they are likely to produce a weak plant with a reduced capacity for reproduction (i.e., lower yield). Once plants are well established, they are less likely to be killed by an attack, particularly in the case of monocotyledonous cereal plants where the growing point is at or below ground level; continuous grazing can be tolerated until stem elongation begins in earnest. Dicotyledonous *Brassica* sp. can also survive being pecked at by pigeons, provided they are not completely mown down to the ground; they have multiple growing points and will compensate by sending out new growth from the leaf axils.

#### Insects

Although it may be stating the obvious, insects have smaller mouths and so prefer more tender parts of plants than larger pests. They cause particular problems for younger plants; the smaller the area of green leaf to begin with, the less damage it takes to destroy it. Insects are either designed for grazing leaves (with jaws) or for piercing leaves then sucking out the sap (with a stylet). Many insects are attracted to flowering canola (oilseed rape) and similar crops in the field where they lay eggs. Once these have hatched, the insect larvae devour the leaves and flowers as they grow. Other insect pests pose a problem in stored grain, particularly weevils, beetles, moths and their larvae.

#### **Nematodes and Molluscs**

In addition to the aerial plant parts, slugs, nematodes, and some insect larvae attack the roots. Of the pest nematodes, some feed on the internal and some on the external parts of plant roots using a stylet in a similar way to aphids.

#### **Disease and Pest Management**

"Control" of pathogens and pests does not tend to be absolute, therefore, "management" seems a more accurate description, and especially when there is a greater emphasis on integrated management. With regular monitoring and careful forward planning, an integrated approach to disease management can be taken to protect the quantity, quality, and safety of the yield. Management of diseases and pests is essentially the prevention of disease and pest epidemics, as the causal organisms are always around somewhere, but must be prevented from building to damaging levels. The disease triangle summarizes the three main factors involved: environment, host, and pathogen (or pest), one at each point of the triangle. If these three elements remain in balance, an epidemic is prevented, if the pathogen/pest becomes too strong, the host becomes vulnerable or the environment favors the pathogen/pest, then an epidemic is likely and control measures must be taken.

#### Husbandry

Physical methods for disease and pest management include the following:

 Soil preparation such as plowing to expose insects to their predators (birds) and to bury the remains of the previous season's crop, which may harbor overwintering stages of pathogens or the eggs of insect pests.

- Informed choice of crop variety to match environmental conditions in which it is to be grown; the variety should be suitable for the soil type and general weather conditions in a given location, with proven resistance to endemic pests and diseases that cannot be effectively controlled by other means.
- The use of crop rotations to prevent a buildup of problem diseases or pests due to providing a continuous succession of host plants.
- Elimination of alternate and alternative (weed) hosts of crop diseases and pests where possible.
- Timing sowing to allow plants to become strong enough prior to insect or disease attack or to delay germination until the threat has receded.
- Encouraging rapid germination and early growth to build up strength quickly and enable prevention of severe damage resulting from initially mild infestation.
- Understanding the life cycles of diseases and pests to be able to manage them, using the results from modeling studies to enable forecasting of likely onset of epidemics.
- Reduction in size of areas in which a single cultivar of a single crop is grown, for example, reduces the pressure on the pathogens to overcome plant resistance.
- Following the rules for notifiable diseases, in order to prevent them from spreading.

#### Resistance

As plants are literally rooted to the spot they grow in, they are not able to escape pests and diseases. They are, however, able to put up some defenses to the attacks that come their way. In addition to the basic structure of plants (i.e., the location of their growing points), integral properties such as thorns, thick cuticles, or chemical composition act as a deterrent, a blockade, or even a toxin to the right invading organism. Other attributes may only become activated once the plant is threatened, e.g., lignification of cell walls surrounding an area being invaded by a pathogen. In the case of genetic resistance to pathogens, resistant plants are able to deny the invader the nutrients or chemical components they require to grow or reproduce. When resistance breaks down, the pathogen has altered to allow it to overcome this.

Genetic resistance may be due to the presence of a single major gene, which confers protection against a matching virulence gene within the pathogen (genefor-gene concept). If a pathogen containing an altered virulence gene tries to infect the host, it is likely to succeed, as the resistance is specific to the initial type of virulence, not this new one. This can be observed at

the seedling stage when new varieties are being screened for their usefulness. This type of resistance is most useful when (1) a new disease problem arises, (2) disease spread is slow, and (3) a single type of pathogen (a single virulence gene) becomes stable within the pathogen population.

Polygenic resistance involves the presence of several resistance genes within a single host cultivar, this confers protection from a range of virulence genes. It is not fully exhibited until the plants mature, so is screened for in the field by looking for a reduced rate of pathogen sporulation.

Biotechnology has allowed improved direction to be used when breeding new crop varieties. In particular, it has been possible to introduce genes that code for Bt proteins into transgenic lines of maize and cotton to confer protection against various insect pests and to remove the need for spraying with insecticides. These crops are widely grown in northern America and have proven themselves to be very effective.

#### **Chemical Control**

The use of pesticides to kill diseases and pests has been exploited in the past in order to meet the market demands for clean, healthy produce. These can be used to accurately target pest organisms by their methods of application: seed dressing, granules, and liquid sprays applied to leaves or soil as appropriate. Contact pesticides cause the death of the pathogen or pest either by direct treatment with spray, dust or vapor or when the pathogen or pest touches the chemical residue on leaves or in soil. In the case of pests, a stomach poison may be used that is only effective when ingested either from the external surface of foliage, within the sap of a plant or when used as a bait. The different types of chemical may be short-lived and require immediate, direct contact with the pathogen or pest, or may be longer-lasting; entering the system of the host plant and remaining there for some time before acting on the attacking organism. The chemical formulation of pesticides appears to be designed to work in more than one way (i.e., on contact and systemically) in order to retain its efficacy over as long a period of time as possible. The overall aim of all pesticides is the same; to prevent the pathogen or pest from growing or multiplying. This is achieved by disrupting the normal function of the invading organism, thus protecting the host plant and allowing it to grow and reproduce.

The development of new, effective pesticides is a long process. An initial product development stage is carried out in controlled environments until suitable formulations are produced. Once the safety of the compound is understood sufficiently, it is tested for efficacy and crop safety by extensive field-testing before approval for use is given. The high cost of development and testing is transferred to the end product, making the use of chemical crop control difficult to justify in some cases.

#### **Integrated Pest Management**

As the agricultural industry has become unable to justify the cost (both monetary and ecological) of using chemical control methods alone, a greater emphasis has been put on integrated pest management. By understanding the epidemiology of the organisms causing the damage, it has been possible to reduce the amount of chemical required for pest control. The strategic use of chemical control when it will be most effective and only when it is absolutely necessary has led to this reduction. Instead of treating a crop when evidence of disease or pests is first observed, conditions are optimized for plant growth and reduced for pathogen and pests. Optimization of crop husbandry to maintain strong plants throughout the season plays a vital role, but the effect that any chemicals may have on naturally occurring beneficial insects or predators must also be taken into consideration.

See also: Barley: Genetics and Breeding. Buckwheat. Cereals: Grain Diseases. Chemicals for Grain Production and Protection. Grain and Plants, Morphology. Organic Growing of Grains. Stored Grain: Invertebrate Pests.

#### **Further Reading**

Agarwal AA, Tuzun S, and Bent E (1999) Induced Plant Defenses against Pathogens and Herbivores. St. Paul, MN: American Phytopathological Society.

Bennett WF (1993) Nutrient Deficiencies and Toxicities in Crop Plants. St. Paul, MN: American Phytopathological Society.

Carlisle WR (1988) New Studies in Biology, Control of *Crop Diseases.* London: Edward Arnold – A division of Hodder and Stoughton.

James WC (1971) A Manual of Assessment Keys for Plant Diseases. St. Paul, MN: American Phytopathological Society.

Jones D and Gareth (1987) Plant Pathology Principles and Practice. Milton Keynes, UK: Open University Press.

Jones RN and Karp A (1986) Introducing Genetics. London: John Murray.

Salisbury FB and Ross CW (1985) Plant Physiology, 3rd edn. Belmont, California: Wadsworth Publishing Co.

Stryer L (1988) Biochemistry, 3rd edn. New York: Free-

Webster J (1980) Introduction to Fungi, 2nd edn. Cambridge: Cambridge University Press.

Wiseman AJL, Finch HJS, and Samuel AM (1993) Lockhart and Wiseman's Crop Husbandry Including Grassland, 7th edn. Oxford: Pergamon.

#### **Relevant Website**

http://www.cropprotection.org.uk - Crop Protection Association (UK).

#### Whole Plant Utilization

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#### Introduction

In subsistence plant husbandry, all parts of the crops were needed for survival and were, therefore, carefully utilized for food and nonfood purposes. Introduction of the monetary economy ended the isolation of the agricultural village. It was integrated into a national and later an international economy, leading to increased competition and specialization.

New technology based on exploitation of the nonrenewable fossil fuels supported this change at an ever-accelerating pace. The local biological and agricultural production chains, which to a great extent were self-sufficient, yet low-producing, were broken up due to new technology, transport, and trade. To cite an example, with the changed production system, the straw, which was left by the new efficient combines as waste in the field, was burnt after grains were harvested. The dramatic change from a local to a more global economy increased production and created competition for each individual class of products. It brought about a specialization, economically favoring farmers who had a local production advantage for a given specific product. Specific monocultures were, thus, regionally favored. At the same time, a range of traditional, agricultural nonfood products were phased out by substitutes produced and supported by gas, oil, and coal.

Since the oil crisis in the 1970s, there has been an increased awareness of the importance of system research and ecological sustainability in the agroindustrial production chains and in the derived agroindustry. Research programs were introduced by governments and international organizations to study renewable production systems that take care of the whole plant in a balanced utilization for food, feed, and nonfood purposes. How would it be possible to

utilize modern technology to re-establish, in a new way, the biological production chains in a recycling effort to conserve fossil energy and avoid an increase of carbon dioxide in the atmosphere, which may contribute to a climatic change? What governmental incentives were necessary for such a change to be driven by the monetary system and by the market?

# Value-Added Fractionation of Whole Grain Crops

The coarse botanical components of the cereal plant can be divided into the internode (stem or stalk), nodes (divisions between internodes at the insert position of leaves), leaves, husks (or cobs from maize), and seeds. Straw is defined as the remains (leaves, internodes, and nodes) that are left in the field after combining. Internodes may contain marrow, which is especially well developed in maize and sorghum. Internodes, nodes, leaves, husks, and cobs are lignocellulose products. These contain large amounts of cellulose and hemicellulose bound to lignin with protein as a minor component.

Each overground grain crop is, in essence, botanically and chemically inhomogeneous and may be fractionated into a range of different components. Thus, straw can be coarsely milled in a simple disk mill to produce flour from leaves, marrow, and chips from internodes and nodes which are separated by a sifting machine yielding fractions with entirely different chemical and physical properties (see Figure 1).

Unfractionated straw has a mediocre value for feed as well as for paper. However, the leaf fraction has improved protein and energy value for feed, and the



**Figure 1** Dried stem of maize separated by disk milling and sieving into a meal fraction from the marrow and into different sizes of chips from the cell walls of internodes and nodes.

internode part has as high a content of  $\alpha$ -cellulose, as wood used for paper. There should, therefore, be an economic incentive for a value-added fractionation of straw, tailored to different uses. The total amount of the world production of cereal grains amounts to over 2 billion tons (Gt) of grains and at least a similar tonnage of straw, which implies  $\sim 1.1$  Gt of internode chips and 0.9 Gt of leaf and marrow meal.

# The Botanical, Chemical Composition, and Productivity of Grain Crops

Plant-breeding efforts have increased cereal seed yield more than the whole crop output, because new varieties have been bred for increased grain-to-straw

**Table 1** Yield and botanical composition of whole cereal crops grain in Denmark<sup>a</sup>

		Grain yield (t ha <sup>-1</sup> )	Straw yield (t ha <sup>-1</sup> )	Grain/straw ratio (w/w)
Spring barley	Mean	5.1	3.9	0.78
	Range	6.3 - 3.6	5.5 - 2.8	1.01 - 0.52
Winter wheat	Mean	6.9	6.1	0.92
	Range	9.1 - 3.8	8.5 - 3.7	1.63 - 0.68
Winter rye	Mean	4.9	6.4	1.31
	Range	5.4 - 4.6	7.5-5.3	1.58-1.10
Spring oats	Mean	4.2	5.7	1.34
. •	Range	5.4-3.1	7.1 - 4.2	1.36-1.32
Maize	Mean	6.2	6.6	1.06
	Range	6.9-5.7	10.6-4.5	1.74-0.72

<sup>&</sup>lt;sup>a</sup> Reproduced with permission from Bjørn Petersen P and Munck L (1993) Whole crop utilization of barley including new potential uses. In: MacGregor AW and Batty RS (eds.) *Barley: Chemistry and Technology*, pp. 437–474. St. Paul, MN: American Association of Cereal Chemists.

ratio, changing the proportions of the whole plant produce by favoring seeds.

In Table 1, indicative Danish data regarding yield and composition of whole crops from barley, wheat, rye, oats, and maize show a variation between extreme samples in grain-to-straw w/w index from 0.52 in barley to 1.74 in maize. The average values indicate that short straw breeding, to favor grain yield under Danish conditions, has apparently been most successful in barley, followed by wheat, maize, rye, and oats. Winter wheat and maize, despite the fact that the latter crop is not fully adapted to the Danish climate, produced most grain (top yield 9.1 and 6.9 t ha<sup>-1</sup>, respectively) and straw (top yield 8.5 and  $10.6 \,\mathrm{t}\,\mathrm{ha}^{-1}$ , respectively). The straw from each sample of the cereal material in Table 1 was hand-dissected into internode, leaf, and node and the fractions were weighed and analyzed (see results in Table 2). The internode is, on an average, the largest component of straw, ranging from 68.7% in rye to 40.4% in maize. The leaf portion ranges from 48.4% in barley to 20.7% in rye, while the node fraction is relatively high in maize (max. 16.6%) and low in barley (min. 1.0%). It is also seen in Table 2 that  $\alpha$ -cellulose is generally higher in the internode (42.2–38.3%) than in the leaf (30.3-28.2%). The leaf fraction has the highest protein content of 3.5-8.3% compared to 2.0-3.7% for internodes. The leaf fraction shows constantly higher values with respect to ash and silicon than internodes. Ash and silicon content depend to a great extent on the soil conditions, with high content in loamy soils compared to sandy. The silicon in the internode fraction in several samples

 Table 2
 Analysis of hand-dissected botanical components of straw from Table 1

		Weight distribution % of total harvested straw w/w			Mean composition (% d.m.)		
		Mean	Max/Min	α-Cellulose	Protein	Ash	Silicon
Spring barley	Internode	50.4	55.1/44.7	38.3	2.0	4.5	0.5
	Leaf Node	41.6 5.4	48.4/33.9 4.0/1.0	28.2	3.9	6.3	1.4
Winter wheat	Internode	55.0	63.0/49.8	42.2	3.0	4.6	1.0
	Leaf	38.7	44.2/31.6	29.3	5.2	8.4	2.3
	Node	4.8	7.0/3.4				
Winter rye	Internode	67.7	68.7/66.7	41.3	3.0	3.7	0.5
•	Leaf	23.9	25.4/20.7	29.8	5.9	5.7	1.2
	Node	5.2	6.5/2.8				
Spring oats	Internode	50.4	53.4/47.3	39.2	2.6	4.6	0.2
	Leaf	42.1	45.5/38.7	30.3	3.5	7.4	1.9
	Node	4.4	5.1/3.7				
Maize	Internode	46.6	52.0/40.4	39.5	3.7	6.3	0.2
	Leaf	42.1	46.4/34.3		8.3	7.9	1.4
	Node	11.9	16.6/8.7				

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grown in sandy soils is below 0.1%, which is a great advantage in the manufacture of chemical paper pulp and paper. In vivo and in vitro digestibility trials give  $\sim 20-30\%$  higher values with cereal leaf compared to internode (e.g., barley in vivo 51.3% versus 41.0%). Leaf meal energy value for fuel varies from 16.8 MJ per kg d.m. in barley to 17.0 MJ per kg d.m. in barley straw pellets compared to 18.4 MJ per kg d.m. in wood.

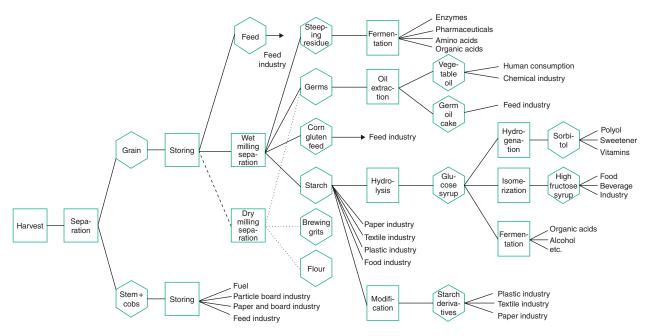
#### **Products from Whole Plant Fractions**

Today, most of the whole plant products are utilized for food and feed. The consumption of grains and grain products for the nonfood sector is relatively small compared to that of food and feed, although in absolute terms, millions of tons of starch, oil, and lignocelluloses are consumed globally by the industry for nonfood purposes.

Research on agricultural, nonfood substitutes for import was high during the Second World War, especially in the USA within the USDA. After the energy crisis in the 1970–80s, research on developing renewable resources for products and energy was again initiated in countries such as USA, Holland, England, and in the Scandinavian countries, as well as in the present European Union. Perhaps the most important driving force was the steadily increasing surplus in cereal production in industrialized countries at the end of the twentieth century. There was, thus, a clear governmental interest in nonfood cereal

products that could eliminate the surplus, while providing income to farmers.

Starch, cellulose, and oil are the major chemical components of industrial interest. Figure 2 gives the whole plant utilization potential of maize as an example. Very large scale industrial units for maize grain utilization is a significant component. These units feature dry and wet (starch) milling of maize, where oil (from germ) and gluten protein (for feed) come as coproducts from starch manufacturing. There is a wealth of possibilities for utilizing the starch polymer after modification by means of organic chemistry or microbiological transformation to, for example, plastic molds, ethanol, acetone, and butanol. Starch from maize, wheat, and potatoes is consumed in large amounts by the paper industry. Here, starch derivatization with ionizing chemicals is used in the form of cation and anion starches as additives to increase paper strength as well as for coating. Graft polymers with vinyl and acrylic monomers can be produced from granular and gelatinized starch for production of plastic films, molded products, etc. Such a solid polymer of starch with acrylonitril can absorb many hundred times its weight in water without being dissolved. These "superslurpers" could be used for applications in hygienic products and medical care, as well as in agriculture for coating seeds and to conserve water under arid conditions. Starch xanthates can be used as carriers for encapsulation of seeds with pesticides.



**Figure 2** Whole plant utilization with maize as an example. (Munck L (1993) On the utilization of the renewable plant resources. In: Hayware MD, Bosemark NO, and Romagosa I (eds.) *Plant Breeding. Principles and Prospects*, pp. 500–522. London: Chapman and Hall.)

The lignocellulosic fractionation options demonstrated in Table 2 regarding stem and leaves are sparsely utilized today. The current world production of paper pulp is in the order of 150 million tons (Mt), of which 12 Mt comes from cereals and grasses corresponding to ~30 Mt of whole straw. Existing fractionation and treatment technology can exploit the lignocellulosic resources of cereals much more effectively than is the case today. Most of the straw produced today is either burnt in the open or collected and used as bedding material for animals and as lowquality feed. Straw can be pelleted into a high-grade fuel. It can be instantly treated with alkali and steamed in a pellet press to loosen the lignin bonds, which greatly improves the physiological energy utilization of feed for ruminants. The same alkali-treated pellet can be used as semi-manufactured material for paper pulp. Short hardwood fibers from birch, etc., and internode fibers are similar in length, but the latter are much more slender. The smaller diameter of the straw fiber seems to have no adverse effect on the technological properties of the paper derived from it. The fiber length from lignocelluloses in the leaf fraction is shorter than that from the internode fraction. The latter is preferable for paper-pulp processing because of its high content of  $\alpha$ -cellulose and low level of silicon. Silicon may be a problem when straw is used as raw material in the paper-pulp industry, because it prevents recycling of the alkali – black liquor – due to gelling and insulation of the cooker.

The use of straw fractions for particle boards is an overlooked application today. The internode fraction from, for example, wheat, rye, maize, and sorghum straw compressed with synthetic glues yields a product strength as high as that of particle boards from wood chips. The texture of this type of boards can be varied by using different size fractions from internodes, making an appealing natural product. Starch can substitute formaldehyde in phenolic polymers, which can be used as binders in particle boards. Bran from phenolrich sorghum varieties can also be used for glue making. Another more elegant solution for glue is enzyme treatment of the lignocellulose matrix of the internode material. This enables the use of the natural component lignin as a binder by introducing free radicals before pressing.

Most of the vegetable oils from grains (e.g., rape, sunflower, and maize) are used in the food sector. There is, however, a significant nonfood market for these vegetable oils in soaps and detergents, paint and varnish (emulsifiers and drying oils), lubricants, greases, cosmetics, and pharmaceuticals. Rapeseed oil for driving diesel engines in, for example, tractors and buses is at present used in the public sector in Europe in an effort to reduce pollution and improve

carbon dioxide recycling. This is more effective per unit of biomass, compared to using ethanol for fuel obtained microbiologically from hydrolyzed starch or lignocellulose, because the fermentation process involves liberation of large amounts of carbon dioxide.

#### Whole Plant Utilization Production Systems – Integrated Plant Conversion in Biorefineries

It may be concluded that there are great opportunities in utilizing whole plants in a variety of food, feed, and nonfood applications. A major obstacle is, however, local organization of the necessary links between decentralized agriculture and centralized industry, for a flexible whole plant utilization of a range of different crops, simultaneously, in accordance with signals from the market. The local cultural role of farms and farmers in society based on traditions has prevented the integration of the local production of vegetative materials and the processing industry. Such an integration has, however, taken place in the forestry industry. Oil palm plantations and the cane and beet sugar industries are examples of industries that are closely connected to the agricultural production. They exercise within a defined crop a whole plant utilization strategy. Since the 1960s, the idea of local agricultural preprocessing stations or biorefineries as a necessary link between agriculture and industry began to emerge in countries such as Denmark, Sweden, Holland, and the United States.

In the following section, the Danish research applicable for northern European conditions is cited as an example. In the 1960s, machine stations and cooperatives for manufacturing of lucerne and grass pellets by high-temperature, oil-heated drum dryers demonstrated the needs and possibilities in changing the structure of local agriculture. Grass and lucerne was harvested as a whole crop by self-propelling choppers (Figure 3a) several times during the growing season. These were then brought in containers (Figure 3b) to the drying station where they were dried and pelleted for sale to the feed industry. A number of very wet harvesting seasons in the 1960s stimulated the use of the whole crop harvesting strategy for other crops such as barley, wheat, broad beans, and maize. The whole biomass was dried in the drum dryer and afterwards separated into seeds and straw. The straw could then be treated in the pellet press of the drying station by steam, sodium hydroxide, and urea to produce high-quality feed for ruminants. In Denmark, in the 1960–70s, a major oil company subsidiary ran this type of biorefinery in a limited sense in Jutland, where whole crop





**Figure 3** The principle of whole plant harvesting exemplified by barley: (a) self-propelling harvesting chopper loading the coarsely chopped plants into a container; (b) when the container is full, it is transported by truck to the biorefinery for separation of the crop components and further processing.

harvesting of lucerne, grass, and cereals from farmers in the area was integrated with a large milking cow unit.

On the island of Bornholm, a demonstration and research biorefinery unit, financed by EU-projects, has been in operation since 1988. It is located adjacent to a feed factory with a drum dryer and a chipboard factory for wood chips. Large-scale trials were performed with fractionation of wheat and rape straw into internodes and leaf meal. High-quality chipboards were produced from internodes chips.

In cooperation with chemical and paper industries, an EU "Cascade" production chain project was implemented, whereby a cation starch product was developed from bran-separated wheat flour in an environmentally closed dry process. The performance of this resource-saving product in paper production was fully comparable to that of cationic wheat starch. The quality factors for performance were monitored throughout the production chain with near infrared spectroscopy (NIR) interpreted by chemometric (multivariate) data analysis. On the basis of the NIR analysis, the quality of wheat optimized for the whole process could be defined and predicted, at the raw material stage. Today, the modified wheat flour product is produced and sold in Germany in competition with modified starch.

Extraction of oil from, for example, rapeseeds normally involves extraction with explosive hydrocarbons in very large factories. A process suitable for local, smaller-scale production was developed by which the rapeseeds were milled and blended in water. Enzyme treatment opened up the oil-filled cells and the oil was extracted from the emulsion by a centrifuge, just as cream and butter are extracted from milk. A high-quality food protein fraction was obtained as a coproduct.

After the oil crisis in 1973, Denmark's lucerne- and grass-drying and pelleting industry, one of the largest in the world at that time, vanished due to high oil prices. Some companies survived by switching to coal. In drying cereals as a whole crop, the pelleted leaf fraction is enough for sustaining the drying process. The implementation of this possibility is dependent on the price of straw. The Danish experience, applicable to the wet and tempered production and harvesting conditions in northern Europe, is contained in the biorefinery concept demonstrated in Figure 4, comprising a machine station, a drum dryer, crop fractionation equipment, and a feed factory serving a few thousand hectares. Compared to the present farm system with individually run units of  $\sim 50-100$  ha, the biorefinery organization would imply the following advantages:

- 1. The economic success of the biorefinery should not be judged on the basis of individual products, but on the integrated total output from a flexible diversified production.
- 2. Because of whole overground plant harvesting (Figures 3a and 3b) and its dryer, the biorefinery organization is independent of harvesting weather. Therefore, a wider selection of whole crops can be safely harvested, separated, and processed as semimanufactured products, ensuring a high quality.
- 3. The diminished monoculture will lead to improved fertility and less costs for pest control.
- 4. The field is cleared in one step and a higher yield of both grain and straw is achieved.
- 5. Harvesting machines and biorefinery equipment will be able to be used most of the whole year, which will improve economy. It will, thus, be able to support a permanent staff, thereby creating local working opportunities.
- 6. The system can be run by locally produced bioenergy.
- 7. Transport costs will be minimized. Locally produced high-quality feed pellets from straw and grass will stay in the area, while surplus grains and internode fractions are sent to larger centralized industries outside the area producing, for

**Figure 4** The biorefinery concept as a link between agriculture and industry for implementing whole plant utilization technology. (Reproduced with permission from Bjørn Petersen P and Munck L (1993) Whole crop utilization of barley including new potential uses. In: MacGregor AW and Batty RS (eds.) *Barley: Chemistry and Technology*, pp. 437–474. St. Paul, MN: American Association of Cereal Chemists.)

- example, wheat flour, starch, and vegetable oil, chipboards, and paper pulp.
- 8. The benefits of the biorefinery principle could only be fully utilized in a network of biorefineries and cooperating industries, which would stimulate sustainable crop utilization and recycling of waste. Local animal and human manure could be collected and used in biogas units, as is done in a few communities in Denmark today. These units could be integrated with the biorefinery. The processed manure products could be recycled to the local agricultural land or sold and exchanged as a hygienic dry product. Thus, the land fertility problem potentially caused by removing too much organic matter (e.g., from straw) from the fields could be solved by re-establishing the biological production chain through recycling the manure.

The whole plant utilization technology included in the biorefinery concept focuses on using agriculture as a vehicle for the development of a differentiated agroindustrial production pattern for food, feed, and nonfood products, optimizing the use of local natural resources in closed biological production chains. The principle can be adapted to widely different cases in industrial as well as developing countries, taking local conditions into account.

#### **Economy**

The cost-effective use of biological material for non-food purposes in the present economy should now benefit from the fact that the price for cereals (e.g., maize) versus oil w/w has decreased sixfold since 1973. The world market price of pure starch is not far from that of crude oil. Synthetic low-density polyethylene from fossil fuels as well as paper pulp (lignocellulose) is in the order of 2–3 times more expensive than starch. The high price of paper pulp today reflects the high energy and machine costs in the forestry industry. There should be considerable energy savings, compared to the forestry industry, by using existing machinery in agriculture to take care of the internode fraction from plants for paper pulp and chipboards.

It should be advantageous for farmers in countries with existing straw-based paper pulp factories to sell the internode fraction to these industries, while retaining the leaf fraction as feed. Agricultural lignocelluloses for paper and chipboards should be a valuable

produce in many developing countries with vanishing forestry resources. Even if several of the previously discussed plant applications are shown to be profitable today, there are economical, structural, and psychological constraints on a system change from fossil products to renewable plant resources.

To stimulate the nonfood option in agriculture oil, prices should increase from the current US\$25 per barrel to reach over US\$50 and be kept at this level for a long time. The world now consumes in the order of 50 million gigawatt hours (GWh) of crude oil annually compared to a global production of cereal starch in grains of ~6 million GWh. The plant utilization option is, thus, not of a magnitude that can match our present utilization of fossil fuels, for example, for driving combustion engines, but should, of course, be reserved mainly for food uses. However, whole plant utilization in many specific applications could locally complement food (and animal feed) production without competing with food production. Nonfood technology such as the utilization of agricultural lignocelluloses could be a vehicle for development of local industries by providing employment and thus creating the necessary purchasing power for food.

# Research for Implementation of Whole Plant Utilization Technology — How Can Markets Recognize the Long-Range Advantages?

Biological systems are characterized by complex, dynamic, synergistic patterns of interrelations between their components and with the environment. Whole plant utilization technology and the biorefinery are concepts in which biological principles, human needs, and environmental limitations are aimed at being organized, thereby approaching a higher level of production organization and greater benefits than at present. In the development of industries in the 1970s, horizontal integration through diversification was favored, while in the year 2004, the opposite paradigm prevails, maximizing profit by focusing on a core product tailored to specific markets. Accordingly, science today is focused primarily on revealing the partite elements of nature and then putting them together by experimental design in the laboratory, rather than exploring them first in nature by specially devised screening methods. It seems as if systemic concepts like whole plant utilization technology are too complex to be recognized by the present strategies for science, economy, and markets, which largely suppress the synergistic element in an attempt at problem reduction. There are at present new promising parallel

developments in the natural, social, and economical sciences, which aim at extending the cognitive limits of human individuals and organizations. This would enable recognition of complex problems in large datasets as patterns on a graphic display by means of new information technology. Grain science is in the forefront here, introducing computerized sensor technology in quality management such as NIR to provide a holistic fingerprint of the physics and chemistry of a sample visualized by multivariate data analytical programs. From huge datasets, specific information may be deduced by comparing samples and by calibration to classic analyses using chemometrics. With such screening methods, it is now feasible to make overviews through large-scale inventories of plant production systems in the field to connect and to tune them to processing chains. For development of whole plant utilization systems, real exploratory data from field inventories and process chains is a must in order to fully understand synergy effects in developing new products and markets from plant raw materials in a sustainable perspective. Thus, biological and electronic circuits can be connected to human selectors. thereby coordinating production, information, and market incentives in a new, more harmonious, and advanced postindustrial production and culture.

See also: Cereals: Protein Chemistry. Grain and Plants, Morphology. Lipid Chemistry. Starch: Chemistry; Modification.

#### **Further Reading**

Bjørn Petersen P and Munck L (1993) Whole crop utilization of barley including new potential uses. In: MacGregor AW and Batty RS (eds.) *Barley: Chemistry and Technology*, pp. 437–474. St. Paul, MN: American Association of Cereal Chemists.

Gomez MI and Munck L (1993) Whole cereal crop utilization. In: Taylor JRN, Randal PG, and Viljoen RH (eds.) *Cereal Science and Technology Impact on a Changing Africa*, pp. 653–688. Pretoria: The Council for Scientific and Industrial Research (The CSIR).

Hugo S, Gooding MJ, Barton SA, and Smith GP (2001) Wheat – still finding alternative uses? A review. *Aspects of Applied Biology* 64: 31–38.

Lynd LR, Wyman CE, and Berngross TU (1999) Biocommodity engineering. *Biotechnology Progress* 15(5): 777–793.

Munck L (1993) On the utilization of the renewable plant resources. In: Hayware MD, Bosemark NO, and Romagosa I (eds.) *Plant Breeding. Principles and Prospects*, pp. 500–522. London: Chapman and Hall.

Munck L (1995) Whole plant utilization by milling and separation of the botanical and chemical components.

In: Dendy DAV (ed.) *Sorghum and Millets*, pp. 223–281. St. Paul, MN: American Association of Cereal Chemists. Munck L, Rexen F, Petersen B, and Bjørn Petersen P (1989) A new agricultural system for Europe?. In: Pomerantz Y (ed.) *Wheat is Unique*, pp. 49–70. St. Paul, MN: American Association of Cereal Chemists.

Munck L and Rexen F (1990) *Agricultural Refineries – A Bridge from Farm to Industry*. Commission of the European Communities. Report EUR 11583 EN. Brussels, Luxembourg.

#### **Relevant Website**

http://www.ienica.net — The website of INFOR-RM-IENICA — the interactive European network for industrial crops and their applications in the changing millennium/industry network for renewable resources and materials. The site contains representative references to websites all over the world.

Protein Chemistry of Cereals see Cereals: Protein Chemistry.

# PROTEIN CHEMISTRY OF DICOTYLEDONOUS GRAINS

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#### Introduction

The protein contents of dicotyledonous grain crops vary widely, from less than 20% of the dry weight in the pseudocereals to  $\sim 50\%$  in some lines of soybean (Table 1). Extraction and separation of "total" protein fractions shows that all comprise highly complex mixtures, with many components being present in small amounts. However, in all cases a small number of components are present in substantial amounts: these correspond to the seed storage proteins and usually account for half or more of the total seed proteins.

Although specialized storage protein types occur in some species, e.g., lectins in some legume seeds, most of the storage proteins present in dicotyledonous seeds fall into three groups which are defined on their solubility and  $S_{20,w}$  values (sedimentation coefficients determined by sedimentation equilibrium ultracentrifugation), which are a measure of their molecular masses. The characteristics of these three types of proteins are discussed, followed by brief descriptions of the storage proteins present in the major dicotyledonous species covered in this encyclopedia.

**Table 1** Protein contents and types of storage proteins in major legume, oilseed, and pseudocereal crops

Species	Protein content (% DW) <sup>a</sup>	Storage proteins
Legumes		
Soybean	30-40	12S glycinin, 7S $\beta$ -conglycinin, 2S albumin
Pea	25	12S legumin, 7S vicilin/convicilin 2S PA1
Peanut	16-36	13S arachin, 8S conarachin, 2S albumin
Lupin	26-42	11–12S conglutin $\alpha$ , 7S conglutin $\beta$ , 2S conglutin $\delta$
Oilseeds		
Oilseed rape/canola	36-44	12S cruciferin, 2S napin
Linseed/linola	20-24	12S globulin
Sunflower	15-20	12S helianthinin, 2S albumin
Safflower	15	12S carmin, 2S albumin
Cottonseed	16–22	11S globulin, 7S globulin, 2S albumin
Pseudocereals		
Quinoa	15	11S chenopodin, 2S albumin
Amaranth	13–18	13S amaranthin, 8S conamaranthin, 2S albumin
Buckwheat	13.5	11S globulin, 8S globulin, 2S albumin

a% Dry weight

The  $S_{20,w}$  values listed are only approximate with a range of values having been reported for some components.

#### **Types of Storage Protein**

#### Globulins

Globulins are defined as proteins that are soluble in dilute salt solutions, usually taken as  $0.5-1.0\,\mathrm{M}$  NaCl. Storage globulins with  $S_{20,w}$  values of  $\sim 7$  and 11 are widely distributed in dicotyledonous plants. However, they have been most widely studied from legume seeds. Hence, 7S globulins are sometimes called vicilins after the Viciae tribe of legumes, and 11S globulins, legumins after the family Leguminosae. The individual types of 7S and 11S globulins in different crops are also often given specific trivial names, as listed in Table 1.

It has long been recognized that members of these two protein groups also vary in their precise  $S_{20,w}$  values, with legumin-like globulins often having values of 12 or more (e.g., the 13S lupin globulin) and vicilin-like globulins  $\sim$ 8 (e.g., in peanut). Furthermore, not all 11S globulins are readily soluble in dilute saline, with notable exceptions being 11S storage globulins of cereals, which are either insoluble in saline (rice) or only soluble at higher concentrations (0.8–1.0 M) of NaCl (oats). Consequently, the term 11S is used in this article to describe storage proteins with a legumin-like structure and the term 7S to describe vicilin-like proteins.

7S globulins 7S globulins typically have molecular masses of  $\sim 150-200 \, \mathrm{kDa}$ , and have a trimeric structure comprising three nonidentical subunits of mass  $\sim 50-70 \, \mathrm{kDa}$ . The subunits contain no cysteine residues, and hence do not form disulfide bonds, and noncovalent forces stabilize the trimeric proteins. However, this simple structure may be modified after protein synthesis in two ways. First, glycosylation (addition of oligosaccharides) may occur on one or two asparagine residues in one or more subunits of the trimer. This results in an increase in mass of the subunits and lower mobility when separated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) with partial glycosylation resulting in additional heterogeneity.

Second, in some species (e.g., pea and field bean), some, but not all, 7S globulin subunits are proteolytically processed at one or two sites, giving rise to polypeptide chains ranging in mass from processed products of  $\sim 12 \, \mathrm{kDa}$  in pea and  $18 \, \mathrm{kDa}$  in bean to unprocessed subunits of  $\sim 50 \, \mathrm{kDa}$ . The processed products remain associated in the trimeric complexes until released by denaturation. The role of this proteolytic processing is not known and it does not occur in some species such as soybean and common or navy bean (*Phaseolus vulgaris*). Although typically trimeric in structure, the 7S globulins may undergo reversible

aggregation into hexamers depending on ionic strength.

11S globulins 11S globulins typically have masses of ~300-400 kDa and are hexamers of six nonidentical subunits. Each subunit is synthesized as a precursor protein which is proteolytically processed to release acidic (also called A or α) and basic (B or  $\beta$ ) polypeptide chains which correspond to the N-terminal and C-terminal parts of the precursor protein, respectively. The masses of the acidic chains vary in the range of 20-60 kDa but are typically  $\sim 40$  kDa, while the basic chains usually have masses of ~20 kDa. Thus, a typical 11S globulin comprises six subunits of mass  $\sim 60 \,\mathrm{kDa}$ , each consisting of 40 kDa and 20 kDa polypeptide chains. The basic and acid chains are covalently linked by a single disulfide bond formed in the precursor protein. Whereas the 7S globulins are rapidly assembled into their mature trimeric structure, the 11S globulin precursors are not immediately assembled into their mature hexameric structure but into an intermediate trimer with the hexamer forming only after proteolytic processing occurs to release the basic and acidic chains.

The polypeptide (i.e., subunit and chain) masses of the 7S and 11S globulins discussed below are summarized in Table 2.

Structures of 7S and 11S globulins Simple comparisons of the amino acid sequences of 7S and 11S globulins show little or no evidence of homology, but

**Table 2** Polypeptide molecular masses ( $M_{\rm r} \times 10^{-3}$ ) of 7S and 11S globulins present in species

	7S	11S acidic chain	11S basic chain
Legumes			
Soybean	50, 80	10-40	20
Pea	12-50, 70	25-40	21-22
Peanut	23-75	14-48	21
Lupin	15-60	25-63	21
Oilseeds Oilseed rape/canola Sunflower		30-40 32-34	20 22–27
Pseudocereals Quinoa Amaranth Buckwheat	15–90	32–39 30–37 32–43	22-23 18-27 20-23

The terms 7S and 11S refer to vicilin-like and legumin-like proteins, respectively, and do not imply that all of the proteins listed have these precise  $S_{20,w}$  values. In fact, the true values vary ( $\sim$ 7–8S and 11–13S, respectively). It should also be noted that widely different values for polypeptide masses have been reported and those presented here have been selected by the author. The 7S polypeptides include whole subunits and chains released by proteolysis. The acidic and basic 11S chains are generated by proteolysis of a single precursor subunit.

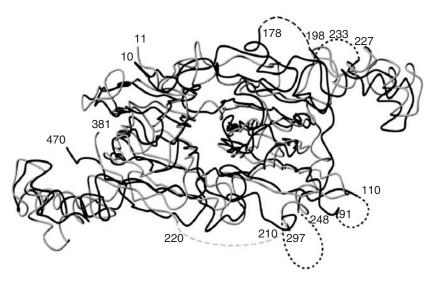
**Figure 1** Hypothetic pathway for the evolution of germins and globulin storage proteins from an ancestral "protogermin" protein. (Reproduced with permission from Shewry PR, Jenkins J, Beaudoin F, and Mills ENC (2003) The classification, functions and evolutionary relationships of plant proteins in relation to food allergens. In: Mills ENC and Shewry PR (eds.) *Plant Food Allergens*. Oxford, UK: Blackwell Science.)

more sophisticated comparisons (in which the properties of the amino acids and their propensity to form similar structures are also considered) do indicate some relatedness, particularly between the C-terminal parts of the 7S subunit and the basic chains of 11S subunits. Similar comparisons also show internal homology between the N- and C-terminal parts of the 7S subunits. These observations, and the more detailed structural comparisons discussed below, indicate that the 7S and 11S globulin subunits have evolved from a single ancestral protein that initially underwent a duplication to give two homologous domains which diverged to form the 7S N-terminus/11S basic chain and 7S C-terminus/11S acidic chain, as shown in Figure 1.

Comparisons of the three-dimensional structures of 7S globulin trimers (phaseolin from common bean, canavalin from jack bean, and  $\beta$ -conglycinin from soybean) and the intermediate trimer of proglycinin (the 11S globulin of soybean) confirm this scheme and show remarkable conservation of structure. Each protein consists of three subunits arranged around a threefold axis (Figure 2), with each subunit consisting of two closely related structural modules, and



**Figure 2** Structure of the 7S globulin canavalin from jack bean (*Canavalia ensiformis*) seeds. The protein is trimeric, with a single subunit shown in lighter gray. Domains 1 and 3 are  $\beta$ -barrels and domains 2 and 4  $\alpha$ -helical. (Reproduced with pemission from Shewry PR, Jenkins J, Beaudoin F, and Mills ENC (2003) The classification, functions and evolutionary relationships of plant proteins in relation to food allergens. In: Mills ENC and Shewry PR (eds.) *Plant Food Allergens*. Oxford, UK: Blackwell Science.)



**Figure 3** Overlay of the backbone structures of single subunits of proglycinin (11S globulin precursor) from soybean (black) and phaseolin (7S globulin) of bean (gray). (Reproduced with permission from Shewry PR, Jenkins J, Beaudoin F, and Mills ENC (2003) The classification, functions and evolutionary relationships of plant proteins in relation to food allergens. In: Mills ENC and Shewry PR (eds.) *Plant Food Allergens*. Oxford, UK: Blackwell Science.)

each comprising an antiparallel  $\beta$ -barrel domain and an  $\alpha$ -helical domain. The subunits of 7S and 11S globulins have similar structures, as shown by the overlay in **Figure 3**, and the mature hexameric 11S globulin protein is considered to be a "dimer of trimers."

The similarity in the structures of the 7S and 11S globulins is consistent with the observation that both proteins can exist in trimeric forms (the mature 7S and intermediate 11S globulins) and in hexameric forms (the mature 11S globulins and the mature 7S globulins under specific conditions). It is also consistent with the fact that the 7S and 11S proteins appear to be stored as a homogeneous mixture in protein bodies in seeds of legumes and other dicotyledonous species.

A final point of interest is that both 11S and 7S globulins may be allergenic, notably in soybean and peanut.

#### **Albumins**

Albumins are defined as proteins that are soluble in water. Consequently, albumin fractions extracted from most plant tissues comprise complex mixtures of largely unrelated components. However, the seeds of many dicotyledonous plants contain a well-defined family of albumin storage proteins with  $S_{20,w}$  values of  $\sim$ 2. These "2S albumins" are major storage proteins in a number of dicotyledonous crops (e.g., oilseed rape/canola and sunflower) and minor components in others (e.g., soybean). Although they have not yet been identified in some dicotyledonous crops, this may reflect lack of study rather than true absence, and they may well prove to be

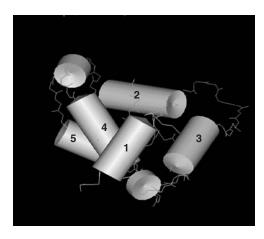
ubiquitous in dicotyledonous seeds. A preliminary report of the presence of 2S albumin storage protein in seeds of one monocotyledonous species (yucca) has not been confirmed by more detailed studies, but they are known to be structurally related to several other families of low-molecular-mass sulfur-rich proteins including nonspecific lipid transfer proteins and trypsin/ $\alpha$ -amylase inhibitors of cereal grain.

A typical 2S albumin consists of two subunits of  $M_{\rm r} \sim 8-10\,{\rm kDa}$  and  $4-5\,{\rm kDa}$ , which are associated by two disulfide bonds. However, these two subunits are initially synthesized as a single precursor polypeptide and hence, by analogy with 7S and 11S storage globulins, they should perhaps be called polypeptide chains. Nevertheless, the term subunit is widely used and will be retained here.

The proteolytic processing to release the 2S albumin subunits may result in the loss of short peptide sequences from the N- and C-termini of the proteins and from between the two subunits ("linker" peptides). Furthermore, this proteolysis only occurs after the proproteins are folded and four disulfide bonds formed between eight highly conserved cysteine residues. Two of these become interchain bonds linking the two subunits and two intrachain bonds within the large subunits. This processing is shown schematically in Figure 4. The mature proteins have a compact tightly folded structure comprising five  $\alpha$ -helices in a right-handed fold (Figure 5).

The vast majority of 2S albumins have two-chain structures similar to that described above. However, single-chain forms (i.e., without proteolytic processing into two chains) also occur, notably in sunflower.

**Figure 4** Schematical sequence of the precursor protein of napin (the 2S albumin of oilseed rape) indicating peptides removed by proteolysis and the large and small subunits of the mature protein.



**Figure 5** Schematic structure of the 2S albumin from oilseed rape. Alpha helices are shown as cylinders and numbered 1–5. (Reproduced with permission from Shewry PR, Jenkins J, Beaudoin F, and Mills ENC (2003) The classification, functions and evolutionary relationships of plant proteins in relation to food allergens. In: Mills ENC and Shewry PR (eds.) *Plant Food Allergens*. Oxford, UK: Blackwell Science.)

Of particular interest in relation to grain nutritional quality is the presence in some species of 2S albumins that are rich in methionine or, less commonly, cysteine, the two sulfur-containing amino acids that are frequently limiting in legume and other dicotyledonous seeds. These albumins have been studied in most detail in Brazil nut and sunflower. Although they were initially considered to provide an attractive target for use in genetic engineering to increase seed sulfur content, it is now known that both are allergenic, as are 2S albumins from a number of other species (e.g., mustards, castor bean, walnut, sesame seeds, and peanut).

#### **Storage Proteins of Legumes**

Many leguminous species store 7S globulin, 11S globulin, and 2S albumin proteins, but the proportions of

these vary and exceptions do occur. For example, *Phaseolus* contains 7S but not 11S globulins. Additional types of storage protein also occur in some species, including various lectins and protease inhibitors, which can have negative impacts on nutritional quality.

Soybean is the most widely studied legume species. It contains major groups of 11S (glycinin) and 7S ( $\beta$ -conglycinin) globulins, in ratios ranging from  $\sim$ 1:1 to 6:1 depending on the genotype, with only small amounts of 2S albumins. In addition, a number of other proteins can each account for up to  $\sim$ 5% of the total protein in some lines, notably Kunitz and Bowman-Birk-type protease inhibitors, lectins, urease, and lipoxygenase. Sedimentation coefficients and masses for glycinin have been reported to range from 11.8S to 13.1S and from 30 to 380 kDa and for  $\beta$ -conglycinin from 6.7S to 8.0S and from 105 to 330 kDa.

Glycinin has a typical hexameric 11S globulin structure, with subunits comprising basic chains of  $M_{\rm r} \sim 20\,{\rm kDa}$  linked to acidic chains varying in mass from  $\sim 10$  to  $40\,{\rm kDa}$ . Glycinin is also unusual in that the hexamer can undergo reversible dissociation into trimers, depending on the ionic strength.  $\beta$ -Conglycinin comprises various combinations of three major subunits  $-\alpha$  ( $M_{\rm r} \sim 80\,{\rm kDa}$ ),  $\alpha'$  ( $M_{\rm r} \sim 80\,{\rm kDa}$ ), and  $\beta$  ( $M_{\rm r} \sim 50\,{\rm kDa}$ ) — resulting in seven major forms. Both 7S and 12S globulins of soybean are also thought to be allergenic.

Pea seeds contain legumins (12S to 13S,  $M_{\rm r}$  330–420 kDa) and vicilins (7S to 8S,  $M_{\rm r}$  150–190 kDa). The legumin subunits consist of acidic chains of  $M_{\rm r}$  ~25 kDa and 40 kDa linked to basic chains of  $M_{\rm r}$  ~20 kDa. The 7S vicilins comprise subunits of  $M_{\rm r}$  ~50 kDa, but proteolysis at one or two sites and glycosylation at a single site may occur, resulting in polypeptide chains varying in  $M_{\rm r}$  (12–50 kDa). Larger subunits of  $M_{\rm r}$  ~70 kDa also occur, which

form a second trimeric 7S protein called convicilin. Pea seeds also contain two major albumins, PA1 and PA2, of which the former may be a 2S storage albumin. PA1 accounts for  $\sim$ 10% of the total seed protein but half of the total sulfur, in the form of cysteine ( $\sim$ 11 mol.%).

The dominant peanut proteins are arachin (13S–14S,  $M_r$  330–400 kDa) and conarachin (8S to 9S,  $M_r$  140–190 kDa). The subunits of arachin comprise acidic chains of  $M_r$  ~14–48 kDa linked to basic chains of  $M_r$  ~21.4 kDa, whereas conarachin comprises polypeptide chains ranging in  $M_r$  from ~23 to 75 kDa. It is probable that both large and typical conarachin subunits are present, as in soybean and pea, with proteolytic processing contributing to the polymorphism. Both proteins also exhibit complex association/dissociation behavior, depending on the ionic strength. The major peanut allergen Ara h 1 is a glycosylated 7S globulin with  $M_r$  ~63.5 kDa, whereas Ara h 3 is an 11S globulin.

Most of the knowledge of peanut albumins comes from the analysis of genomic clones and is related to their role as allergens (Ara h 2, 4, 6, and 7). They show high homology to 2S albumins from other legumes (soybean, lupin) and are presumed to have a typical hetrodimeric structure.

The salt-soluble (i.e., albumin and globulin) proteins of lupins are called conglutins. The major component is usually the 7S conglutin  $\beta$ , with slightly less conglutin  $\alpha$  (11S globulin) and  $\sim$ 25% of the 2S albumin conglutin  $\delta^-$ . Conglutin  $\alpha$  has a mass of  $\sim$ 336 kDa and readily dissociates to a trimeric form (7.1S,  $M_r$  170 kDa) at low ionic strength or high pH (8.8). It comprises acidic chain varying in  $M_r$  (within and between species) from 25 to 63 kDa linked to basic chains of  $M_r$   $\sim$ 21 kDa. Conglutin  $\alpha$  is unusual among 11S legumin-type globulins in that it is glycosylated on a single site. Conglutin  $\beta$  comprises polypeptides that range in mass between and within species from  $\sim$ 15 to 60 kDa.

Little detailed work has been carried out on the other legumes/pulses discussed in this article. Both chickpea and lentil contain 11S and 7S storage globulins (in a ratio of  $\sim$ 6:1 in chickpea). In addition, chickpea contains a major albumin of  $M_{\rm r} \sim$ 20 kDa, which comprises subunits with  $M_{\rm r}$  10 and 12 kDa.

Adzuki bean is rich in 7S globulins with major components of  $M_{\rm r} \sim 55 \, \rm kDa$ , but small amounts of 11S globulins and 2S albumins are also present.

#### **Storage Proteins of Oilseeds**

The two most intensively studied oilseeds are sunflower and oilseed rape (canola). Both contain predominantly 11S-type globulins with some 2S albumins.

The 12S globulins (cruciferin) of oilseed rape account for  $\sim$ 40% of the total seed proteins and have  $M_{\rm r}$  of 30–360 kDa. They have a typical hexameric structure with acidic chains of  $M_{\rm r}$  30–40 kDa linked to basic chains of  $M_{\rm r}$  20 kDa. The 2S albumins of oilseed rape (napins) also have a typical structure. The major napins have masses of  $\sim$ 14.5 kDa (subunit  $M_{\rm r}$  of  $\sim$ 9 kDa and 4 kDa) but minor forms of  $M_{\rm r}$   $\sim$ 12.5 kDa also occur. Napin processing includes the loss of N-terminal, C-terminal, and linker peptides, as discussed above.

Sunflower, a member of the family Compositae, contains 12S globulins (helianthinin) and 2S albumins in a ratio of  $\sim 2:1$ . The  $M_r$  300 kDa helianthinin comprises acidic chains of  $M_r$  32-34 kDa ( $\alpha$  and  $\alpha$ 'chains) linked to basic chains of  $M_r$  22–27 kDa ( $\beta$  chains). Sunflower 2S albumins comprise  $\sim 11$ 13 components with masses of 10-18 kDa. All are single chain proteins (i.e., they lack a proteolytic cleavage site between the putative large and small subunits), but most are synthesized as pairs, two albumins being released from a single precursor protein by proteolysis. One sunflower albumin which is synthesized from a separate precursor protein is called SFA8. This contains 16 methionine and 8 cysteine residues in a mature protein of 103 residues (i.e., a total of 23 mol.% of sulfur-containing amino acids). Safflower, a related member of the family Compositae, also appears to contain major groups of 12S globulin (called carmin) and 2S albumin storage proteins but little detailed work has been carried out.

Cottonseed is unusual among nonleguminous dicotyledonous species in containing 7S globulin storage proteins as well as 11S globulins and 2S albumins, with most of the knowledge being based on DNA-derived sequences with little direct analysis of the seed proteins. Linseed (and linola) has not been widely studied but the major seed protein is reported to be a 12.7S globulin of  $M_{\rm r} \sim 250-300\,{\rm kDa}$ . SDS-PAGE showed six nonidentical subunits.

#### **Storage Proteins of Pseudocereals**

Pseudocereals are not true cereals but three dicotyledonous crops that produce small grain-like seeds. Consequently, their seed proteins are similar to those of other dicotyledonous plants rather than related to cereal seed storage proteins.

Seeds of amaranth (*Amaranthus* spp., Amaranthaceae) contain 13S legumin-type globulins (amaranthin) and 8S vicilin-type globulins (conamaranthin). In addition, albumin proteins with  $S_{20,w}$  values of  $\sim$ 2 are present, with SDS-PAGE showing major bands of  $M_r$  9 kDa and 4 kDa. This is consistent

with the presence of typical 2S albumins. Two methionine-rich proteins with  $M_{\rm r} \sim 18\,{\rm kDa}$  have also been reported. Quinoa (*Chenopodium quinoa*, Chenopodiaceae) seeds also contain 11S globulins (chenopodin), of  $M_{\rm r} \sim 320\,{\rm kDa}$  and 2S albumins, the latter being rich in cysteine (15.6 mol.%) but low in methionine.

In buckwheat (*Fagopyrum esculentum*, Polygonaceae) the major 13S legumin-like globulin is located in the cotyledons with smaller amounts of putative 8S globulins in the perisperm. 2S storage albumins, which are rich in methionine (~9 mol.%), are also present.

#### **Conclusions**

The 2S albumins, 7S globulins, and 11S globulins are major groups of storage proteins that are widely distributed in seeds of dicotyledonous plants, although their relative amounts vary and other types of storage proteins also occur in some species. Consequently, they largely determine the nutritional quality and processing properties of the seeds. A detailed knowledge of their structures and properties is therefore essential in order to elucidate structure—function relationships. This will facilitate the optimization of seed quality for traditional and novel products with improvement using classical genetic or genetic engineering approaches.

See also: Amaranth. Beans. Buckwheat. Canola: Processing. Cereals: Protein Chemistry. Chickpea: Overview. Lupin: Overview. Oilseeds, Overview. Pea: Overview. Protein Synthesis and Deposition. Pseudocereals, Overview. Pulses, Overview. Quinoa. Soybean: Soy Concentrates and Isolates. Sunflower. Taxonomic Classification of Grain Species. Wheat: Grain Proteins and Flour Quality.

#### **Further Reading**

Bewley JD and Black M (1999) Seeds Physiology of Development and Germination. New York: Plenum.

Black M and Bewley JD (2000) Seed Technology and its Biological Basis. Sheffield: Academic Press.

Boulter D and Croy RRD (1997) The structure and biosynthesis of legume seed storage proteins: a biological solution to the storage of nitrogen in seeds. *Advances in Botanical Research* 27: 1–84.

Salunkhe DK, Chavan JK, Adsule RN, and Kadam SS (1992) World Oilseeds Chemistry, Technology, and Utilisation. New York: Van Nostrand Reinhold.

Shewry PR (1995) Plant storage proteins. *Biological Reviews* 70: 375–426.

Shewry PR and Casey R (eds.) (1999) *Seed Proteins*. Dordrecht: Kluwer.

Shewry PR, Jenkins J, Beaudoin F, and Mills ENC (2003)
The classification, functions and evolutionary relationships of plant proteins in relation to food allergens.
In: Mills ENC and Shewry PR (eds.) *Plant Food Allergens*. Oxford, UK: Blackwell Science.

### PROTEIN SYNTHESIS AND DEPOSITION

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#### Introduction

The protein content of seeds varies widely, from  $\sim 10\%$  of the dry weight in cereals to  $\sim 40\%$  in soybean. The number of individual protein components present is vast, but all seeds contain well-defined groups of storage proteins, which account for half or more of the total proteins. The storage proteins are largely responsible for the nutritional quality and processing properties of the grain and will, therefore, be the focus of this article.

Four types of storage proteins occur widely in seeds, which were initially defined based on their solubility properties and molecular masses measured by sedimentation equilibrium ultracentrifugation. The latter allows the calculation of sedimentation coefficients  $(S_{20,w})$  values) with higher values indicating greater mass. Alcohol-soluble prolamins form the major storage protein fraction in most cereal grain and two types of globulins (7S and 11S), the major fractions in most dicotyledonous species, including legumes and oilseeds. However, many of the latter also contain 2S albumin storage proteins. Similarly, all cereals also contain storage globulins, with proteins related to the 11S globulins of dicotyledonous plants forming the major storage protein group in oats and rice. The characteristics of these four types of proteins

Table 1 Summary of the characteristics of albumin, globulin, and prolamin storage proteins

2S albumins	Soluble in water Molecular mass ( $M_r$ ) typically $\sim 10-15$ kDa Processed post-translationally to give large and small subunits Two intrachain bonds within the large subunit and two interchain disulfide bonds Not glycosylated Specific components are rich in methionine
7S globulins	Soluble in dilute salt solutions Typically trimeric proteins of $M_r$ 150–190 kDa Subunit $M_r$ varies from $\sim$ 40 to 80 kDa but is typically $\sim$ 50 kDa Subunits may be proteolytically processed and glycosylated Contain little or no cysteine and methionine
11S globulins	Soluble in dilute salt solutions Typically hexameric proteins of $M_r$ 300–450 kDa. Subunits typically of $M_r$ 60 kDa are post-translationally processed to give $M_r$ 40 kDa (acidic) and $M_r$ 20 kDa (basic) chains associated by one interchain disulfide bond Low in cysteine and methionine
Prolamins	Vary widely in structure, with subunit $M_r$ ranging from $\sim$ 10–100 kDa Include monomeric forms and high $M_r$ polymers stabilized by interchain disulfide bonds Presence of repeated sequences and regions (domains) rich in specific amino acids results in unusual amino acid compositions Rich in proline and glutamine, poor in lysine and, in some cases, tryptophan, threonine, and methionine No glycosylation or proteolytic processing Soluble in alcohol/water mixture when native and/or reduced

Reproduced with permission from Shewry PR (2000) Seed proteins. In: Black M and Bewley JD (eds.) Seed Technology and Its Biological Basis, pp. 42–84. Sheffield: Blackwell Science and Sheffield Academic Press.

are summarized in Table 1 and the trivial names used for different species in Table 2. This article will focus on their synthesis and deposition.

# Synthesis, Folding, Processing, and Deposition of Seed Storage Protein

The vast majority of the proteins present in the mature cereal grain, including all of the major groups of storage proteins, are encoded by genes present on the chromosomes in the nucleus. Smaller genomes are also present in two organelles, the mitochondrion and plastid, but these do not encode any major seed proteins. Initiation of gene transcription is regulated by the interactions between promoter elements located upstream of the gene-coding regions and transcription factors, which are proteins that interact with the promoter elements and with the RNA polymerase enzyme that catalyzes the synthesis of messenger RNA (mRNA) from the DNA template in the gene. The mRNA is initially synthesized as a precursor form which is processed in the nucleus prior to being transported through the nuclear membrane to the cytoplasm for translation.

Protein synthesis involves the translation of the nucleotide sequence of the RNA (which is initially derived from that of the genomic DNA) into the amino acid sequence of the protein. This occurs on structures called ribosomes, which comprise complexes of ribosomal RNA and proteins. Transfer RNAs bearing

**Table 2** Types of major storage proteins present in seed crops and their trivial names

Species	Protein type	Trivial name
Cereals		
Wheat (Triticum spp.)	Prolamin	Gliadin/glutenin
Barley (Hordeum vulgare)	Prolamin	Hordein
Rye (Secale cereale)	Prolamin	Secalin
Corn (Zea mays)	Prolamin	Zein
Sorghum (Sorghum bicolor)	Prolamin	Kafirin
Oats (Avena sativa)	Prolamin 11S globulin	Avenin
Rice (Oryza sativa)	Prolamin 11S globulin	Glutelin
Legumes		
Soybean (Glycine max)	7S globulin	$\beta$ -Conglycinin
	11S globulin	Glycinin
Broad bean (Vicia faba)/	7S globulin	Vicilin
pea ( <i>Pisum sativum</i> )	11S globulin	Legumin
French bean ( <i>Phaseolus vulgaris</i> )	7S globulin	Phaseolin
Peanut (Arachis hypogaea)	7S globulin 11S globulin	Conarachin Arachin
Oilseeds	2S albumin	
Cottonseed	11S globulin	Gossypin
(Gossypium hirsutum)	7S globulin 2S albumin	Congossypin
Sunflower (Helianthus annuus)	11S globulin 2S albumin	Helianthinin
Oilseed rape/canola (Brassica napus)	11S globulin 2S albumin	Cruciferin Napin

Modified from Shewry PR (2003) Plant proteins. In: Thomas B, Murphy D, and Murray B (eds.) *Encyclopaedia of Applied Plant Science*, pp. 889–896. London, UK: Elsevier Science.

**Table 3** Summary of the major events in protein synthesis from nuclear genes

Genomic DNA in the chromosomes of the nucleus consists of a linear sequence of four nucleotides (bases). Individual genes comprise protein-coding sequences flanked by regulatory sequences. Coding sequences specify the amino acid sequences of proteins in series of three bases called codons. The correspondence of codons to amino acids is called the genetic code. Regulatory sequences control gene expression by interactions with regulatory proteins (transcription factors) and RNA polymerase enzymes.

Transcription of gene gives messenger (m) RNA with codon sequence reflecting that in the gene.

mRNA is processed in the nucleus and transported via nuclear membrane to cytoplasm.

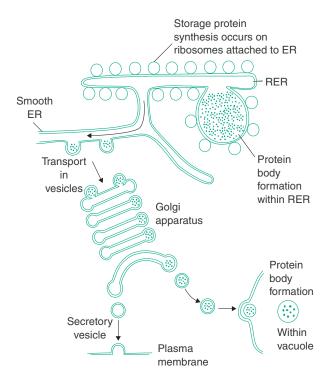
mRNA binds to ribosome in the cytoplasm. Transfer RNAs bearing amino acids recognise and bind to specific codons allowing the amino acids to be linked in a specific sequence by peptide bonds. Thus the sequence of codons determines the amino acid sequence of the protein.

Translation of a signal peptide directs the ribosome to the endoplasmic reticulum (ER) allowing translocation of the nascent protein into ER lumen.

amino acids and mRNAs are brought together on the ribosome resulting in the formation of peptide bonds between adjacent amino acids leading to the synthesis of the polypeptide chain.

Attachment of the mRNA to the ribosome occurs in the cytoplasm and the translated protein may subsequently be released into the cytoplasm. However, many proteins are synthesized with an N-terminal signal peptide, which directs them into the endomembrane system. This is because the translation of the signal peptide causes the ribosome to attach to the outer surface of the endoplasmic reticulum (ER), allowing the signal peptide to direct the nascent (newly synthesized) protein through the ER membrane into the lumen.

The major events in protein synthesis and the way that the sequence of nucleotides in the genomic DNA ultimately determines the sequence of amino acids in the protein, are summarized in Table 3. More detailed and highly readable accounts of the various stages of protein synthesis are given in Buchanan, Gruissem, and Jones (see Further Reading).



**Figure 1** Schematic summary of storage protein synthesis, processing, trafficking, and deposition in the endomembrane system of the cell.

### **Seed Storage Proteins Are Products of** the **Endomembrane System**

Seed storage proteins are present in the cells in dense protein bodies. These originate from the endomembrane system of the cell, which comprises the ER and structures derived from this, including the Golgi apparatus and various types of vesicle, which transport components to and from specific cellular destinations. The parts of the endomembrane system that are relevant to storage protein synthesis and deposition are shown schematically in Figure 1 while the processing events are summarized in Table 4.

It should be noted that trafficking of proteins through the endomembrane systems is a highly regulated process with the destinations of individual proteins being determined by the presence of specific sequences or structural features. In the absence of such signals, the protein is secreted from the cell, by fusion of "secretory vesicles" derived from the Golgi apparatus with the plasma membrane. Hence, secretion is often referred to as the default destination.

# Synthesis, Folding, and Disulfide Bond Formation Occur Simultaneously on the ER

Seed storage proteins are synthesized by mRNA associated with polyribosomes present on the rough ER.

	ER	Golgi apparatus	Vacuole
2S albumins	Folding, disulfide bond formation		Proteolytic processing
7S globulins	Folding, N-glycosylation, formation of trimers	Glycan modification	Glycan trimming, proteolytic processing
11S globulins	Folding, disulfide bond formation, formation of intermediate trimers		Proteolytic processing to give acidic and basic chains, formation of hexamers

Table 4 Processing of seed storage protein in the endomembrane system of the cell

Folding, disulfide bond formation,

polymer assembly

The protein translated from the mRNA differs from that deposited in protein bodies in the presence of a short N-terminal extension (the signal sequence which is usually  $\sim$ 20 amino acids) whose role is to lead the newly synthesized (nascent) polypeptide through the ER membrane into the lumen, thus entering the endomembrane system. This occurs by specific interaction of the signal sequence with a complex of proteins called the translocon. Once the nascent polypeptide emerges into the ER lumen the signal sequence is removed by a specific enzyme (a signal peptidase) and the polypeptide chain commences to fold into its three-dimensional structure. These events, translocation, signal peptide cleavage and folding, occur when the protein is still undergoing synthesis and hence are termed cotranslational. Protein folding is almost certainly assisted by a complex of proteins and other factors, the most well known of which is the "molecular chaperone" BiP (binding protein). In addition to assisting protein folding, BiP also binds to malfolded proteins to prevent their escape from the ER. Thus, it is often present in higher amounts in mutants in which seed protein synthesis is affected (e.g., floury-2 and defective endosperm -B30 of maize) as well as during the period of maximum storage protein synthesis and deposition.

**Prolamins** 

Most proteins synthesized on the ER also contain interchain and/or intrachain disulfide bonds. These bonds also form in the ER as an integral part of protein folding and this may be assisted by the enzyme protein disulfide isomerase (PDI) which catalyzes the formation, exchange or reduction of disulfide bonds, depending on the substrate and conditions.

# **Storage Albumins and Globulins Are Deposited** in Storage Vacuoles

The 2S albumin and 7S and 11S globulin storage proteins are transported via the ER lumen, Golgi apparatus, and vesicles to specialized storage vacuoles where they form dense deposits. These vacuoles may subsequently divide to form protein bodies. However, all three types of storage protein may also undergo modification, either in the vacuole or

during their passage through the ER and Golgi apparatus.

The 2S albumins are synthesized as precursor proteins, which undergo folding and disulfide bond formation in the ER lumen. The precursors are then proteolytically processed in the vacuole to generate the mature two-subunit structure. This processing involves cleavage adjacent to specific asparagine residues and the loss of one or more short peptide fragments (from the protein N-terminus, C-terminus, and between the two subunits).

The 7S and 11S globulin subunits are also folded in the ER lumen with the formation of a single intrachain disulfide bond in the latter (7S globulins lack cysteine residues and hence have no disulfide bonds). Both types of subunits are then assembled into trimeric structures stabilized by noncovalent forces. There has been considerable debate about the extent to which globulin storage proteins are modified by the addition of oligosaccharides (glycosylation). It is now established that this rarely, if ever, occurs for 11S globulins but that some 7S globulin subunits are modified by glycosylation of specific asparagine residues (N-glycosylation). This occurs in several stages, with the initial attachment of a high mannose sugar occurring cotranslationally (i.e., concurrent with folding and disulfide bond formation). Subsequently, enzymes present in the ER and Golgi apparatus may act to remove or add further sugar residues to give a more complex structure with final "trimming" occurring within the storage vacuole. Once within the vacuole, the 11S globulins are proteolytically cleaved at an internal asparagine residue to give acidic and basic chains that remain associated by the single disulfide bond formed in the ER. After this proteolytic processing, the "intermediate timers" formed in the ER are able to assemble to form the mature hexameric structure.

The 7S globulin subunits may also be proteolytically processed in the vacuole, but this depends on the species and the precise protein subunit. For example, in bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) the 7S globulin subunits do not undergo any proteolysis, while in pea (*Pisum sativum*) some

subunits are processed at one or two sites to give polypeptide chains ranging in mass from  $\sim$ 12 to 35 kDa, in addition to unprocessed chains of mass  $\sim$ 50 kDa. However, the trimeric structure is not affected by these processing events.

#### Cereal Prolamins Are Deposited in Two Types of Protein Body

Cereal prolamins do not undergo glycosylation or proteolytic processing (except for cotranslational removal of the signal sequence), but some subunits are assembled into high molecular mass polymers stabilized by interchain disulfide bonds. In wheat, these glutenin polymers may have masses of between 1 and 10 MDa and it is not known how such complex multisubunit structures are assembled. However, it is probable that assembly and interchain disulfide bond formation occur rapidly after synthesis within the ER lumen. Subsequently, prolamins are deposited in two discrete types of protein body.

In rice and maize (and probably also in sorghum and millets), prolamins accumulate directly within the lumen of the ER to form a population of protein bodies surrounded by a membrane of ER origin which may remain studded with ribosomes. In contrast, it appears that two separate pathways occur in wheat, barley, and rye, with some prolamins being trafficked through the ER and Golgi apparatus and deposited in storage vacuoles (as described above for albumin and globulin storage proteins) while others are retained in the ER as in maize and rice. It has been suggested that the polymeric prolamins are preferentially retained in the ER and monomeric forms transported to vacuoles, but it seems likely that the division is not so clear-cut. It is also probable that the relative amounts of prolamins deposited in the two types of protein body vary with the rate of protein synthesis and with the developmental state of the tissue. Thus, it can be envisaged that high levels of protein synthesis would lead to a high level of accumulation within the ER lumen and that this route would also be favored as the cells become distended with deposits of starch and proteins. In wheat, the individual protein bodies collapse during the later stages of seed maturation forming a continuous matrix in the cells of the mature grain.

# What Determines the Destination of Storage Proteins in the Endomembrane System?

As discussed above, trafficking through the endomembrane system is a highly regulated process with the destinations of proteins being determined by their sequences and structures.

The retention of storage proteins within the ER or their transport to the vacuole should, therefore, result from the presence of specific signals. A number of proteins are permanently resident in the lumen of the ER, including PDI and BiP. In these cases, retention results from the presence of a specific ER retention signal, which is a tetrapeptide located at the protein C-terminus. The two most widespread tetrapeptides are KDEL and HDEL, which correspond to the amino acids, lysine or histidine, aspartic acid, glutamic acid, and leucine. There is no evidence that such a retention signal is present at the C-termini of prolamins that are retained in the ER and a different mechanism must therefore be sought. It has been suggested that BiP plays a role in rice, by transiently binding to prolamins and retaining them in the ER to allow assembly into protein bodies. However, this mechanism has not so far been shown to be applicable to other cereals. An alternative explanation is that retention results from the insolubility of the proteins in aqueous environments (i.e., the ER lumen) and their propensity to form aggregates under such conditions. It can be envisaged that such aggregates would accumulate directly in the ER lumen and then be "budded off" to form protein bodies.

Some proteins are targeted to the vacuole by the presence of specific "prosequences" which are removed by post-translational proteolysis once the protein arrives in the vacuole. These prosequences may be at the protein N-terminus, as in the barley cysteine proteinase aleurain and the sweet potato storage protein sporamin (which is stored in tubers) or at the C-terminus as in lectins. None of the 2S albumins, 7S globulins, 11S globulins or prolamins that are deposited in vacuolar protein bodies has cleavable prosequences that are involved in vacuolar targeting. However, it has been proposed that aggregation of 7S and 11S globulins into electron-dense aggregates in the Golgi is a prerequisite for ensuring that they are sorted into dense vesicles and ultimately deposited into the vacuole, whereas other proteins are sorted into different types of vesicles with different destinations. This sorting may require the presence of specific hydrophobic residues, which mediate the proteinprotein interactions. However, the precise mechanisms that determine the final destination of seed storage proteins, whether to ER-derived or vacuolar protein bodies, are still poorly understood.

#### Organization of Storage Proteins in Protein Bodies

The 7S and 11S globulins have similar three-dimensional structures with the 11S hexamers essentially corresponding to dimers of the trimeric 7S

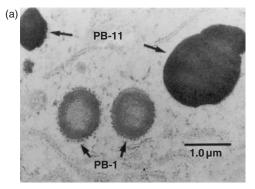
proteins. This similarity presumably facilitates their efficient packaging in the same protein bodies. However, protein bodies may also contain separate phases, or inclusions, containing specific proteins or other components. For example, the protein bodies of castor bean consist of a matrix of 2S albumins and other proteins (including lectins) with crystalline inclusions of 11S globulins and noncrystalline globoid inclusions consisting of phytin (calcium and magnesium salts of myoinositol hexaphosphoric acid). Phytin globoids are also present in protein bodies of other dicotyledonous seeds and in aleurone cell protein bodies of cereals. The different routes, taken by prolamins and glutelins (11S globulin homologues) in rice, lead to the formation of two separate populations of protein bodies called PB-I (ER-derived containing prolamins) and PB-II (vacuolar-derived containing glutelins). However, in wheat and oats these two types of protein are located in the same protein body, with prolamin inclusions in a globulin matrix in oats and vice versa in wheat (Figure 2). This segregation has been suggested to result in oats from the fusion of ER-derived protein bodies containing prolamins with vacuolar protein bodies containing globulins.

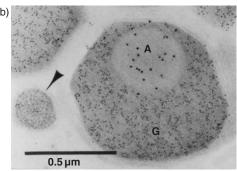
It has also been shown that different types of prolamin (zein) are concentrated in the outer and inner parts of protein bodies of maize, although in this case the location appears to result from the pattern of development of the bodies in the endosperm cells.

### Regulation of Storage Protein Synthesis

#### **Tissue Specificity**

Storage protein synthesis in seeds is strictly regulated with respect to timing, tissue specificity and, to a lesser extent, nutrient availability. In addition to the embryo, which is a diploid zygotic tissue that gives rise to the seedling, all seeds also contain an endosperm, which is triploid and is derived from a second fertilization event. However, the size of the endosperm varies greatly, forming 80–90% of the mature cereal grain but being largely broken down during development in soybean and many other seeds. Similarly, whereas some seeds such as quinoa and amaranth contain a perisperm (derived from the maternal nucellar tissue), this fails to develop and is absorbed in most species. In all seeds, at least one of these three tissues functions as a storage tissue, and in some cases more than one. The central part of the endosperm is the major storage tissue in cereals, accumulating protein (prolamins with varying amounts of 11S-type globulins) and starch. However, 7S globulins and oil are also stored in the outer cells of the endosperm





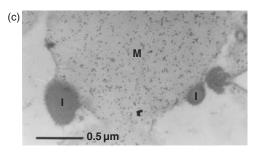


Figure 2 Protein bodies in developing starchy endosperm cells of cereals. (a) Rice at 7 d after flowering, showing two populations of protein bodies. PB-I are spherical vesicles delimited by a single unit membrane derived from the ER and contain prolamins. PB-II are amorphous, derived from vacuolar deposition and contain globulins/glutelins. (b) Oats at 8 d after anthesis showing lightstaining deposits of prolamin (labeled with 10 nm colloidal gold) as inclusions in protein bodies containing globulins (5 nm colloidal gold). (c) Wheat at 11 d after flowering showing inclusions of triticin (dark staining, labeled I) within a matrix of prolamin (M). The bars are 1.0 µm in (a) and 0.5 µm in (b) and (c). Part (a) is reproduced with permission from Yamagata H and Tanaka K (1986). The site of synthesis and accumulation of rice storage proteins. Plant and Cell Physiology 27: 135-145. Part (b) is reproduced with permission from Lending CR, Chesnut RS, Shaw KL, and Larkins BA (1989) Immunolocalization of avenin and globulin storage proteins in developing endosperm of Avena sativa L. Planta 178: 315-324. Part (c) is reproduced with permission from Bechtel D, Wilson JD, and Shewry PR (1991) Immunocytochemical localization of the wheat storage protein triticin in developing endosperm tissue. Cereal Chemistry 68: 573-577.

(the aleurone layer) and the single cotyledon (scutellum) of the embryo. The cotyledons of the embryo form the major storage tissue in grain legumes (including soybean, peanut, lupin, and pea), sunflower and oilseed rape/canola while proteins and oil are stored in the endosperm and embryo of quinoa with starch being stored in the perisperm.

It should also be noted that the synthesis of seed storage proteins has never been detected in nonseed tissues of the plant, whether by determining gene expression (i.e., mRNA populations) or protein accumulation.

#### **Temporal Regulation**

Seed development proceeds through a series of genetically programmed stages, starting with cell division and differentiation to establish the basic structure of the component organs and tissues (i.e., the embryo, endosperm etc.). The synthesis and accumulation of storage products, including proteins, occurs during the subsequent expansion of the cells of the storage tissue(s) and ceases when these cells mature and desiccate. The onset and duration of the phases varies widely between species and genotypes and is greatly affected by the environmental conditions. In small grain cereals (wheat, barley) grown in the UK, storage protein synthesis is usually first detected in appreciable amounts at  $\sim 12-14$  days after pollination and is most active over the next 2-3 weeks. However, synthesis may be initiated as early as 6 days in plants grown in hot, dry climates. In maize (corn) grown in North America, storage protein synthesis occurs between  $\sim$ 3 and 7 weeks after pollination.

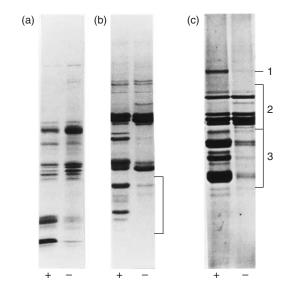
Storage protein fractions are, of course, not single proteins but mixtures of components, which may be encoded by several multigene families. In general, these genes show highly coordinated patterns of expression, with only small differences in their timing and relative levels of expression during development. For example, in sunflower the expression of genes for 11S globulin storage proteins (helianthinins) is detected slightly before that of genes for 2S albumins, but in wheat and the barley transcripts for all groups of prolamin storage proteins show similar expression patterns.

#### **Environmental Control**

Environment has a major impact on seed development, including storage protein synthesis, with higher temperatures generally increasing the rate of development. In addition, specific heat-shock effects may occur when temperatures exceed  ${\sim}35^{\circ}\mathrm{C}$  – these can result in detrimental effects on the quality of wheat grown in Australia and some other parts of the world. The most dramatic effect of environment on storage protein synthesis is that of nutrition. It is well established that the protein content of seeds is regulated by the availability of nitrogen, with storage

protein acting as a sink for nitrogen, which is not required by the plant for other purposes. For example, increasing the total nitrogen content of barley grain from  $\sim 1\%$  to 3% is associated with an increase in prolamin (hordein) storage proteins from  $\sim 35\%$  to 50% of the total grain nitrogen. This effect can be exploited to grow grain with defined protein contents for specific end uses (e.g., low protein for malting barley, high protein for bread-making wheat).

However, almost all storage proteins also contain sulfur in the form of the amino acids cysteine and methionine. Many species are able to adjust to variation in the relative availabilities of nitrogen and sulfur by having specific types of seed storage proteins with high and low contents of sulfur amino acids. Thus, the 2S albumin storage proteins of oilseed rape and sunflower have higher contents of cysteine and methionine than the globulin storage proteins and the synthesis of the former is therefore reduced under conditions of low sulfur availability (Figures 3a and 3b). Similarly, restricted availability of sulfur favors the synthesis of the sulfur poor C hordeins of barley (Figure 3c) and  $\omega$ -gliadins of wheat over other more sulfur-rich prolamins, which can have adverse consequences for grain quality in the latter species.



**Figure 3** The effect of sulfur on the total seed proteins of oilseed rape (a) and sunflower (b), and on the prolamin (hordein) proteins of barley (c). Note the presence of S-rich low molecular mass proteins (probably 2S albumins) in oilseed rape and sunflower (bracket) and of S-poor C hordeins (2) in barley. Parts (a) and (b) are reproduced with permission from Spencer D, Rerie WG, Randall PJ, and Higgins TJV (1990). The regulation of pea seed storage protein genes by sulfur. *Australian Journal of Plant Physiology* 17: 355–363. Part (c) is reproduced with permission from Shewry PR, Franklin J, Parmer S, Smith SJ, and Miflin BJ (1983) The effects of sulfur starvation on the amino acid and protein compositions of barley grain. *Journal of Cereal Science* 1: 21–31.

#### **Regulation of Storage Protein Gene Expression**

The regulation of seed storage protein genes usually occurs at the level of gene transcription, although some "fine tuning" can occur at the level of translation of the mRNA into proteins. Consequently, interest has focused on the identification of regulatory sequences present upstream of the gene sequences that encode storage proteins (called 5' upstream sequences) and of specific proteins that bind to these (transcription factors). This has led to the identification of short conserved nucleotide sequences (often called boxes) that regulate expression of the genes.

Comparison of the 5' upstream sequences of a number of genes encoding 11S storage globulins has led to the identification of a 28 base pair (bp) "legumin box." Deletion of a central 7-nucleotide motif from the center of this box resulted in a drastic reduction in the gene expression in transgenic plants, demonstrating its importance. Related "boxes" are also present in the 5' upstream sequences of 7S globulin and 2S albumin genes. However, further studies have demonstrated the presence of several additional upstream regulatory sequences that may modulate storage globulin gene expression.

Similar studies have shown that many prolamin genes also contain a conserved sequence of  $\sim 30 \,\mathrm{bp}$ located about 300 bp upstream of the gene-coding region. This is called the "endosperm box" or "prolamin box" and is unrelated in sequence to the "legumin box." The "prolamin" box actually comprises two conserved sequences separated by a more variable region. One of these conserved sequences is thought to be responsible for conferring endosperm-specificity while the other may play a role in the regulation of the gene. However, additional regulatory sequences are undoubtedly also present including a short sequence (the E motif) in the  $\alpha$ -zein genes of maize, which binds the protein encoded by the Opaque 2 locus. Mutation of this locus results in reduced  $\alpha$ -zein synthesis and a "high lysine" phenotype. Although several other proteins that bind to regulatory sequences of storage protein genes have also been identified, the precise mechanisms of storage protein gene regulation remain poorly understood.

#### **Conclusions**

It is clear that we still have much to learn about the precise mechanisms of storage protein synthesis, trafficking, and deposition, although the broad principles are now understood. A detailed understanding of these mechanisms is important as interactions (i.e., between proteins and with other grain components) established during synthesis and deposition may

ultimately affect the functional properties of the proteins in food products. Furthermore, it is becoming clear that the synthesis, assembly, deposition, and interactions of seed proteins may be affected by environmental factors that ultimately impact on seed quality. Identifying the major sites of these effects and the critical stages of seed development should allow them to be predicted and ultimately manipulated give a higher level of stability.

It is also important to understand the mechanisms of seed protein synthesis, trafficking, and deposition in order to facilitate the application of genetic engineering to improve grain quality. It is essential that any novel or mutant proteins expressed in transgenic plants should be "accepted" by the secretory system as authentic and transported to their correct destination. Any errors in folding or failure to contain the correct targeting information could lead to protein turnover or accumulation in incorrect compartments (e.g., ER instead of storage vacuoles in dicotyledonous seeds) with adverse effects on grain development, quality, and yield.

See also: Cereals: Grain-Quality Attributes; Protein Chemistry. Protein Chemistry of Dicotyledonous Grains.

#### **Further Reading**

Bechtel D, Wilson JD, and Shewry PR (1991) Immunocytochemical localization of the wheat storage protein triticin in developing endosperm tissue. *Cereal Chemistry* 68: 573–577.

Bewley JD and Black M (1995) Seeds Physiology of Development and Germination, 2nd edn. New York: Plenum Press.

Buchanan BB, Gruissem W, and Jones RL (2000) Biochemistry and Molecular Biology of Plants. Rockville, MD: American Society of Plant Physiologists.

Larkins BA and Vasil IK (1997) Cellular and Molecular Biology of Plant Seed Development. Dordrecht: Kluwer Academic.

Lending CR, Chesnut RS, Shaw KL, and Larkins BA (1989) Immunolocalization of avenin and globulin storage proteins in developing endosperm of *Avena sativa L. Planta* 178: 315–324.

Muntz K and Shutov AD (2002) Legumains and their functions in plants. *Trends in Plant Science* 7: 340–344.

Shewry PR (2000) Seed proteins. In: Black M and Bewley JD (eds.) *Seed Technology and its Biological Basis*, pp. 42–84. Sheffield: Blackwell Science and Sheffield Academic Press.

Shewry PR (2003) Plant Proteins. In: Thomas B, Murphy D, and Murray B (eds.) *Encyclopaedia of Applied Plant Science*, pp. 889–896. London: Elsevier Science.

Shewry PR and Casey R (1999) *Seed Proteins*. Dordrecht: Kluwer Academic.

Shewry PR and Halford NG (2002) Cereal seed storage proteins: structures, properties and role in grain utilization. *Journal of Experimental Botany* 53: 947–958. Shewry PR, Franklin J, Parmer S, Smith SJ, and Miflin BJ (1983) The effects of sulphur starvation on the amino acid and protein compositions of barley grain. *Journal of Cereal Science* 1: 21–31.

Spencer D, Rerie WG, Randall PJ, and Higgins TJV (1990) The regulation of pea seed storage protein genes by sulphur. *Australian Journal of Plant Physiology* 17: 355–363.

Yamagata H and Tanaka K (1986) The site of synthesis and accumulation of rice storage proteins. *Plant and Cell Physiology* 27: 135–145.

#### **PROTEOMICS**

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#### Introduction

Elucidation of grain composition at the molecular level is needed to permit ongoing genetic improvements to grains with respect to their efficient production and processing. This information is especially needed concerning the proteins of the grain, because they are the primary molecules in implementing the "directions" from the relevant genes, under the further control of environmental conditions. There is thus an important interaction between genotype (the variety, the set of genes) and growth conditions (Figure 1), and this interaction determines the synthesis of the individual proteins that are needed to conduct all the functions of the plant cells.

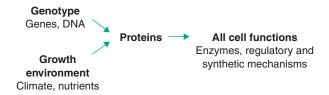
#### **Background to Proteomics**

The study of proteins and their functions has long been an accent of biochemical research, but it has been only in the 1990s that the concept of "proteomics" has been introduced to this study. Proteomics is a powerful approach to the analysis of gene expression in biological systems, with the term "proteome" indicating the full complement of proteins expressed by the genome of an organism (e.g., wheat) in a tissue (e.g., leaf, endosperm, or embryo) at a particular stage of development and under specific growth conditions. Studies of the proteome and the genome complement each other (see Genomics). The genotype is defined by the complete set of genes that

constitute the genome of an organism (a grainproducing plant for current discussions). Although the complete genome is present in every cell of the plant, only those genes appropriate to a specific tissue are active there, e.g., in the roots or in the leaves. The specific genes that are active are also determined by the growth conditions, as shown in Figure 1.

The first activity of the genes is transcription, i.e., the production of messenger ribonucleic acid (mRNA), with a nucleotide sequence based on that of the stretch of DNA that constitutes the particular gene. Analysis of the mRNA (the "transcriptome") provides information about which proteins are to be synthesized in the next stage of translation (see Protein Synthesis and Deposition), as shown in Figure 2. Routinely, analysis of the proteins (the proteome) is preferred over study of the mRNA, because the mRNA molecules are transitory, being degraded after their role in protein synthesis is completed. Therefore, the mRNA transcripts may not be a true indicator of protein expression, and often do not correlate with protein levels.

The transcriptome and the proteome are thus subsets of the wider range of mRNA and protein molecules that might have been produced, given the full extent of the genome. In summary, study of the proteome tells us about the range of proteins that relate to the specific tissue under study (root or shoot or mature grain), as it was grown under the particular



**Figure 1** The interaction of genotype and growth conditions to affect the protein composition.

environmental conditions existing at the time for the genotype involved.

#### **Methods of Proteome Analysis**

The various aspects comprising proteome technology are illustrated in Figure 3 – including, the preparation and extraction of proteins, the fractionation of proteins using the "core" separation technique of two-dimensional gel electrophoresis (2-DE), image analysis and subsequent characterization, and identification of proteins using micro-analytical techniques combined with protein-sequence database interrogation.

Figure 4 provides an example of a proteome map. This shows the proteome of the floury endosperm of Wyuna immature wheat grain (mid-development, 17 days postanthesis; DPA), obtained by extracting the proteins from the sample of grain endosperm, fractionating them in the first dimension according to their isoelectric points in two parts. The seconddimension fractionation resolved the proteins according to their sizes (largest at the top). Each spot represents a single protein (polypeptide). Numbered spots have been cut out of the second-dimension gel, eluted from the gel piece, and analyzed to characterize their N-terminal amino-acid sequence, which was then used to interrogate protein sequence databases to determine their likely identity. Table 1 shows the results of identifying some of the protein spots. Figure 4 and Table 1 illustrate the three main steps of proteome analysis, namely, sample preparation, protein fractionation, and component characterization and identification.

#### **Preparing Samples for Two-Dimensional Electrophoresis**

Sample preparation is critical in obtaining reproducible and high-resolution gels. Prior to the extraction and solubilization of proteins, tissues are often treated for the removal of cell components, which can exert deleterious effects during extraction (such as salts, lipids, organic acids, phenolic compounds, and pigments). An example of this treatment is the precipitation of proteins from plant tissues using trichloroacetic acid (TCA) and acetone, resulting in the removal of most of these undesirable components.

Proteome studies of wheat have involved the extraction and solubilization of proteins in a buffer consisting of 7 M urea, 2 M thiourea, 2 mM trybutyl phosphine (TBP), 4% 3-((3-cholamidopropyl)dimethylammonio)-1-propane sulfonate (CHAPS), 1% carrier ampholytes, 40 mM tris, and a trace amount of Bromophenol blue dye. The urea and

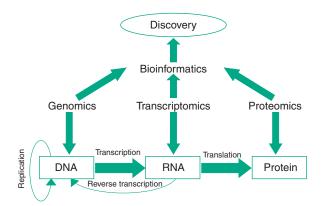


Figure 2 Newly emerging technologies to elucidate genome and proteome interactions.

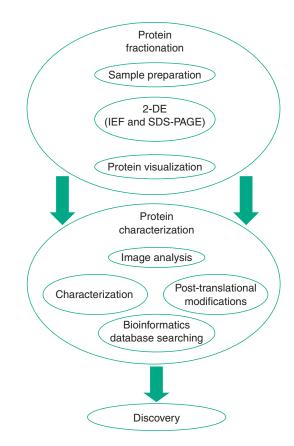
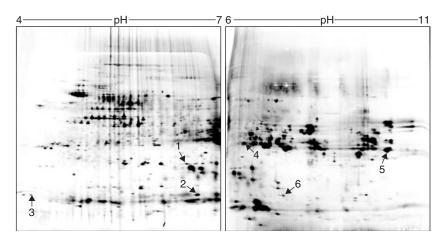


Figure 3 The various aspects of proteome technology, from the fractionation of proteins, through to characterization and identification, and ultimately discovery. (Reproduced with permission from Walsh BJ, Molloy MP, and Williams KL (1998) The Australian Proteome Analysis Facility (APAF): assembling large scale proteomics through integration and automation. Electrophoresis 19: 1883-1890. See http://www.publish.csiro.au/nid/40.htm for more details.)

thiourea are chaotropic agents, denaturing the protein molecules. The zwitterion detergent (CHAPS), combined with the chaotropic agents, aids in the solubilization of proteins. The reducing agent TBP



**Figure 4** The acidic and basic proteome maps of immature (17 DPA) wheat-grain endosperm of the soft Wyuna variety. Protein spots were characterized by N-terminal amino acid sequencing and identified from protein-sequence database interrogation. Protein identities are described in **Table 1**.

Table 1 N-terminal amino acid sequence characterization of immature wheat endosperm proteins

Spot no.	N-terminal sequence	Gene product	Identity (%)	Matched organism	Comments and accession no.
1	XATFTLPD	Superoxide dismutase (EC 1.15.1.1)	87.5% in 8 aa	Wheat	N-terminal sequence starts at residue 22 of O82571 with residues 1–21 possibly being a signal sequence or truncated. Also matches to P93606 and Q96185
2	SGPWMCYV	α-Amylase inhibitor WDAI-3	100% in 7 aa	Wheat	P10846 (fragment). Also matches other α-amylase inhibitors P01084 and P01085
3	MKLIAAYL	60 S acidic ribosomal protein P2 (ribosomal protein "A")	100% in 8 aa	Wheat	Subunits P1 and P2 exist as dimers at the large ribosomal subunit P05390
4, 5	NMQVDPSG	γ-Gliadin	100% in 8 aa	Wheat	N-terminal sequence starts at residue 20 of P21292 as signal sequence of precursor is residues 1–19
6	DILRSDQP				No close matches

Protein sequences were used to interrogate SWISS-PROT and TrEMBL databases via FASTA algorithm.

is noncharged and breaks the disulfide bonds existing in many proteins, producing individual polypeptides. TBP does not migrate out of the first-dimension immobilized pH gradient (IPG) strip during the course of isoelectric focusing (IEF), allowing the proteins to remain in their reduced state as separate polypeptides. The trace amount of Bromophenol blue dye is used as an indicator of current during IEF, migrating off the IPG strip due to the negative charge of the dye.

#### **Two-Dimensional Gel Electrophoresis**

The resulting protein extracts are fractionated to provide a 2-DE protein map. The first dimension involves IEF, in which proteins are fractionated across a specific pH range using commercially available IPG strips. Proteins in the IPG strip migrate and resolve to the point at which they have a zero net charge, which

is known as their isoelectric point (pI). The second-dimension fractionation resolves the proteins on the basis of molecular mass, using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The SDS detergent binds to the denatured proteins, overriding any intrinsic charge differences, to provide a uniform charge-to-mass ratio, allowing proteins to resolve according to their respective sizes.

The 2-DE proteome map in Figure 4 represents the proteins of immature wheat grain flour. It shows the polypeptides extracted from the immature endosperm of the soft wheat variety "Wyuna," the endosperm being the floury part of the grain, excluding the germ, embryo, and bran layers (*see* Grain and Plants, Morphology). The proteome map of Wyuna appears in two parts, reflecting two different pH ranges used in IEF. Protein extracts were fractionated in both acidic (pH 4–7) and basic ranges (pH 6–11). The

proteome pattern is thus a map of isoelectric point (pI), in the horizontal dimension increasing from left to right, and of molecular size in the vertical dimension. SDS-PAGE provides fractionation on the general basis of the size of the protein molecules, from largest (top) to smallest (bottom). After 2-DE fractionation, the proteins in the gels must be visualized. There are various stains available for protein visualization, including "silver staining," but the more common and routinely used stains now are fluorescent dyes, such as SYPRO Ruby protein stain. This stain is commercially available, ready-to-use, ultrasensitive and compatible with subsequent protein characterization methods.

The two proteome maps in Figure 4 show that a large number of polypeptide spots has been resolved. About 1300 protein spots can be counted using specialized image analysis software. There are many more that are faint or difficult to discern with certainty, due to their low concentration or poorer resolution, or because of the presence of overlapping components. The number of proteins actually visualized also depends on the affinity of the individual proteins for the dye used.

#### Characterization and Identification of Protein Spots

This procedure of 2-DE has been available for some decades. The relatively novel aspect of proteome analysis is the subsequent stages of postseparation microcharacterization of the individual protein spots. The ability to characterize and identify proteins in a high-throughput manner is a critical factor in proteome projects. Two widely used protein-characterization methods are N-terminal amino acid sequencing and mass spectrometry (MS), with the latter being the preferred method of choice used in proteome projects.

N-terminal amino-acid sequencing has been a popular method for characterizing proteins in the past. The most common form of this method is the traditional Edman degradation chemistry, which can be used to sequence proteins from gels, or it can be carried out on proteins electroblotted from the proteome gel to a polyvinylidene difluoride (PVDF) membrane. In either case, Edman chemistry involves the reaction of phenylisothiocyanate with the free amino group of the protein; derivatives are cleaved, then converted to phenylthiohydantoins, which differ in their amino-acid side chain, allowing them to be characterized by retention time using reversed-phased high-performance liquid chromatography. This is not the preferred method in high-throughput largescale proteome projects, due to the relatively low number of samples that can be processed in parallel (generally only three or four samples), its low sensitivity, and the time and cost for analysis. This method of characterization is useful when studying proteins from poorly defined genomes. Examples of proteins characterized and identified by N-terminal sequencing and protein-sequence database interrogation are provided in Table 1.

Due to the recent emphasis on high-throughput protein characterization, traditional amino-acid sequencing has been superseded by mass spectrometry (MS). Generally, MS can be used to (1) determine the composition of the polypeptide analyte (when run in MS mode), by measuring the mass values of peptides generated by enzymatic digestion of the protein from the gel, and (2) determine the primary structure of selected peptides, by fragmenting a selected peptide in order to deduce the amino-acid sequence, a technique known as tandem MS (MS/MS). When running in the MS mode, two particular techniques are commonly used for protein characterization. The first technique is matrix-assisted laser desorption/ ionization (MALDI) MS, and the second is electrospray ionization (ESI) MS. In the MALDI-MS method, ions are generated from solid-phase samples in a high vacuum by a short laser pulse. These ions are accelerated by an electric field into a time-of-flight (TOF) analyzer, giving rise to the term MALDI-TOF MS.

In this technique, the flight time of an analyte is proportional to the mass-to-charge ratio. Therefore, the mass-to-charge ratio of an unknown analyte can be deduced by calibrating the instrument with an analyte with known mass. In the case for ESI-MS, the ions are generated from a liquid phase, which is thought to occur by residual solvent evaporation or field desorption. In this technique, the mass analyzer is usually an ion trap, quadrupole or an ion cyclotron analyzer. Both MALDI and ESI are the preferred methods for the ionization of peptides and proteins. When running MS/MS mode, the analyte is measured in terms of mass, and then selected for fragmentation, usually by collision-induced dissociation (CID) within the mass spectrometer. This method produces primary structure information, which can then be used to identify the protein of interest from proteinsequence databases.

Routine protein identification in high-throughput proteomic projects is usually accomplished using MALDI-TOF MS peptide-mass fingerprinting (PMF), which involves the enzymatic digestion of proteins, giving rise to a unique subset of peptide masses. This can be achieved using a range of enzymes, the most common being trypsin, cleaving specifically at the C-terminal side of lysine and arginine amino-acid residues. The generated subset of polypeptides can

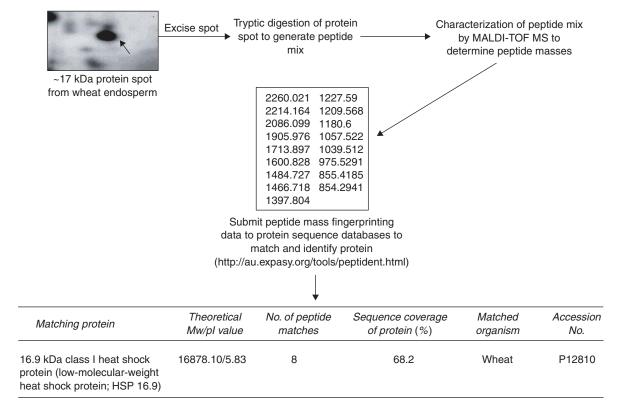


Figure 5 An example of protein characterization and identification using peptide mass fingerprinting.

then be used to interrogate theoretically derived peptide-mass fingerprints of proteins in protein-sequence databases. If the protein is not present in the databases, or the PMF data are not sufficient for an unambiguous identification, tandem MS is used to generate amino-acid sequence information to search protein databases. Furthermore, MS strategies have been developed to detect post-translational modifications of proteins, such as glycosylation and phosphorylation of proteins. An example of protein characterization and identification using PMF is described in Figure 5.

# The Potential Value of Proteomics to Grain Science

The great promise of proteome analysis is that we can identify the individual proteins that are responsible for specific aspects of the phenotype, i.e., the many different characteristics of the plant, and of the grain produced from it. Take the example of a plant breeder who wishes to select for genotypes that have resistance to a specific pathogen, e.g., the rust fungus that attacks the plant, thereby reducing yield of grain. Conventionally, the breeder grows up a large number of genotypes as plants, which must be inoculated with the rust spores. Those that demonstrate

resistance are selected. This process is time- and labor consuming. If, on the other hand, we knew the specific proteins that are responsible for conferring rust resistance, analysis of protein composition might be used to identify the resistant genotypes much more quickly and efficiently. To achieve this outcome, proteome analysis might be applied to the leaf proteins of a set of genotypes of defined resistance or susceptibility, searching for specific proteins that might serve as markers of resistance. The added value of this approach would be to use the information in selecting suitable parent lines for ongoing breeding. The approach would also be integrated with complementary studies at the genome level, searching to identify the genes responsible for the synthesis of the marker proteins. Some of these concepts are ideas that have not yet achieved reality, whereas there are many cases where proteome technology has already proved valuable.

### Relationship between Proteome and Transcriptome Results

In one particular study, the relationship between the wheat grain endosperm transcriptome (mRNA transcripts) and the proteome (proteins) was investigated. In this study, total RNA extracted from grain, harvested at 4, 6, 8, 10, 12, 15, 18, 21, and 25

days after flowering (days post-anthesis, DPA), was analyzed, after size separation and transfer to nylon membrane, to determine the timing of mRNA transcription from a range of genes during seed development. In particular, the accumulation of the high-molecular-weight glutenin subunit mRNA transcripts and gliadin mRNA transcripts was investigated (see Cereals: Protein Chemistry). This analysis indicated that significant levels of mRNA from seed storage proteins do not accumulate until after 12 DPA. Gene expression was also investigated for a number of enzymes associated with starch and protein biosynthesis. As an example of this, protein disulfide isomerase, an enzyme involved in protein folding, showed significant transcription as early as 6 DPA, with transcript levels declining significantly by 18 DPA.

As a result of these analyses, a cDNA library was constructed from mRNA isolated from endosperm tissue at 8, 10, and 12 DPA. The total number of active genes in the 8-12 DPA endosperm tissue was estimated. The minimum and maximum limits from these analyses were 4500 and 8000 genes being active. Thus, the average number of different proteins being expressed in the endosperm tissue was 6250. By contrast, proteome analysis of Wyuna endosperm tissue at this same stage of development showed that  $\sim$ 1700 protein spots could be detected from 2-DE gels. These findings suggest that  $\sim 27\%$  of the genes expressed in the wheat endosperm could be detected from 2-DE proteome gels. Many reasons may account for the fact that only a portion of the expressed genes could be visualized on 2-DE gels. These include the poor extractability of the more hydrophobic proteins (such as membrane proteins), the fact that some proteins can remain in the IPG strips and do not migrate into the second dimension SDS-PAGE gel, and the sensitivity limitations of protein visualizing stains, in terms of the lower abundance proteins.

# **Characterization of Wheat Endosperm Proteins during Development and Maturity**

Proteomic technology has proved to be a valuable tool in the characterization of protein composition in the developing (17 DPA) and mature (harvested, 45 DPA) wheat grain endosperm. In such studies, many proteins (over 300) from the immature endosperm were characterized by N-terminal amino-acid sequencing and identified from protein-sequence databases, resulting in a detailed proteome map for this wheat cultivar. From these analyses, 55% proteins were identified from database matches, 17% proteins were not matched (they were previously unidentified proteins), and 28% of the proteins produced no

sequence information. The most abundant endosperm proteins identified in this study at 17 DPA belong to the seed-storage gliadin and glutenin families,  $\alpha$ -amylase inhibitor and α-amylase/trypsin inhibitor families, and the protein disulfide isomerase families. Different subclasses of proteins were observed in some of these protein families. The abundance of these particular families is to be expected at this stage of development. A significant finding of this study was the identification of multiple forms of proteins in these abundant families, as well as in other less abundant protein families. These multiple forms may be attributed, at least in part, to post-translational modifications, although the hexaploid nature (designated A, B, and D genomes) of common bread wheat cultivars increases the complexity of the situation (see Wheat: Genetics).

The synthesis of seed storage proteins commences at  $\sim$ 10–12 DPA, the proteins being deposited in the endosperm, to provide a source of nutrients and energy for the new plant. The mechanism of deposition is important for elucidation of the dough-forming properties of the storage proteins, given the importance of disulfide-based polymerization of glutenin subunits of the gluten complex. At 17 DPA, protein disulfide isomerase was abundant in wheat endosperm; this enzyme catalyzes the formation of interand intra-chain disulfide bonds, required for the correct folding of newly synthesized proteins (see Protein Synthesis and Deposition). Two forms of protein disulfide isomerase were expressed, which differed in the number of alanine residues in the N-terminus (EEAAAAEE and EEAAAAAEE). These differences may be explained by the control of two separate genes for PDI being expressed at 17 DPA, which could signify the crucial importance of this enzyme to the synthesis and deposition of proteins in the endosperm. Figure 6 depicts the major classes of proteins identified in immature wheat grain endosperm studies so far.

Protein expression within the endosperm at both immature and mature stages of grain growth were compared to gain an insight into developmental changes in protein composition. An example of these developmental changes can be described in the case of the protein disulfide isomerase enzyme. This enzyme was characterized and identified in the immature grain endosperm of 17 DPA, and expression of this enzyme was monitored at maturity (Figure 7). At 17 DPA, when the wheat plant is actively synthesizing many proteins, abundant isoforms of protein disulfide isomerase were present in the endosperm. With the decrease in protein synthesis at maturity, some of the isoforms of protein disulfide isomerase were no longer detected in the endosperm.

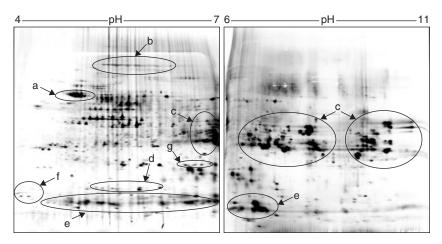
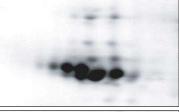


Figure 6 Proteome maps of immature Wyuna endosperm proteins at 17 DPA, including acidic (pH 4-7) and basic (pH 6-11) ranges. Particular protein regions, based on previously reported data, were designated on the 2-DE gels. These protein regions correspond to (a) PDI isoforms, (b) high-molecular-weight glutenin subunits, (c) gliadins, (d) small heat shock proteins, (e) α-amylase/trypsin inhibitors, (f) acidic ribosomal proteins, and (g) superoxide dismutase isoforms. (Reproduced with permission from Cornish GB, Skylas DJ, Siriamornpum S, Bekes F, Larroque OR, Wrigley CW, and Woottom M (2001) Grain proteins as markers of genetic traits in wheat. Australian Journal of Agricultural Research 52: 1161-1171.)

PDI enzyme isoforms Immature wheat grain endosperm 17 DPA



PDI enzyme isoforms Mature wheat grain endosperm (harvest)



Wyuna cultivar

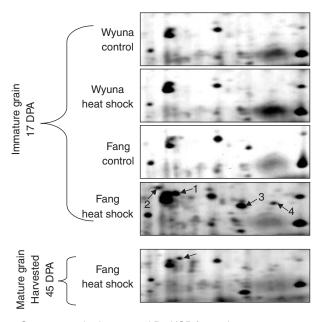
Figure 7 Comparison of PDI expression in immature (17 DPA) and mature (harvested) wheat grain endosperm.

This approach provided extensive information about protein composition in the endosperm, and about changes that occur during development.

## **Identification of Endosperm Proteins Associated** with Heat Tolerance

Another example showing the value of proteomics is in the identification of proteins associated with tolerance to stress conditions during growth. Proteome analysis shows the results of the interaction between the genes (the genome) and the growth environment (e.g., climatic conditions). As mankind has extended the cultivation of grains into climates not ideally suited to them, there have been attempts to breed genotypes (varieties) with tolerance to the stresses imposed. Such stresses include heat (a few very hot days or prolonged heat), frost and drought, as well as a range of pests and pathogens.

In this study, proteome technologies were used to determine the effects of heat shock on endosperm protein expression for the heat-susceptible Wyuna and heat-tolerant Fang cultivars. Wheat plants were grown at day/night temperatures of 24/18°C during development, with stressed plants subjected to heat shock at day/night temperatures of 40/25°C for 3 days in duration, namely, at 15, 16, and 17 DPA. Grain samples were taken during development (17 DPA) and at maturity (harvested grain at 45 DPA). Comparisons were made between these two wheat cultivars differing in heat tolerance, on the basis of rheological properties of dough prepared from the cultivars. Heat-susceptible Wyuna and heat-tolerant Fang cultivars were selected based on previous assessments of these wheats, in terms of heat tolerance and dough rheology. Proteome analysis was conducted on normal and heat-stressed endosperm tissue at the immature (17 DPA) stages of grain development. The heat-shocked Fang cultivar induced or up-regulated the expression of a small number of proteins, which were not detected in the heat-shocked Wyuna cultivar. Four of these expressed protein spots (indicated in Figure 8) were matched to plant small heat-shock proteins (HSP) by searching proteinsequence databases. Proteome analysis was also conducted on endosperm tissue from heat-shocked mature grain (45 DPA) in order to monitor the expression of the candidate marker proteins that were identified at the immature 17 DPA stage of development. As shown in Figure 8, protein spot number 1,



Spot #1 matched to a 16.9 kDa HSP from wheat Spot #2 matched to a 18.1 kDa from *Medicago sativa* (alfalfa) Spot #3 matched to a 16.9 kDa HSP from wheat Spot #4 matched to a 16.9 kDa HSP from wheat

**Figure 8** Comparison of control and heat-shocked immature wheat endosperm for the Fang (tolerant) and Wyuna (susceptible) wheat varieties. Candidate marker proteins for heat tolerance, identified at 17 DPA, were monitored at maturity for the heat-shocked Fang variety.

characterized and identified from heat-shocked Fang immature grain, is still detected at maturity. Consequently, this protein would be the best candidate, based on this study, to further investigate as a marker protein, in order to indicate whether or not a grain sample had been exposed to some form of heat stress in the field, and also to indicate if the grain is from a heat-tolerant wheat genotype.

#### **Cultivar Discrimination**

The grain proteome has also been investigated as a basis for devising more efficient methods of discrimination between varieties (see Variety Identification of Cereal Grains). Four wheat cultivars (Halberd, Cranbrook, CD87, and Katepwa) were selected on the basis of differences in their quality, in terms of dough-processing attributes that can suit one cultivar better than another for specific types of industrial utilization. Three particular regions of the proteome were compared in close detail, representing three different classes of proteins: high-molecular-weight polypeptides of glutenin, gliadins, and small heat-shock proteins (based on previous protein identifications). Cultivar-specific proteins were observed in each of these regions, providing information on the extent of grain protein polymorphisms in commercial wheats.

# Improving on Current Proteome Technology

At present, 2-DE is the core technology for arraying proteins in proteome projects. However, there are limitations to proteome studies at all stages of the 2-DE process. These limitations need to be overcome, or at least minimized, for this technology to be applied in a more effective manner for discovery in proteome analysis. Technological advances are required, in the areas of sample preparation and 2-DE, to increase the number and type of proteins visualized, so as to include polypeptides that may now be excluded, such high-molecular-weight, membrane and lowabundance proteins. Recent improvements in increasing the levels of proteomes visualized on the 2-DE gels are a result of the continual development of sequential protein extraction methods and the commercial availability of narrow range IPG strips. A combination of sequential protein extractions and broad and narrow IPG strips allows for a "subproteomic" analysis, in which many more, especially low-abundance and hydrophobic proteins, have a better chance of being visualized using 2-DE gels.

## **Future Prospects**

Further potential in applying the proteome approach to cereal-based research is enormous, with the possibility of future work being based on genotype and environmental interactions, and how these interactions effect the wide range of quality attributes that are relevant to marketing and the many forms of processing. These studies would probably take the form of identifying and establishing protein-marker-based systems, for the selection of desirable genotypes for processing requirements.

With the completion of the first plant-genome sequencing initiative for *Arabidopsis thaliana*, and progress with other plant-genome projects, such as the rice-genome sequencing initiative, plant proteomics is now set to explode on a global scale. With increased development of high-throughput proteome technologies, and the availability of completely sequenced plant genomes on publicly accessible databases, there is now a real opportunity for plant scientists and agricultural biotechnology companies to contribute to, and capitalize on these developments for discovery.

See also: Cereals: Protein Chemistry. Genomics. Grain and Plants, Morphology. Protein Synthesis and Deposition. Variety Identification of Cereal Grains. Wheat: Genetics.

## **Further Reading**

Dunn MJ (2000) From Genome to Proteome. London: Wilev.

Pennington SR and Dunn MJ (2001) Proteomics: From Protein Sequence to Function. Oxford UK: BIOS Scientific Publishers.

Skylas DJ, Mackintosh JA, Cordwell SJ, et al. (2000) Proteome approach to the characterisation of protein composition in the developing and mature wheat-grain endosperm. Journal of Cereal Science 32: 169–188.

Theillement H, et al. (1999) Proteomics for genetic and physiological studies in plants. *Electrophoresis* 20: 2013-2026.

Walsh BJ, Molloy MP, and Williams KL (1998) The Australian Proteome Analysis Facility (APAF): assembling large scale proteomics through integration and automation. Electrophoresis 19: 1883–1890.

Westermeier R and Naven T (2002) Proteomics in Practice: A Laboratory Manual of Proteome Analysis. Weinheim: Wiley-VCH Verlag GmbH.

### Relevant Websites

http://www.proteome.org.au — Australian Proteome Analysis Facility website.

http://au.expasy.org — Proteomic tools.

http://wiley-vch.de - Proteomics is a scientific journal specializing in articles on proteomics.

## **PSEUDOCEREALS, OVERVIEW**

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### Introduction

Most human foods come from plants. Roots, underground storage or propagation organs, stems, petioles, shoots, flowers, or fruits are eaten. Fruits in the form of grains or seeds are the major form of human food.

Almost all of the grains eaten by humans are cereals. A cereal grain contains an embryo and usually a quantity of endosperm, enclosed within a seedcoat. Cereals are one type of monocotyledonous plant (all of which present a single shoot on germination); pseudocereals are noncereals, comprising noncereal monocotyledonous plants as well as dicotyledonous plants; the latter have grains containing no endosperm but an embryo enclosing perisperm.

The usefulness of any grain as human food (its functionality) depends on the quantity and quality of protein, starch, and lipid (fats and oils) present in the tissues of the grain (embryo, endosperm and/ or seedcoat).

Grain functionality depends principally on the genetic and environmental influences on protein, starch, and lipid.

The proportion of embryo, endosperm, or perisperm within any grain is primarily determined by a plant's genetic make-up. The eventual composition of the grain can be influenced by the natural growing conditions and the manner in which the crop and its product have been managed. Important environmental factors not only include the growing conditions but also the method of harvesting, the manner in which the grain was stored, the nature of any processing carried out, including the extraction method used, and the presence of any antinutritional factors.

Grains used in the human diet come from surprisingly few crops.

Such crops are collectively referred to as the cereals (including crops such as wheat, rice, maize, barley, oats, rye, etc.), pulses (including bean, chickpea, cowpea, pigeonpea, lentil, lupine, etc.), oilseeds (including canola, sunflower, safflower, sesame, coconut, cottonseed, flaxseed, mustard, etc., as well as some pulse crops, such as soybean and peanut) or pseudocereals.

There can be some confusion with the use of such crop groupings, since each grouping is not mutually exclusive; for example, some pulse crops are used as oilseed crops and the term pseudocereal is sometimes used to refer to crops more commonly identified as oilseeds or legumes.

#### **Definition**

The American Heritage Dictionary of the English language defines a pseudocereal as "any of several plants, such as quinoa, that do not belong to the grass family but produce fruits and seeds used as flour for bread and other staples."

The Oxford English Dictionary defines the term cereal as "a name given to those plants of the family Graminaceae [now Poaceae] or grasses which are cultivated for their seed as human food ... sometimes extended to cultivated leguminous plants." The term pseudo is defined as "false, pretended, counterfeit, spurious, sham, falsely so called or represented." When combined with another element, such as the term cereal, pseudo is defined as "indicating close or deceptive resemblance to the thing denoted by the second element, without real identity or affinity with it."

The term pseudocereal is best used to refer to a plant which is grown as a crop to produce starchy grain suitable for human food, excluding plants already classified in a grouping such as the cereals (those species from the grass family Gramineae), pulses (those species from the legume families Leguminosae-Caesalpinioideae, Leguminosae-Mimosoideae, and Leguminosae-Papilionoideae), oilseeds (those species described in terms of the commercially useful lipid or tricylglycerol content), or nuts.

## **Current Role of Pseudocereals**

The pseudocereals are relatively unimportant on a global scale today. However, they have been significant contributors to the human diet in certain defined regions in the past and could be developed again as important new crops. For example, pseudocereals may play a role in human nutrition for those who have allergies to traditional cereals; for the primary producer, pseudocereals can play a role in cereal rotations, reducing the buildup of grass weeds, pests, or diseases.

The three best-known pseudocereal crops are grain amaranth (*Amaranthus caudatus*, *A. cruentus*, *A. hypochondriacus*; family: Amaranthceae; *see* **Amaranth**), quinoa (*Chenopodium quinoa* subsp. *quinoa*; Chenopodiaceae; *see* **Quinoa**), and buckwheat (*Fagopyrum esculentum*; Polygonaceae; *see* **Buckwheat**). Each is briefly described below, from the viewpoint of functionality. A range of potential pseudocereals from other plant families is then presented, with dicotyledonous families followed by monocotyledonous families.

#### Weeds

Many of the species listed as potential pseudocereals below are currently regarded, in some areas, as weeds; one of them, *Amaranthus retroflexus*, could be regarded as the world's most cosmopolitan weed.

A weed is defined as a plant which is growing in the wrong place. If some of the potential pseudocereals listed below can be commercialized, they would no longer be regarded as weeds.

#### Warning

The historic use of any obscure plant by a culture for medicinal or similar personal use does not mean that the plant is safe for use as a human food. Poisonous or antinutritional factors exist in many plants and their historic use was often complemented by elaborate preparation to reduce the effect of any poison. As an example, Australian aborigines were able to consume cycad seeds and yams but only after extensive preparation, which reduced the toxicity of the material.

Regular use of a plant over a lifetime by a group of people may also render a plant relatively safe for that group of people, whereas a newcomer who has never eaten the food can suffer serious effects on eating it for the first time.

#### **Amaranthaceae**

Amaranthus caudatus, A. cruentus, A. hypochondriacus (grain amaranth) Amaranthus caudatus (Inca wheat, love-lies-bleeding) is native to the northern higher-altitude regions of Bolivia, Peru, and Ecuador, whereas Amaranthus cruentus (Purple amaranth) and Amaranthus hypochondriacus (Prince's feather) are native to Guatemala and Mexico. The species were grown by pre-Colombian civilizations, Aztecs, and indigenous US tribes but have mostly been replaced by cereal crops.

Depending on the species, grain amaranth seeds are sprouted, parched, toasted, ground into flour, baked, mixed with sugar to make confectionery items, rolled into balls, cooked as porridge or popped.

Grain amaranth has enjoyed a resurgence in recent years, especially through the health food market, since its crude protein is high (14–19%), with a high lysine (up to perhaps 6% of the protein) and tryptophan content. It appeals to the modern consumer since these essential amino acids are low in cereals.

Grain amaranth is being developed as an energy food to be combined with traditional cereal grains in breakfast food, bread, multigrain crackers, pastes, pancake mixes, or popped as a snack food product. Popping can, however, affect its nutritional quality.

The grain is easy to digest and heat processing improves its digestibility.

Amaranth starch (up to 69% of the grain) is principally amylopectin; granules are relatively small

 $(1-3\,\mu\text{m})$  compared with cereals  $(3-30\,\mu\text{m})$  and have a higher solubility and gelatinization temperature, rendering a distinctive gel. The seed can comprise as much as 10% oil; this oil contains squalene, which is used in cosmetics manufacturing. Antinutritional trypsin inhibitors occur in concentrations up to twice that observed in wheat.

# Potential pseudocereals in the Amaranthaceae family

- Amaranthus dubius (spleen amaranth, khada sag), Amaranthus frumentaceous (poong keeray, tola kura), Amaranthus tricolor (tampala), Amaranthus graecizans (spreading pigweed), Amaranthus blitum (livid amaranth), Amaranthus quitensis (yuyo colorado, sangorache), Amaranthus retroflexus (redroot, pigweed), Amaranthus spinosus (spiny amaranth): the seeds are eaten raw in India and other places or mixed with other grains or processed into flour or other products.
- Achyranthes aspera (devil's horsewhip, prickly chaff-flower): the seeds have been used in desert areas in India as a famine food.
- Celosia argentea var. argentea (red-fox), Celosia argentea var. cristata (cockscomb): the seeds of these species have been used as famine foods.

#### Chenopodiaceae

Chenopodium quinoa subsp. quinoa (quinoa) Chenopodium quinoa subsp. quinoa is native to the border areas between Peru and Bolivia. It held a special position in the agricultural and ceremonial life of the Inca people and was grown in Colombia, Peru, the southern areas of Bolivia, the northern areas of Argentina, and the central areas of Chile.

Quinoa grain has a protein content ranging as high as 22.8%, a carbohydrate content up to 77.4%, a fat content up to 9.5% (and relatively stable), and a fiber content up to 5.8%. The grain is higher in protein, fat and fiber, and lower in carbohydrate content than most comparable cereals, due principally to the proportional size of the embryo within the grain (up to 30% of the grain's gross weight, compared with 1% for most cereals).

The proteins in quinoa are principally albumins and globulins, which are at higher concentrations than is normally found in the major cereals (wheat, rice, or maize). The amino acid balance (higher in histidine, lysine, isoleucine, methionine, and cysteine content) and mineral content (calcium, magnesium, phosphorus, potassium, and iron) of quinoa grain is superior to most cereals. However, there

are saponins in the seedcoat which have antinutritional properties.

# Potential pseudocereals in the Chenopodiaceae family

- Chenopodium album (fat hen, lamb's quarters). The seed is sprouted or ground into flour for breads, pancakes, muffins, and biscuits in Russia and China; the grain is reported to have 16.1% protein, 6.9% fat, 48.9% carbohydrate, and 5.8% ash.
- Chenopodium ambrosioides (wormseed, Mexican tea). In the Himalayas, the seed is ground into flour and mixed with warm water or roasted or added to alcoholic beverages; care needs to be taken with its preparation and use.
- Chenopodium berlandieri subsp. nuttalliae (southern huauzontle). The seed is ground to a meal and used for bread or gruel (a light form of porridge produced by boiling meal in water or milk).
- Chenopodium murale (nettle-leaf goosefoot, sow-bane). The seed is popped or parched and ground to make gruel.
- Chenopodium pallidicaule (canihua). The seed is toasted and ground into a flour and eaten as a breakfast food, mixed with wheat flour in baked products or drunk as a beverage.
- Chenopodium sp. (California Yokuts lamb's quarters). This selection was used as a semi-domesticated grain crop by Yokuts Indian villages, in the Central Valley, California.
- Atriplex canescens (fourwing saltbush). The seed is ground into meal and used as flour, or as a beverage.
- Atriplex confertifolia (shadscale). The seed is ground into meal and used for bread or mush (a heavier form of porridge than gruel, produced by boiling meal in water or milk).
- Atriplex hortensis (orache). Flour is prepared from the seeds and used in soup and muffins; the seed is reportedly rich in vitamin A.
- Atriplex lentiformis (quail bush) and Atriplex polycarpa (all scale). The seeds are eaten by the Native American Pima group, located in Arizona.
- Atriplex patula (halberd-leaved saltbush). The seed is ground and mixed with corn and steamed as meat balls by the Zuni Indians.
- Atriplex prostrata (hastate saltplant). The seed is ground into flour for use in baked products.
- Haloxylon sadicornicum. The seeds are mixed with other grains for bread making in India.
- Suaeda corniculata and Suaeda heteroptera. The seeds are eaten raw in Manchuria.
- Tecticornia verrucosa. The seeds are ground to flour by Australian aborigines and baked.

#### **Polygonaceae**

Fagopyrum esculentum (buckwheat) Buckwheat originated from south central China.

This crop is harvested with its green pericarp intact; once the pericarp (hull) has been removed, the groat can be coarsely milled into grits for breakfast food or roasted and sold in whole or a granulated form to be boiled, steamed, or baked. Buckwheat flour itself is used in soba-style Japanese noodles, polenta (a form of porridge), desserts, ice cream cones, dietetic foods, and canned meat or vegetable products and as a component (30–40%) of the flour used in pancakes, breads, pasta products, cakes and dumplings, often combined with wheat flour.

It is expected that buckwheat flour could be used more readily in specialty breads, pasta, extruded snack food, and ready-to-eat cereals.

The proteins in buckwheat grain are principally globulins with a small proportion of prolamins; buckwheat protein has twice the lysine content of wheat or white rice. The grains are relatively high in potassium, magnesium, phosphate, iron, and vitamins  $B_1$  and  $B_2$ .

## Potential pseudocereals in the Polygonaceae family

- Fagopyrum cymosum (perennial buckwheat) or Fagopyrum tataricum (Tartary buckwheat). In the Himalayas, the seeds are eaten or ground into flour.
- Calligonum polygonoides (phog, phogalli). The seeds are eaten raw in India.
- Polygonum aviculare (knotgrass, gooseweed). The seeds are eaten or ground into flour for use in cookies or pancakes; they are sometimes mixed with wheat; care needs to be taken with their preparation and eating.
- Polygonum glabrum (sauri arak, jioti, aatlaria).
  The seeds are parched and made into a kind of Indian "sattu" (a traditional weaning food mix often eaten by adults, usually based upon roasted Bengal gram, wheat, and sugar with spices added and served in a number of ways, including as a porridge).
- Polygonum orientale (Prince's feather). The seeds are eaten in China.
- Polygonum plebeium (chimtee sag, raniphul, machichi). The seeds are crushed on stones, cooked, and eaten in the form of a damper; the dry seed contains 17.4% protein, 50.4% carbohydrate, 3% fat, and 16.2% fiber.
- Rumex acetosa (sorrel dock). The seeds are used as
  a base for bread in Scandinavian countries; care
  needs to be taken with their preparation and eating.

- Rumex acetosella subsp. acetosella (sheep sorrel). The seeds are ground into flour to make a flat bread called sygrasbrod.
- *Rumex crispus (curled dock)*. The seeds are ground into meal or flour and used in pancakes; care needs to be taken with preparation and eating.

# Potential Pseudocereals in Other Dicot Families

Other dicotyledonous families with potential pseudocereal species producing starchy grains include: Bixaceae, Cactaceae, Cannabidaceae, Caryophyllaceae, Chloranthaceae, Cistaceae, Portulacaceae, and Trapaceae. Species of potential interest are named in the following.

There are many other minor dicotyledonous families with plants producing starchy grains which will not be referred to again; these include: Ancistrocladaceae, Basellaceae, Buddlejaceae, Desfontainiaceae, Dioncophyllaceae, Droseraceae, Erythroxylaceae, Frankeniaceae, Gelsemiaceae, Leitneriaceae, Lennoaceae, Molluginaceae, Nepenthaceae, Nyctaginaceae, Orobanchaeae, Pentaphragmataceae, Phytolaccaceae, Retziaceae, Sarcolaenaceae, Sargentodoxaceae, Saururaceae, Schisandraceae, Stegnospermataceae, and Tamaricaceae. Most of these families have very limited numbers of species.

### **Bixaceae**

Bixa orellana (annatto). The seeds are used as coloring and flavoring for meat, poultry, or fish; the seed-coat contains the carotenoid, bixin, which is used as a tasteless coloring for cheese, butter, margarine and chocolate and for color in soap and skin products.

#### Cactaceae

- Carnegiea gigantea (giant cactus). The seeds are ground into flour and used to make soup, paste, and other products by the Native American Papago group.
- Ferocactus wislizeni (fishhook cactus). The seeds are ground, parched, and used for bread and gruel.
- Opuntia clavata (ishikana). The seeds are roasted by Native Americans of the Acoma and Laguna groups; however, the mucilage in the fruit can render the food objectionable.
- Opuntia phaeacantha (bastard fig). The seeds are dried, parched, and ground into meal for gruel and cakes.
- Opuntia soehrensii (ayrampo). A violet dye is extracted from the seeds and used as a food coloring.

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- Pachycereus pecten-aboriginum (cardon hecho hecho). In Mexico, the seeds are ground and made into cakes.
- *Pachycereus pringlei (cardon)*. The seeds are toasted, ground, and fashioned into balls.
- Stenocereus stellatus (xoconochtli, joconostle).
   The seeds have been eaten.

#### Cannabidaceae

*Cannabis sativa (hemp)*. The seeds are parched, fried as cakes, used in asanomi (in Japan), in the spice mix shichimi, in ale or are eaten as sprouts.

## Caryophyllaceae

- *Lychnia segetum*. The seeds have been eaten as a famine food in France.
- *Spergularia arvensis (corn spurrey)*. The seeds are used for bread flour in Norway and Sweden.
- *Stellaria media (chickweed)*. The seeds are used as bread or as a soup thickening in India.

## Chloranthaceae

Sarcandra glabra (tea scent). The seeds are roasted and used as a sesame substitute.

#### Cistaceae

Cistus ladanifer (labdanum). The seeds are ground and used for cakes and breads.

#### Portulacaceae

Portulaca oleracea (purslane). The seeds are sprouted or ground for use in gruel, cake, bread, or pancakes; the content of glycine and tyrosine in the seeds is reportedly high; care needs to be taken with their preparation and eating.

## **Trapaceae**

- Trapa natans (water caltrop, water truffle). The seeds are eaten raw, roasted, boiled, fried, or ground into flour for bread and sweet puddings.
- Trapa bispinosa. The seeds are cooked and eaten in India.

## Potential Pseudocereals in Monocot Families

Monocotyledonous families with potential pseudocereal species include: Araceae, Cannaceae, Commelinaceae, Cyperaceae, Marantaceae, Musaceae, Palmae, Pandanaceae, Pontederiaceae, Typhaceae, and Zingiberaceae. Species of potential interest are named in the following.

There are also many minor monocotyledonous families with plants that produce starchy grains which will not be referred to again; these include: Alismataceae, Anarthriaceae, Aponogetonaceae, Bromeliaceae, Burmanniaceae, Butomaceae, Centrolepidaceae, Costaceae, Cyanastraceae, Cyclanthaceae, Eriocaulaceae, Flagellariaceae, Haemodoraceae, Eriocaulaceae, Hydrocharitaceae, Joinvilleaceae, Juncaceae, Juncaginaceae, Lemnaceae, Mayacaceae, Melanthiaceae, Najadaceae, Philydraceae, Potamogetonaceae, Rapateaceae, Rhipogonaceae, Scheuchzeriaceae, Sparganiaceae, Triuridaceae, and Velloziaceae.

#### **Araceae**

Peltandra virginica (arrow arum, tuckahoe). The seed produces a bread similar to corn bread, with a cocoa flavor.

#### Cannaceae

Canna edulis (Queensland arrowroot). Immature seeds are cooked in tortillas or cakes; the plant is used as a source of arrowroot in Colombia.

#### Commelinaceae

- Commelina benghalensis (keng, bokna, mu-Kengeria), Commelina forskailii (kansura, kanshura). The seeds are ground into flour for bread.
- Commelina communis (spider wort, day flower), Commelina obliqua (kena, keni, kana, kanjura). The seeds are eaten.
- Cyanotis axilaris (vichaka, narido, damro, soltra). The seeds are ground into flour for bread or bhadku, a mixture of flour and salt.

## Cyperaceae

- Cyperus bulbosus (theg, motha, mothabasa). The seed is ground into flour and made into bread or "ghes" or "rab" in India; Australian aborigines ate the seed raw or roasted.
- *Mariscus sieberianus (tall sedge)*. The seeds are made into flour in China.
- Scirpus lacustris (great bulrush). The seeds are ground and mixed with meal for bread, mush, or pancakes.
- *Scirpus maritimus (chid, dila)*. The seeds are eaten raw or pounded, made into flour and mixed with millet in India.
- Scirpus validus (tall bulrush). The seeds are eaten.

#### Marantaceae

Thaumatococcus danielli (miracle berry). The seeds are used as a sweetener but the extract does not withstand heat.

#### Musaceae

Ensete ventricosum (Abyssinian banana). The endosperm of the seed is eaten in Africa.

#### Palmae/Arecaceae

Hyphaene thebaica (gingerbread palm). The kernels are crushed and mixed with porridge; the edible portion of the kernel has 2.8% protein, 74.7% carbohydrate, 0.4% fat, and 11% crude fiber.

#### **Pandanaceae**

Pandanus tectorius (Nicobar breadfruit, screwpine). The seeds are edible but only after careful preparation.

#### **Pontederiaceae**

Pontederia cordata var. cordata (lance-leaf pickerel weed). The seeds are eaten raw, boiled or parched, and ground into flour for bread.

## **Typhaceae**

Typha angustifolia (narrow-leaf cattail). The seeds are roasted and eaten.

#### **Zingiberaceae**

- Aframomum angustifolium (Madagascar cardamom). The dried seeds are used like pepper or added to coffee.
- Aframomum melegueta (Melegueta pepper). The seeds are used as flavoring for beverages, meats, ice cream, candy, and bread.
- Amomum compactum (round cardamom). The seeds are used in cakes in Malaysia.
- Amomum subulatum (Indian cardamom). The seeds are used for flavoring in char masala, in Afghanistan.
- Amonum xanthioides (bastard cardamom). The seeds are used as flavoring in liqueurs in China.
- *Elettaria cardamomum (cardamom)*. The seeds are used as flavoring.
- Etlingera elatior (Phillipine wax-flower, ondje). The seeds are eaten raw in Malaysia.

### Conclusion

In the past, the pseudocereals have represented an important component of the diet of many people in a wide range of cultures. The representative list of potential pseudocereals included above demonstrates the opportunities for future development of functional foods that exist amongst the pseudocereals, based on the usefulness of these foods in the past. In addition, the reputation of so many of the species as weeds indicates that they will probably have growth characteristics which should assist with their development into modern commercial production systems.

See also: Amaranth. Buckwheat. Quinoa.

## **Further Reading**

Belton PS and Taylor JRN (2002) *Pseudocereals and Less Common Cereals*. Berlin: Springer.

Crawford M (2000) Edible Plants for Temperate Climates. Totnes, Devon: Agroforestry Research Trust.

Facciola S (1998) Cornucopia II. A Source Book of Edible Plants. Vistas, CA: Kampong Publications.

Hegarty MP, Hegarty EE, and Wills RBH (2001) Food Safety of Australian Plant Bushfoods. Rural Industries Research and Development Corporation, Canberra, ACT (http://www.rirdc.gov.au/reports/NPP/01-28.pdf).

Mabberley DJ (1997) *The Plant-Book. A Portable Dictionary of the Vascular Plants*, 2nd edn. Cambridge: Cambridge University Press.

Wiersema JH and Leon B (1999) World Economic Plants. A standard reference. Boca Raton, Florida: CRC Press. Williams JT (ed.) (1995) Cereals and Pseudocereals. London: Chapman and Hall.

## **Relevant Websites**

http://www.biodiversity.uno.edu — The families of flowering plants: descriptions.

http://www.newcrops.uq.edu.au - Australian new crops website, University of Queensland, Gatton.

http://www.ibiblio.org - Plants for a future.

http://www.hort.purdue.edu — Center for new crops and plant products, Purdue University and Famine Foods database.

## **PULSES, OVERVIEW**

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#### Introduction

Then said Daniel to Melzar, whom the prince of the eunuchs had set over Daniel, Hananiah, Mishael, and Azariah, prove thy servants, I beseech thee, ten days; and let them give us pulse to eat, and water to drink. Then let our countenances be looked upon before thee, and the countenance of the children that eat of the portion of the king's meat: and as thou seest, deal with thy servants.

Daniel 1:11–13 (RSV)

Grain legumes are plants belonging to the legume family (*Leguminosae*) that produce seeds used directly for human food. Humans particularly value this group of plants for the protein contributed by their seeds to our diets. Grain legumes are commonly subdivided into pulses, which, in addition to protein, store high levels of carbohydrate (>60%) and low amounts of lipid (<6%) in their dry seeds, and leguminous oilseeds which boast higher lipid, but lower carbohydrate levels than pulses (Tables 1 and 2). In

this organizational structure, soybean (*Glycine max*) and peanut (groundnut, *Arachis hypogaea*) are typically categorized as oilseeds while common beans (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*) are considered pulses. Pulses also contain high levels of dietary fiber (~20 g fiber per 100 g compared to 12 g fiber per 100 g for wheat, 9 g fiber per 100 g for soybean, and 3 g fiber per 100 g for rice).

Pulses are ancient foods that help fulfill the ageless need to sustain body and soul, as exemplified by Daniel's insistence on a simple plant-based diet over the meat-rich diet provided by King Nebuchadnezzar. The word "pulse" arrived in modern English from the Greek "poltos" meaning "porridge of meal," by way of Latin ("puls," "pultis"), Old French ("pols"), and Middle English ("puls").

Gathering plant parts for food is a reliable way (in comparison to episodic hunts for animal prey) of feeding oneself in a hunting and gathering society. Gathering pulse seeds is particularly convenient compared to parts of other plants. Pods from grain legume plants are handy and easily grabbed compared to subterranean roots and tubers. No tools other than fingers and palms are needed to grab and hang on to the large seeds. Keeping both feet firmly on the ground is less daunting than climbing trees for aerial fruits

**Table 1** Names and regions of greatest genetic diversity for major pulse species

Botanical name	Common and vernacular names	Center of domestication
Cajanus cajan	Pigeon pea, angola pea, congo pea, dhal, no-eye pea, red gram	India
Cicer arietinum	Chickpea, garbanzo bean, gram	Southeast Turkey
Macrotyloma uniflorum	Horse gram	
Lathyrus sativus	Grasspea, chickling vetch	Southern Europe, Southwest Asia
Lens culinaris	Lentil, split pea, red dhal, masur, lenteja, lentille, manssor, burssum	Eastern Mediterranean, Fertile Crescent (Iraq, Iran)
Phaseolus vulgaris	Common bean, dry bean, kidney bean, frijol, caraota, poroto, habichuela, haricot bean, snap bean	Mexico and Peru
Phaseolus lunatus	Lima bean	Peru, Central America, Caribbean
Pisum sativum	Field pea, garden pea, Guisante, muttar, pois, arveja, Alaska pea	Fertile Crescent (Iraq, Iran), Turkey, Greece
Vicia faba	Broad bean, faba bean, horse bean, Windsor bean, haba, feve	Eastern Mediterranean, West Asia
Vigna radiata	Green gram, golden gram, mung bean, oregon pea, chickasano pea	India, Southeast Asia
Vigna mungo	Mung bean, black gram, urd, kambulu, uride	India
Vigna unguiculata	Cowpea, catjang, Hindu cowpea, kaffir bean, black-eyed pea, frijol, caupi	Sub-Saharan Africa

Adapted from Sinha SK (1977) Food Legumes: Distribution, Adaptability and Biology of Yield. Rome, Italy: Food and Agriculture Organization of the United Nations. Based on Sinha (FAO) and Adams MW and Pipoly JJ, III (1980) Biological structure, classification and distribution of economic legumes. In: Summerfield RJ and Bunting AH (eds.) Advances in Legume Science, pp. 1–16. Kew, UK: Royal Botanic Gardens.

and nuts. The dense seeds are more filling than leaves, and will satisfy hunger in the absence of meat if hunting is unsuccessful. As a bonus to those concerned about times of scarcity, the seeds can be dried and stored for a period of months or more without loss of food value when eaten, or viability when planted. Pulse seeds are large relative to cereals, so they are convenient to handle for food preparation and planting. Because of their convenience, availability, and composition, wild pulses were an accessible, nutritional contributor to early hunting and gathering societies across the world.

The wild progenitors from which pulses were domesticated have been identified, with the exception of Vicia faba. The archeological record for some pulses, particularly *Phaseolus* species and *Lens culinaris*, is more complete than for others such as Vigna unguiculata and Cajanus cajan. In whatever manner they were developed, the domesticates quickly spread across regions and continents, particularly to regions lacking a wide range of highly desirable, locally domesticated food crops such as Europe and North America. Over time, domestication, selection, and trade resulted in a wide variety of pulses grown, contributing protein to human diets across the globe. Pulse and cereal grain domestication appear to have taken place at roughly the same time in human history, which suggests that these two foods may be ancient partners in providing human nutrition. While cereals provided most of the energy and much of the protein requirements of humans, pulses

**Table 2** Protein and other nutritional constituents of pulses and other reference foods (g constituent 100 g food<sup>-1</sup>)

Food	Protein	Lipid	Carbohydrate	Water
Pulses				
Common bean	22.2	1.1	61.5	11.5
Lima bean	21.5	0.7	63.4	10.7
Chickpea	19.3	6.0	60.7	11.5
Green gram	23.9	1.1	62.6	9.0
Cowpea	23.8	2.1	59.6	11.1
Oilseed legumes				
Soybean	36.5	19.9	30.2	8.5
Peanut	25.8	49.2	16.1	6.5
Cereals				
Wheat, hard white	11.3	1.7	75.9	9.6
Brown rice, raw	7.5	2.7	76.2	12.4
Animal products				
Beef, lean ground	20.0	10.0	0.0	69.5
Pork, ground	16.9	21.2	0.0	61.0
Fish, cod	17.8	0.7	0.0	81.2
Milk, cow's 3.25% fat	3.3	3.3	4.7	88.0
Egg, hen's	12.5	10.0	1.2	75.3

Based on data from FAOSTAT Database - http://www.fao.org/ag/.

played a strong supporting role as a contributor of concentrated dietary protein. Concurrent pulse and cereal production and their dietary consumption resulted in more sustainable farming systems, as well as better nutritional balance, than would have been derived from production and consumption of cereals alone.

## Consumption

Current pulse consumption in many parts of the Old and New World appears to be affected by cultural inertia; past consumption patterns in those regions are echoed in the present. While pulses are common components of diets around the world, the extent to which they contribute to the diet correlates closely with the historic availability and acceptance of animal protein. Ancient civilizations that consumed more pulses were those where animal protein was less available or acceptable. They included those that maintained high human population densities and overwhelmed local supplies of wild and domestic animal protein (East Asian Old World civilizations), those where few animals were domesticated (Aztec and Mayan New World civilizations), and those where predominant religious practices excluded animal protein consumption (Hindu veneration of the cow in South Central Asia). Even today, those regions of the world consume more pulses than elsewhere.

Besides influencing the amount of pulse contribution to the diet, cultural inertia expresses a parallel effect on the pulse species consumed within a region. Those pulses with long records of use in a particular country or region of the world continue as dietary preferences today (Table 3).

The 40 day fast of Jesus in the desert became the standard reference early in the Common Era for the custom of mortification of the body by monks belonging to the Roman Church. The fasting monks had to be engaged in conducting monastery's daily functions; thus, the elders of the monastic community began, in the fourth century, to standardize the fast diet, in order to ensure that their workforce received at least the nutrition required to keep body and soul intact, but without arousing earthly sensuality in monks eating flavorful food. A satisfyingly dull puree of pulses, accompanied perhaps by a vegetable soup, became typical of the single meal allowed during fasts. Monks certainly had their fill of pulses as they contemplated eternity; the fifth century monasteries scheduled ~230 fast days per year.

Before widespread adoption in seventeenth century, Europe of cuisine innovations such as leavened bread and three-a-day meals, people of medieval

Table 3 Pulses consumed in various world regions

Country or region	Pulse species commonly consumed
Mexico	Phaseolus vulgaris, P. coccineus, P. lunatus, P. acutifolius, Vicia faba
North America	Phaseolus vulgaris, P. lunatus
Brazil	Phaseolus vulgaris, P. lunatus, Cicer arietinum, Vigna unguiculata
Caribbean Islands	Phaseolus vulgaris, P. lunatus, Vigna unguiculata, Cajanus cajan
India	Vigna radiate, V. mungo, Cajanus arietinum, Cajanus cajan, Pisum sativum, Lens culinaris, Lathyrus sativus
Africa	Vigna unguiculata, Phaseolus vulgaris, P. lunatus, Vicia faba
Mediterranean and Europe	Vicia. faba, Pisum sativum, Phaseolus vulgaris

Adapted from Smart J (1990) Grain Legumes: Evolution and Genetic Resources. Cambridge, UK: Cambridge University Press.

Table 4 Protein per capita consumption in two decades by country groupings (g protein per day)

Protein source Developed cou		ed countries	Developing countries		Low income countries <sup>a</sup>		
	1961-70	1991–2000	1961–70	1991–2000	1961-70	1991–2000	
Total plant	45.5	43.1	41.9	48.2	42.0	46.2	
Cereals	31.9	29.1	28.4	34.4	29.1	33.5	
Pulses	2.2	1.9	6.2	4.0	7.0	5.2	
Total animal	46.6	55.4	9.4	18.2	7.8	11.5	
Meat	19.7	25.9	3.8	8.1	2.5	3.3	
Milk	15.8	16.9	2.7	4.1	3.1	4.9	
Total	92.2	98.5	51.3	66.5	49.7	57.7	

<sup>&</sup>lt;sup>a</sup>Countries with per capita annual income below a ceiling (\$750 USD in 1999 and 2000). Based on data from FAOSTAT Database – http://www.fao.org/ag/.

societies typically ate warm servings of porridge twice daily. These plates or bowls of boiled gruel incorporated readily available and affordable cereal grains such as barley and wheat, and also pulses such as the wild-growing or domesticated lentils first found in the Eastern Mediterranean and West Asia. Meat consumption declined with increasing population, decline of wild gamestocks, and movement of people into towns and cities. World trade, natural sciences, and the influence of aristocratic culture began to influence diets in the late 1600s. Three-a-day meals became common across socio-economic groups and cookbooks spread food culture throughout the urban and rural society. New foods - including spices, potatoes, and a thickener made by browning flour in fat known as roux – made their appearance and elbowed the cereal-and-pulse porridge out of popularity. The reformation de-emphasized fasting which further reduced the consumption of sturdy pulse porridges.

## Composition

Pulses, with roughly double the protein content of cereals, are damned with faint praise as the protein of the poor. They contribute excellent nutrition to all, but because they are inexpensive relative to meat, they

are particularly beneficial to subsistence farmers and those with low disposable income in food-deficient countries. In such circumstances, pulses can account for  $\sim 10\%$  of daily protein intake and 5% of the daily energy in the human diet (Table 4). The praise, however, is faint and damning because this association with poverty, a connection perhaps firmly established with the porridges of the Middle Ages, stigmatizes pulse foods among those with or aspiring to higher income who increasingly favor meat protein. Scarcely, 2% of dietary protein in developed countries comes from pulses. In more affluent settings, pulses remain an underused alternative to high-priced animal protein. Interestingly, at least one segment of affluent society, lifestyle- or health-conscious eaters such as vegetarians or those seeking the nutritional benefits of high dietary fiber, still treasures these plants. Pulse consumption is declining on a per capita basis, regardless of economic status. The increased protein consumption recorded in developing countries is due to inclusion of additional cereals, meat, and milk in the diet. One aggravating reason for low consumption is flatulence, a well-known side effect of pulse consumption, and intestinal fermentation of complex sugars and dietary fiber. If gassiness causes the consumer discomfort or embarrassment, pulse foods will be avoided when other choices are available.

Table 5 Pulse domestic production and utilization in two decades by country groupings (% of total domestic supply)

Source	Develope	ed countries	Developing countries		Low income countries <sup>a</sup>	
	1961-70	1991–2000	1961-70	1999–2000	1961-70	1991–2000
Domestic production	97.9	111.6	101.5	98.3	101.5	98.7
Imports	9.0	25.2	1.7	8.4	0.4	4.8
Exports	6.5	35.0	3.0	7.1	1.9	3.6
Feed use	53.8	64.0	7.1	9.9	7.8	11.3
Food use	30.9	26.4	80.2	77.9	79.2	76.1

<sup>&</sup>lt;sup>a</sup> Countries with per capita annual income below a ceiling (\$750 USD in 1999 and 2000). Based on data from FAOSTAT Database – http://www.fao.org/ag/.

**Table 6** Indispensable amino acid requirements in dietary protein and the amino acid composition of selected foods (mg amino acid per g protein)

Amino acid	Infant requirement	Adult requirement	Hen's egg	Cow's milk	Whole wheat flour	White bean flour
Histidine	23	17	24	28	22	28
Isoleucine	57	23	63	60	40	42
Leucine	101	52	88	98	63	76
Lysine	69	47	70	79	26	72
Methionine + cysteine	38	23	56	34	35	19
Phenylalanine + tyrosine	87	41	98	96	81	77
Threonine	47	24	49	45	27	39
Tryptophan	18	6	16	14	11	10
Valine	56	29	72	67	43	46

Based on data from Anonymous (2002) Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids (Macronutrients). Chap. 10, p. 71. Washington, DC: National Academies Press (Online version available on p. 535 at the URL http://www.nap.edu/books/0309085373/html/).

Developed countries are currently net exporters of pulses, while developing and low income countries are net importers (Table 5). Trade in pulses has grown substantially since the 1960s. During that period the rate of growth in pulse imports was greatest in the poorest countries, and export growth was the greatest in the most affluent. The poorest countries also had the greatest rate of growth in pulse utilization for livestock feed.

#### **Protein Complementation**

Understanding amino acid balance is key to recognizing the special contribution made by pulses to protein complementation in cereal-based diets. Different sources of plant protein are made up of characteristic concentrations of indispensable (essential) amino acids, and therefore contribute upon digestion a different balance of amino acids for human nutrition. Amino acid balance in a particular protein source can be scored against that of a reference protein, typically hen's egg (regarded as well balanced in relation to adult human metabolic needs) or milk (Table 6). No plant-based protein mimics the indispensable amino acid balance present in egg, so it follows that each type of plant protein must exhibit a characteristic set of limiting amino acids. Whole

wheat flour and white-seeded common beans generally have lower proportions of the indispensable amino acids than do egg or milk. In particular, the amino acid lysine limits efficient utilization of wheat protein in human metabolism, while the indispensable sulfur-containing amino acid methionine limits bean protein. The indispensable amino acid balance of wheat and bean is characteristic of other cereal grains and pulses. Cereal grains are typically deficient in lysine, but high in methionine, while the opposite is the case for pulses: high in lysine, low in methionine. Cysteine, a dispensable sulfur-containing amino acid synthesized from methionine and serine, is in many references listed along with methionine as a limiting amino acid of pulses.

Plant protein complementation, accomplished by consuming protein from more than one plant source, can provide humans with a balanced diet of amino acids. Pulses, with their high concentration of lysine relative to lysine-deficient cereals, and cereals, with their high concentration of methionine and cysteine relative to that found in pulses, dance as ideal dietary partners, because the amino acids making up their proteins are very good complements. For example, at 0.7 g per kg body mass day<sup>-1</sup>, an 85 kg adult would require ~60 g of protein per day containing at least 2820 mg lysine. This requirement could be

satisfied by consumption of 288 g wheat (Tricicum aestivum) and 124 g bean (Phaseolus vulgaris) (Tables 2 and 6). To meet the lysine requirement through consumption of wheat alone, the same adult would need to eat 960 g wheat containing  $\sim$ 108 g total protein. The intentional combination of cereals and pulses at meals offers inexpensive (in comparison to meat) plant-based diets with balanced amino acid composition.

While the central theme of this overview is the enormous contribution made by dry pulse seeds to the human diet, pulses have other food uses. Some species are used as vegetables either as green mature or immature seeds or pods, as sprouted seeds, or even as stocks for fermentation. Fermented pulses - primarily involving black gram (Vigna mungo), Bengal gram (Cicer arietinum), and green gram (Vigna radiata) - are components of diets particularly in India as "idli," "dhokla," and "khaman." Fermentation, sometimes in combination with cereal grains, causes desirable reduction in beany flavor, aroma, and consistency, and improves digestibility.

Nonfood uses include dry seeds used as animal feed, forage from pulse vegetation, in aquaculture feeds, and even as ornamentals. In a Southeast Asian setting, well-established shrubs of pigeon pea, a short-term perennial grown on field banks or as an intercrop, as well as dry-season grown horse gram and cowpea, can be intermittently grazed or cut for hay. N concentration of the hay varies between 2.5% and 4.5%, but livestock may need a few days to be accustomed to the feed. Dairy cattle are reported to similarly consume and utilize faba bean silage and grass-legume silage. From a coolseason perspective, seeds of pea, lentil, faba bean, and chickpea can be used as a protein source in livestock feed. Oil and fiber contents of the seeds affect the metabolizable energy values (MEn) of pulses when fed to various types of livestock. For example, the relatively low-oil, high-fiber pea has a low (2570 kcal kg<sup>-1</sup>) MEn when fed to poultry, while for swine the pea MEn is much higher (3740 kcal kg<sup>-1</sup>). Pulse seed amino acid profile for monogastric feeds, while good, is inferior to soybean. Pea and faba bean protein is highly digested, but some livestock feeding studies have shown an increased excretion of endogenous protein, which may be due to seed antinutrients. Dry-roasting the whole seeds has been shown to improve digestibility and protein utilization. Pulses have great potential for use in aquaculture diets, but in this application are hampered by their carbohydrates. However, field peas (Pisum sativum), faba beans (Vicia faba), chickpeas (Cicer arietinum), and vetch (Vicia sativa) seeds can be used in aquaculture feeds for juvenile silver perch following de-hulling, which removes much of the undesirable carbohydrate fraction.

#### Antinutritional Factors

Low digestibility hampers full utilization of pulse protein. In part, the problem may result from the more rapid discharge, relative to other foods, of digesting pulses from the intestinal tract and reduced protein hydrolysis by gut enzymes. However, antinutritional factors, compounds in pulses that interfere with their digestion and metabolism, also play a major role in restricting dietary utilization in some pulse species. These compounds generally include proteinaceous molecules such as protease inhibitors (particularly trypsin inhibitors), and lectins, and also nonproteinaceous compounds such as tannins.

Most of the wild relatives of pulses contain toxins and antimetabolites. One of the key features of pulse domestication from wild forms is human selection for lower levels of these compounds. Traditional foodpreparation techniques used for pulse seeds result in reduction or elimination of these metabolic impediments. Some modern food pulses today, in particular the *Phaseolus* species, can still contain sufficiently high levels of antinutritional factors in their dry seeds to cause digestive difficulties if eaten without proper processing. For this reason, pulse seeds are not eaten raw, but processed in a way (moist heat >100°C, sprouting, fermentation) that inactivates the antinutritional factors.

Protease inhibitors, a major class of antinutritional factors in pulses, often inhibit the digestive enzyme trypsin, but may act more broadly by inhibiting chymotrypsin and other serine proteases. The role of these inhibitors in the plants that produce them is uncertain, but may involve defense against disease and insects. An alternative explanation is that these compounds might simply serve as protein reserves high in sulfur-containing amino acids such as cysteine and coincidentally inhibit human digestion. The primary concern regarding raw pulse consumption is with very young humans and other young animals that are more susceptible to the effects of protease inhibitors than adults. In vitro studies suggest that protease inhibitors can impair nutrient utilization and reduce growth rates in these groups. In vivo animal studies indicate that interference with growth occurs primarily when the level of dietary protein itself is limiting. Pancreas activity can be affected when dietary protein digestion is inhibited and high levels of undigested protein interfere with appropriate pancreatic regulatory control. Since pulses are used in some human infant diets, cooking must be done with

particular care to ensure that protease inhibitors are inactivated.

Lectins, another major class of antinutritional factors in pulses, are proteins that bind to carbohydrates or to molecules containing carbohydrates. This binding capacity allows them to agglutinate red blood cells (lectins are sometimes called phytohemaglutinins) of different animal species depending on the specific receptors on the cell membrane surface. This agglutination specificity provides a method of rapid detection and classification. Lectins differ in the severity of their impact. Some (mainly found in Phaseolus vulgaris) are classified as toxic, others are considered only growth inhibitory (*P. lunatus*) and still others essentially nontoxic (Pisum sativum, Lens culinaris, Vicia faba). The role of lectins in the plant is not well established, although they appear to help the plant defend itself against specific bacteria, fungi, and insects. They may also aid processing and movements of glycosylated storage materials during seed maturation, assist in establishment of the symbiotic relationship of legumes and rhizobia, and promote cell growth and division. Or, like protease inhibitors, lectins may simply serve as storage proteins.

The effect of ingested lectins on human and other animal metabolism varies according to the lectin type, the species ingesting the protein, and the age, nutritional, and health status of that human or animal. To affect human metabolism, plant lectins first must bind to epithelial cells in the gut. This binding requires the presence of particular carbohydrate groups on the lumen of the gut epithelial cells. Dietary lectins strongly resist proteolytic degradation in the gut. Toxic dietary lectins can modify hormone balance, lipid, and muscle metabolism. These modifications deplete stores of lipid, glycogen, and protein and lead to weight loss. Some nontoxic lectins bind to the gut epithelium, but have no deleterious effect on metabolism, while others exhibit only limited binding capacity. Deleterious lectin effects appear to be reversible.

Tannins can form strong cross-linked complexes with dietary proteins and enzymes. The antinutritional impact of tannins on digestion and metabolism is not fully understood.

Protease inhibitors and lectins are heat labile and rendered innocuous by usual methods of cooking. Moist heat treatment — such as the traditional soaking of beans followed by cooking in boiling water — is generally considered to be sufficient to inactivate protease inhibitors. Soaking may help leach out some tannins, but heat treatment will not reduce the impact of that remaining in the seeds.

Particular pulse species accumulate additional classes of antinutrients. For example, *Lupinus albus* 

seeds may contain alkaloids. Neuro- and osteo-lathyrism, the former a reversible paralysis and the latter a skeletal abnormality, have been linked to excessive consumption of grasspea (Lathyrus sativus) seed. Broadbean (Vicia faba) antinutrient levels are low and do not require moist heating or fermentation to inactivate, but can induce favism, a hemolytic anemia, in individuals with congenital deficiency of glucose-6-phosphate dehydrogenase. Pulses of Mediterranean origin – such as peas (Pisum sativum), chickpeas (Cicer arietinum), and lentils (Lens culinaris), generally have low toxic and antinutrient levels. While protease inhibitors have been found in cowpeas (Vigna unguiculata), they are apparently not present in serious levels, nor have harmful quantities of cowpea lectins been reported. V. mungo and V. radiata seeds likewise contain little in the way of antinutritional factors.

## **Agronomy**

The Leguminosae family is enormous, with an estimated 16 000–19 000 species in ~750 genera. If the raw number of species were to determine the greatness of a family, Leguminosae would be eclipsed only by the Orchidaceae and Compositae. If instead the economic value of a family were considered, legumes would be second only to the Graminae. Taxonomists place pulses in the legume subfamily Papilionoideae, mostly in tribe Phaseoleae, but a few in the tribes Vicieae, Cicereae, and Genisteae.

Cropping systems develop in response to the demand for particular crops, soil nutrient status, soil physical structure, and biotic competition and are constrained by available resources (including climate) and knowledge. Pulses in temperate cropping systems are commonly planted in monocultures as part of a rotational sequence with maize (Zea mays), small grain cereals, or occasionally forages or periods of fallow in dryland areas. Since in these cropping systems pulses are typically cash crops and occasionally feed crops, their inclusion in the rotation and total acreage from year to year depends on the status of cash markets and economic benefits of growing that pulse crop relative to other crops rather than with maintaining a set crop rotation, maintaining particular soil characteristics or addressing biotic competition. Higher-value crop alternatives may be readily substituted for pulses in the rotation in some climates. National farm strategies with strong economic impact, such as government subsidies or deficiency payments, can affect pulse production by inclusion in or exclusion of particular pulse crops from the programs. For example, the United States Food Security and Rural Investment (FSRI) Act, governing USA

farm legislation for the period 2002–07, includes as program crops — dry peas, lentils, and small chickpeas — for the first time, but common beans are not included. While not a big producer of any of these pulse crops, the USA is an exporter, and additional production stimulated by the FSRI could affect prices in other pulse exporting or importing countries.

Leguminosae will collaborate with Rhizobium bacteria in an ecologically unique symbiotic relationship. This symbiosis supports biological nitrogen fixation and frees legumes from the need to access soil sources of fixed nitrogen. Rhizobia invade the legume root hairs and reside in cortical root swellings (nodules) where nitrogen from the atmosphere is fixed for eventual transport within the plant and assimilation into protein. Even though a legume can support the symbiotic relationship, it will not occur unless the rhizosphere contains compatible rhizobia. Strains of rhizobia differ according to the particular legume hosts that they will inoculate, and are classified according to this host range.

Cropping systems in developing countries typically rely on intercropping where pulses are planted along with other species, commonly cereals and vegetables. The intercropping strategy can provide subsistence farmers with several essential foods from one plot of land, produce higher protein yield per hectare, combine complementary canopy structures (low stature pulses with high stature sorghum (Sorghum bicolor) and maize (Zea mays), balance utilization of soil moisture following rainy periods, or reduce crop competition by weeds and insects. It is unlikely that a nonlegume companion crop of similar growth period will benefit directly from the pulse crop's nitrogen fixation, but perennials, crops with longer growth periods, or subsequent crops may benefit from the nutrients released through decomposition of pulse crop litter. The agronomic impact of a legume crop on the intercrop will depend on the crop species (and even the cultivar) in the intercrop, the extent of its inclusion in the plot, tillage, and soil type.

Pulses can assist in soil improvement, particularly when used as green manure crops when the whole plant is plowed down into the soil. Fixed nitrogen and in some cases phosphorus is made available to subsequent crops, and the added organic matter contributes to improved soil structure. Also, the deep tap roots of some crops such as pigeon pea reduce soil erosion and mine deep soil for nutrients that are returned to the surface through leaf drop.

In sustainable shifting cultivation systems of intercropping, a few years of crop production, are followed by a decade or more of bush fallow where cropping is suspended and native vegetation regrows. Cultivated pulses grown during the cropping years provide concentrated protein for human diets using nitrogen fixed from the atmosphere, and help maintain the soil nitrogen status by not depleting fixed soil nitrogen. During the bush fallow stage, indigenous legumes, including trees, contribute to the restoration of soil nitrogen. When human population density is low relative to available land, and bush fallow periods are sufficiently long to restore soil nutrients, shifting cultivation can be sustained. With increased population or decreased land base, the cropping period must be extended, bush fallow period reduced, and as a result soil nutrient status and crop productivity decline.

Since in most cases pulse crops are not alternate hosts for cereal crop diseases, their use in crop rotations will help reduce cereal disease innocula. However, pulses are certainly not immune to pests; they are attacked by many fungal, bacterial, and viral diseases and a range of sucking, boring, and chewing insects. Also, pulses are typically poor competitors against weeds, except perhaps under stress conditions such as drought conditions where annual weeds are also checked.

At harvest time, pulses often disappoint and frustrate growers by failing to meet yield potential. Pulses are often considered to be extremely sensitive to environmental stress, relative to the more robust cereals, a sensitivity which may in part be associated with maintaining the symbiotic relationship with Rhizobium. The Rhizobium-Leguminosae symbiosis requires energy supplied by the host plant to support the metabolic needs of the bacterial colonies in return for assimilated nitrogen. In the case of the pulses, which are typically grown as annual crops, the symbiotic relationship must be re-established with every new crop. Farmers watching the color of the growing annual pulse crops in their fields can clearly see the lag time between planting and the establishment of functioning nodules. For example, a temperate common bean crop planted in a soil with compatible rhizobia will tend to have a deeper shade of green about a month after planting, as fixed nitrogen becomes available to the plant. During the early portion of the growing season, the host plant pays the costs of establishment and only later receives the benefits. Plant-generated energy used to support the symbiotic relationship is not available to the plant for dry matter production, so some amount of yield penalty should be anticipated. Effective nodulation and nitrogen fixation depends on favorable interactions between the host and appropriate Rhizobium genotypes and the environment. If these interactions falter and nitrogen fixation lags behind demand, dry matter production will suffer.

#### **Final Words**

Declining per capita pulse consumption, particularly given the nutritional value of pulses and their affordability, is cause for concern. Although some regions in developed countries now benefit from new pulse export opportunities and are increasing production, this expansion reflects changing markets and increased population, not increased dietary utilization. Much more global impact could be accomplished by addressing constraints to consumption, including the social stigma associated with eating poor pulses compared to rich meat, flatulence, yield variation, and agronomic constraints to product quality and stable high yield. Reversing the decline in pulse consumption in developing and low income countries may be impossible given the strength of the human drive toward diets associated with affluence. Perhaps the greatest initial gains could occur among consumers in developed countries currently with very low pulse consumption. The keys to higher consumption in those countries may involve increasing the availability of value-added pulses such as those produced by certified organic methods or food quality assurance programs, and highlighting their inclusion in national and regional cuisine initiatives. In tandem, these efforts to add value and visibility could impart some cache to pulses and improve their appeal.

So he consented to them in this matter, and proved them ten days. And at the end of ten days their countenances appeared fairer and fatter in flesh than all the children which did eat the portion of the king's meat. Thus Melzar took away the portion of their meat, and the wine that they should drink; and gave them pulse.

Daniel 1:14-16 (RSV)

See also: Cereals: Protein Chemistry. Consumer Trends in Consumption. Fermentation: Foods and Nonalcoholic Beverages. Nutrition: Guidelines for Grain-Based Foods; Effects of Food Processing.

## **Further Reading**

- Adams MW and Pipoly JJ, III (1980) Biological structure, classification and distribution of economic legumes. In: Summerfield RJ and Bunting AH (eds.) *Advances in Legume Science*, pp. 1–16. Kew, UK: Royal Botanic Gardens.
- Allen ON and Allen EK (1981) The Leguminosae: A Source Book of Characteristics, Uses and Nodulation. Madison, WI: University of Wisconsin Press.
- Booth MA, Allan GL, Frances J, and Parkinson S (2001) Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: IV. Effects of dehulling and protein concentration on digestibility of grain legumes. *Aquaculture* 196: 67–85.

- Dembińska M (1986) Fasting and working monks: regulations of the fifth to eleventh centuries. In: Fenton A and Kisbán E (eds.) *Food in Change*, pp. 152–160. Edinburgh: John Donald Publishers.
- Duke JA (1981) Handbook of Legumes of World Economic Importance. New York: Plenum Press.
- FAO (2002) *Agriculture 21*. Agriculture Department, Food and Agriculture Organization of the United Nations. Rome, Italy: FAO Publishing and Multimedia Service (http://www.fao.org/ag/).
- Huisman J and van der Poel AFB (1994) Aspects of the nutritional quality and use of cool season food legumes in animal feed. In: Muehlbauer FJ and Kaiser WJ (eds.) Expanding the Production and Use of Cool Season Food Legumes, pp. 53–76. Dordrecht: Kluwer Academic.
- Jansman AJM, Hill GD, Huisman J, and van der Poel AFB (eds.) (1998) Recent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed. Wageningen, The Netherlands: Wageningen Pers.
- Labuza TP and Erdman JW, Jr (1984) Food Science and Nutritional Health: An Introduction. St. Paul, MN: West Publishing.
- Marquardt RR and Bell JM (1988) Future potential of pulses of use in animal feeds. In: Summerfield RJ (ed.) World Crops: Cool Season Food Legumes, pp. 421–444. Dordrecht: Kluwer Academic.
- Reddy NR, Pierson MD, and Salunkhe DK (eds.) (1986) Legume-Based Fermented Foods. Boca Raton, FL: CRC Press.
- Reeds PJ and Beckett PR (1996) Protein and amino acids. In: Ziegler EE and Filer LJ, Jr (eds.) *Present Knowledge in Nutrition*, 7th edn., pp. 67–86. Washington, DC: ILSI Press.
- Sinha SK (1977) Food Legumes: Distribution, Adaptability and Biology of Yield. Rome, Italy: Food and Agriculture Organization of the United Nations.
- Smart J (1990) Grain Legumes: Evolution and Genetic Resources. Cambridge, UK: Cambridge University Press.
- Summerfield RJ and Roberts EH (eds.) (1985) *Grain Legume Crops*. London: Collins Professional and Technical Books.
- 't Mannetje L and Jones RM (eds.) (1992) *Plant Resources of Southeast Asia. No 4. Forages.* Wageningen: Pudoc Scientific Publishers.
- Toussaint-Samat M (1992) (translated from the French by Bell A) *History of Food*. Cambridge, MA: Blackwell Publishers.

## **Relevant Websites**

http://www.fao.org – FAOSTAT Database, a comprehensive resource for agricultural production data, including than on pulse crops.

http://www.icarda.org — The website of the International Center for Agricultural Research in the Dry Areas, based in Syria, has useful information on pulse agronomy and utilization.



## **QUINOA**

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## Introduction

Quinoa is a typical crop of the Andean region. It has been cultivated since ancient times, and was a staple food of the Inca empire. However, production of quinoa was almost completely reduced after the Spanish conquest; foreign grains were widely produced instead.

During the 1970s, initial interest in the crop increased. At the same time, higher plantation areas for cultivation of quinoa in South America and new markets for the grain in the USA and Europe were developed. Thus, quinoa is now of great interest to the scientific world, and it may become a food for the future.

The origin of quinoa and its distribution in the Andean region have been reviewed, as well as its actual distribution in North and South America and Europe. It is important to mention the strong and dedicated interest in cultivating this grain outside the borders of the former Inca empire. Classification of the grain, cultivation practices, and morphology of the plant have been described.

Quinoa has been recognized as an extremely nutritious grain all over the world, thanks to both the high quantity and good quality of its protein content as regards its essential amino acid content. Thus, an extensive review of the chemical composition and nutritional value of quinoa has been made, including its main nutritionally disadvantageous factors.

Finally, several traditional and new forms of using quinoa have been described. Some future perspectives with regard to the germplasm collection, agronomic and agricultural practices, postharvest and industrial uses of this promising grain, which has been called the "mother grain," have been included.

## **Origin and Distribution**

Quinoa (*Chenopodium quinoa*, Willd) is an indigenous crop of the Andean region of South America. It is one of the oldest crops of the American continent. Archeological findings in northern Chile showed that quinoa was used prior to 3000 BC. In Ayacucho, Peru, evidence has been shown that quinoa was cultivated before 5000 BC.

The quinoa plant was widely cultivated in the entire Andean region, in Colombia, Ecuador, Peru, Bolivia, and Chile, before the Spanish conquest. However, habits and traditional foods of natives were substituted by foreign crops such as wheat and barley. Therefore, quinoa was cultivated either in small plantations in rural areas for domestic consumption or as borders for other crops such as potatoes or maize. For that reason, it was classified as food for poor people.

Quinoa is grown in the highlands from 5°N in southern Colombia to 30°S in northeastern Argentina. At sea level, it is cultivated between 36°S and 40°S, in central Chile.

In the late 1970s, the main production areas of quinoa were widely described in Colombia, Chile, the Andean valleys in Peru, Altiplano in Bolivia and Peru, and the highlands of Ecuador. In Peru and Bolivia, this crop has been of great importance. It is cultivated not only for domestic consumption but also for export. Common efforts made by governments and research institutes have contributed to increase the production of quinoa in Andean countries. The cultivation of quinoa has now spread from the Andean region to several countries in the world. In the 1980s, in the USA, quinoa was cultivated in the Colorado Rockies. Nowadays, it has became a commercial crop. In the UK, in 1989, quinoa was grown commercially. In Manitoba, Canada, quinoa was grown for domestic consumption; farmers expected to have a viable crop for Canada. Quinoa was introduced to Denmark in 1984. Further improvements in the crop could make quinoa a promising new crop for European agriculture.

In order to promote the regional interchange of the excellent genetic material of quinoa among research

institutes and universities, an American and European trial of quinoa was undertaken in the late 1990s. In the project, sponsored by the Food and Agriculture Organization (FAO: regional office for Latin America and the Caribbean), 25 cultivars selected from ten different countries were tested: eight from Peru, four from Bolivia, two each from Ecuador, the UK, Denmark, and Chile, and one each from Argentina, Brazil, Colombia, and The Netherlands. The best cultivar from each country taking part in the experiment was used as a local control. Therefore, it is expected to be able to identify, evaluate, and select promising genotypes of quinoa with high yield, and to provide the technological knowledge of cultivation practices and production of quinoa suitable for the agroecological and food requirements of the producer and national consumer.

### Classification

Quinoa belongs to the Chenopodiceae family, genus Chenopodium. Its botanical name is Chenopodium quinoa, Willd. Common names used in the Andean region are: "quinua," "kiuna," "parca" (Ecuador, Peru, Bolivia); "supha," "jopa," "jupha," "jiura," "aara," "ccallapi," "vocali" (Bolivia); "quinhua" (Chile); and "suba," "pasca" (Colombia).

The classification of quinoa was first made from the color of the plant and fruits. Subsequently, it was based on the morphological types of the plant. Despite the wide variation observed, quinoa is considered to be one single species. For practical reasons, quinoa, like maize, was classified as a race.

The most extensive collection of different races of quinoa belongs to Peru and Bolivia; each has over 2000 ecotype samples. However, other collections

do exist in Argentina, Colombia, Chile, Ecuador, England, the USA, and the Former Soviet Union.

Quinoa collected in Ecuador, Peru, and Bolivia has been classified into 17 races; however, more races may exist. Two types of inflorescence are described:

- 1. glomerulates small groups of flowers (glomeruli) originate from tertiary axes;
- 2. amaranthiformes have glomeruli originating mainly from secondary axes.

According to this, the races of quinoa are classified as follows: first, glomerulate inflorescence: Cajamarca, Copacabana, Cuzco, Challapata, Cochabamba, Sicuani, Junin, Ancash, Glorieta, and Dulce; second, amaranthiforme inflorescence: Achacachi, Puno, Real, Potosi, Puca, Sucre, and Pichincha.

Quinoa grows from sea level to the Andean highlands. Thus, one of the most useful classifications is that describing five ecotypes: sea level, valley, subtropical, salar, and altiplanic (Table 1).

## **Cultivation Practices**

The cultivation of quinoa is related to the crop rotation seen in potatoes. This is the usual practice that improves quinoa yield and preserves soil fertility. Moreover, the biological cycle of several pathogenic microorganisms is broken down. Together with residues of fertilizer previously applied to the crop, nitrogen is sometimes applied. Cultivation of the quinoa plant requires loose soil that can retain an adequate amount of moisture.

Quinoa tolerates a wide range of pH conditions of the soil, from pH 6.0 to 8.5. The plant is not affected by temperature from around  $-1^{\circ}$ C. However, it tolerates high temperatures not above 35°C. Quinoa

Table 1 General categories of quinoa

Ecotypes	Location	Growth altitude (m)	Varieties	Characteristics
Sea level	South of Chile	< 500	Chilean varieties	Unbranched, long day plants, yellow, bitter seeds
Valley	Andean valley	2000-4000	Blanca de Junín, Rosada de Juní, Amarilla de Matangani, Dulce de Quitopamba, Dulce de Lazo	Big plants, branched, short growth period
Subtropical	Subtropical area of Bolivia (Yungas)	2500-3000		Plants with intense green color that turn orange as they mature; small seeds, white or orange
Salar	Bolivian Salares	3700-3800	Real	Plants adapted to salty and alkalinic soils; bitter seeds; high saponin content
Altiplanic	Area around Lake Titicaca	3500-4000	Cheweca, Kancolla, Blanca de Julí	Short plants with straight stems; short growth period; resistant to frost

is frost-resistant when the frost occurs before flowering; after that significant damage may occur. Quinoa flowers are sensitive to frost. Quinoa is droughtresistant. It is able to develop even in regions where annual rainfall is in the range 200-400 mm.

The planting season varies from August, in the Andean highlands, extending through December, and in some areas from January to March. Seeds may be spread, but weed control and mechanized practices become difficult. Quinoa is planted in rows (row spacing range 40-80 cm) when mechanized agricultural practices are used. In dry areas, seeds can be deposited at the bottom of the furrows; once planted, the seeds are covered with soil. When rain is more abundant, seeds are deposited on the top of the ridge.

Sowing density may vary according to the region. It has been reported from 0.4 to 0.6 g m<sup>-2</sup> in Bolivian Altiplano, from 0.5 to 2.3 g m<sup>-2</sup> in Puno, and from 0.8 to 1.4 g m<sup>-2</sup> in Ecuador. A density of 1.2 g m<sup>-2</sup> has been recommended in Puno for mechanical drilling. However, sowing density could be related to the climatic conditions of the region where it is cultivated.

At physiological maturity, quinoa is harvested. The grains become dry and hard, making it difficult to break them with a finger nail. Physiological maturity may be reached within 70-90 days after flowering. Depending on the variety, it takes between 5 and 8 months for a plant to mature.

Traditional harvesting is done manually. The plants are either pulled or cut with a sickle, then placed in windrows to dry completely. Threshing is performed by rubbing the panicles by hand against a stone or using threshing on the floor with sticks, animals, or vehicles followed by winnowing. Mechanical threshers have been applied using stationary threshers, some of which are adapted from those used for cereals. The yield of quinoa can be in the range of  $45-500 \,\mathrm{g\,m^{-2}}$  depending on the variety and growing conditions.

The most important fungus disease is downy mildew (Peronospora farinosa), which requires high humidity and temperature as ideal conditions to grow. However, it succeeds in low humidity and low temperature  $(6-10^{\circ}\text{C})$  found in the north Altiplano. The main symptom is chlorotic lesions on the upper surfaces of the leaves, with a white or purple mycelium on the lower surfaces.

The disease brown stalk rot is produced by *Phoma* exigua var. fovaeta. Low temperature, high humidity, and wounds in the plant, such as those produced by hail, favor the growth of pathogen. Dark brown lesions with a vitreous edge (5-15 cm) on the stem and inflorescence are the main symptoms. The stem is often shrunken, the plant may become chlorotic, and progressive defoliation towards the apex may occur.

Kcona kcona (Scrobipalpula sp.) is probably the most serious pest of quinoa. When drought periods and high temperatures are present, insects attack intensely. Larvae first destroy leaves and inflorescence. Later on, when the plant is mature, larvae destroy the panicle and grains. Sometimes, a white powder around the base of the plant is seen as a result of grain destruction. Treatment is performed just before harvest to prevent contamination of seeds and consequently postharvest losses.

## Morphology of the Plant

Quinoa is not a true cereal grain: it is a pseudocereal, which is dicotyledonous. In contrast, cereals are monocotyledonous. In spite of that, the composition of cereals and quinoa is similar as regards the main components.

Quinoa, as a plant, grows 1-3 m high (Figure 1). The seeds can germinate very fast, in a few hours after being exposed to moisture. Roots can reach depths of up to 30 cm. The stem is cylindrical (3-5 cm diame)ter); it can be either straight or with branches, and its color is variable. Depending on the variety, it changes from white, yellow, or light brown to red. Leaves are shaped like a goose's foot. They are formed by petioles and lamina; petioles are long-channeled on their upper side. Lamina is polymorphous in the same plant; rhomboidal or triangular in the lower lamina of leaves, triangular or lanceolate in upper leaves.



Figure 1 Quinoa plant. (Courtesy of Ing. Carlos Nieto C. Pronaleg-Iniap.)

The flowers are incomplete; they do not have petals. Quinoa has both hermaphrodite flowers, located at the distal end of a group, and female flowers located at the proximal end. Quinoa inflorescence is full of bunches (racemose), which emerge on the upper part and do not have branches. The arrangement of flowers in raceme is considered to be the panicle; the length of the panicle varies from 15 to 70 cm. Flowers can be clustered in different forms — either amaranthiforme or glomerulate.

Quinoa is a fruit of the Chenopodium family. The fruit of quinoa is an achene. It produces small, circular-shaped seeds, about 2 mm diameter (250–500 seeds per gram: Figure 2). It is covered by perigonium, which is the same color as the plant: white, yellow, gray, light brown, pink, black, or red. It is easily removed when it is dried. Another two layers enclose the seed. Pericarp adheres to the seed; it contains saponins which confer the bitter taste characteristic of quinoa. Episperm encloses the cylindrical seed as a thin layer. The embryo can be up to 60% of the seed weight. It forms a ring around the perisperm. The high protein content in quinoa, unlike cereals, is explained by the high proportion of embryo.

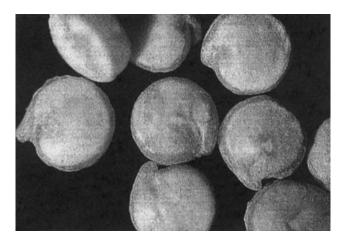
## Chemical Composition and Nutritional Value

The diet of ancient inhabitants of the Inca empire has generated interest due to its extremely nutritious quality. Quinoa seeds contain carbohydrates, protein, fat, minerals, and vitamins. The chemical composition of quinoa depends on the variety and the environment of its cultivation.

#### **Quinoa Seeds**

Protein The protein content of quinoa seeds varies from 8% to 22%, which on an average is higher than common cereals such as rice, wheat, and barley (Table 2). However, it presents less than 50% of the protein found in most legumes. In quinoa, most of the protein is located in the embryo.

In pseudocereals, such as quinoa, albumins and globulins are the major protein fraction (44-77%) of total protein), which is greater than prolamins (0.5-7.0%). Using a modified Osborne's method, protein fractions of quinoa were reported to be 75.1% of albumins + globulins and 19.4% of glutelins (insoluble); no prolamins were found. Thus, quinoa is



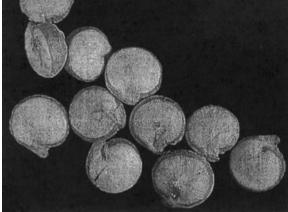


Figure 2 Quinoa seeds. (Courtesy of Silvia Valencia Ch.)

Table 2 Chemical composition of quinoa and some cereals and legumes (g per 100 g dry wt)

	Quinoa	Barley	Maize	Rice	Wheat	Oat <sup>b</sup>	Rye <sup>b</sup>	Bean	Lupine	Soy
Protein	16.5	10.8	10.22	7.6	14.3	11.6	13.4	28.0	39.1	36.1
Fat	6.3	1.9	4.7	2.2	2.3	5.2	1.8	1.1	7.0	18.9
Fiber	3.8	4.4	2.3	6.4	2.8	10.4	2.6	5.0	14.6	5.6
Ash	3.8	2.2	11.7	3.4	2.2	2.9	2.1	4.7	4.0	5.3
Carbohydrates	69.0	80.7	81.1	80.4	78.4	69.8	80.1	61.2	35.3	34.1
kcal 100 g <sup>-1a</sup>	399	383	408	372	392	372	390	367	361	451

<sup>&</sup>lt;sup>a</sup>kcal 100 g<sup>-1</sup>:  $4 \times (\%$ protein + carbohydrates) +  $9 \times (\%$ fat).

<sup>&</sup>lt;sup>b</sup> Source: Kent N (1983) Chemical composition of cereals. In: *Technology of Cereals*, 3rd edn., pp. 27–48. Oxford: Pergamon Press; Koziol MJ (1992) Chemical composition and nutritional evaluation of guinoa (*Chenopodium guinoa* Willd). *Journal of Food Composition Analysis* 5: 35–68.

considered to be a gluten-free grain because it contains very little or no prolamin. Quinoa provides a nutritional, economical, easy-to-prepare, flavorful food source which is of particular relevance for people with gluten intolerance, such as those with celiac disease.

Quinoa has a good balance of the amino acids that make up the protein. It is exceptionally high in lysine (Table 3), an amino acid which is not overly abundant in the vegetable kingdom. It is also a good complement for legumes, which are often low in methionine and cystine.

The nutritional evaluation of quinoa protein has been reported in several studies. The protein efficiency ratio (PER) in raw debittered quinoa was 78-93% that of casein. These figures increased when quinoa was cooked, and became 102-105% of those of casein. Similar results were found when guinoa from the San Luis Valley of Colorado was used. Thus, the quality of protein in quinoa matched that of the milk protein casein.

Carbohydrates The major component in quinoa is carbohydrates, which varies from 67% to 74% of the dry matter. Starch is about 52-60%. The starch compound is located in the perisperm of the seed; starch can be present as simple units or as spherical aggregates. The amylose content is about 11%, which is lower than in cereals, for example, rice (17%), wheat (22%), and barley (26%).

The diameter of quinoa starch granules is in the range of 0.4-2.0 µm. Starch granules in quinoa are smaller than those reported for maize (range  $1-23 \,\mu\text{m}$ ) and for wheat (2-40  $\mu\text{m}$ ). Small-granule starches often exhibit a higher gelatinization temperature; for quinoa the gelatinization temperature range is 57-64°C. Other carbohydrates are found

Table 3 Essential amino acids in quinoa and other foods (g per 100 g protein)

	Quinoa	Maize	Rice	Wheat	Bean	Milk	FAO <sup>a</sup>
Histidine	3.2	2.6	2.1	2.0	3.1	2.7	2.6
Isoleucine	4.9	4.0	4.1	4.2	4.5	10.0	4.6
Leucine	6.6	12.5	8.2	6.8	8.1	6.5	9.3
Lysine	6.0	2.9	3.8	2.6	7.0	7.9	6.6
Methionine <sup>b</sup>	5.3	4.0	3.6	3.7	1.2	2.5	4.2
Phenylalanine <sup>c</sup>	6.9	8.6	10.5	8.2	5.4	1.4	7.2
Threonine	3.7	3.8	3.8	2.8	3.9	4.7	4.3
Tryptophan	0.9	0.7	1.1	1.2	1.1	1.4	1.7
Valine	4.5	5.0	6.1	4.4	5.0	7.0	5.5

<sup>&</sup>lt;sup>a</sup> As reported by FAO. Food and Agriculture Organization, Koziol MJ (1992) Chemical composition and nutritional evaluation of quinoa (Chenopodium quinoa Willd). Journal of Food Composition Analysis 5: 35–68. <sup>b</sup> Methionine + cystine.

in small amounts, such as monosaccharides (2%) and disaccharides (2.3%), crude fiber 2.5-3.9%, and pentosans 2.9-3.6%.

Fat, vitamins, and minerals Quinoa contains from 2% to 10% fat. Quinoa and soy oils exhibit similar fatty acid composition; thus, quinoa is a rich source of essential fatty acids such as linoleic (18:2*n*-6: 52%) and linolenic (18:3*n*-6: 4%). Quinoa is a good source of minerals. It contains more calcium, magnesium, iron, and zinc than common cereals, and the iron content is particularly high (Table 4). Polishing and washing quinoa seeds reduces the mineral content to some extent, 12-15% in the concentration of iron, zinc, and potassium, 27% loss of copper and 3% loss of magnesium. Quinoa contains more riboflavin ( $B_2$ ) and  $\alpha$ -tocopherol than rice, barley, or wheat (Table 4). Quinoa seeds can be a source of vitamin E.

Nutritional disadvantages Saponins and phytic acid are the main disadvantageous factors in quinoa. Other inhibitors, trypsin inhibitor, and tannins are present in low levels.

Trypsin inhibitor in eight varieties of quinoa (range  $1.36-5.04 \,\mathrm{TIU \, mg^{-1}}$ ) was lower than for soybean (24.5 TIU mg<sup>-1</sup>). Trypsin inhibitor is a thermolabile compound which is inactivated by heat treatments.

Polyphenols (tannins) are present in small amounts (0.53 g per 100 g in whole quinoa seeds), which are reduced after scrubbing and washing with water  $(0.23 \,\mathrm{g}\,\mathrm{per}\,100 \,\mathrm{g}).$ 

Table 4 Mineral composition (mg per kg dry wt) and vitamin concentration (mg per 100 g dry wt) of guinoa and some cereals

	Quinoa	Wheat	Rice	Barley			
Minerals (mg per kg)							
Ca	1487	503	69	430			
Mg	2496	1694	735	1291			
K	9267	5783	1183	5028			
Р	3837	4677	1378	3873			
Fe	132	38	7	32			
Cu	51	7	2	3			
Zn	44	47	6	35			
Vitamins (mg per 10	00 g)						
Thiamin (B <sub>1</sub> )	0.38	0.55	0.47	0.49			
Riboflavin (B <sub>2</sub> )	0.39	0.16	0.10	0.20			
Niacin (B <sub>3</sub> )	1.06	5.88	5.98	5.44			
Ascorbic acid (C)	4.00	0	0	0			
α-Tocopherol	5.37	1.15	0.18	0.35			
$\beta$ -Carotene	0.39	0.02	NR	0.01			

NR. not reported.

Source: Koziol MJ (1992) Chemical composition and nutritional evaluation of quinoa (Chenopodium quinoa Willd). Journal of Food Composition Analysis

<sup>&</sup>lt;sup>c</sup>Phenylalanine + tyrosine.

Saponins are glycoside compounds which occur in two groups. According to the nature of the sapogenin moiety, they are conjugated with hexoses, pentoses, or uronic acids. The sapogenins are steroids (C27) or triterpenoids (C30). Using a gas chromatography method, the sapogenins oleanolic acid, hederagenin, 30-o-methylspergulagenate, and phytolaccagenic acid are identifiable in sweet and bitter genotypes of quinoa.

Quinoa saponins are soluble in methanol or water. They have strong detergent properties which form very stable foam in water solutions, and reduce the superficial tension of aqueous solutions. They also show hemolytic activity and are in general toxic to cold-blooded animals which obtain oxygen directly from water. Saponins are also present in common foodstuffs such as peanuts, asparagus, garlic, onion, and spinach.

The amount of saponins present depends on the variety of quinoa. It is higher in bitter-flavor varieties than in sweet, or low-saponin, varieties. Quinoa comprises saponins from 0.1% to 5%.

The saponins of quinoa seeds are reduced to low levels after dry polishing and washing with water. These levels are apparently nontoxic to humans.

The reduction of plasma cholesterol and bile salt concentration has been attributed to the presence of certain saponins in the diet. However, some saponins can form insoluble complexes with minerals, such as zinc and iron, which make the minerals unavailable for absorption in the gut.

Phytic acid Phytic acid (myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) is found in most cereals and legumes at concentrations of 1–3% dry matter. It is also found in some fruits and vegetables.

In cereals, phytic acid is located in the germ. In quinoa seeds, phytic acid is located in the external layers as well as in the endosperm. It has been reported that mean (value) phytic acid concentration, in five varieties of quinoa, was 1.18 g per 100 g.

Studies *in vivo* and *in vitro* have shown that phytic acid interferes with mineral absorption in the gut of humans, because of its ability to form insoluble complexes with divalent minerals such as iron, zinc, and calcium. Even small amounts  $(0.5 \, \mu \text{mol g}^{-1})$  of inositol hexaphosphate or pentaphosphate may reduce the solubility of iron.

Inositol hexaphosphate (IP<sub>6</sub>) was mainly found in varieties from Ecuador: sweet INIAP-Tunkahuan (11.3  $\mu$ mol g<sup>-1</sup>) and bitter INIAP-Ingapirca (8.6  $\mu$ mol g<sup>-1</sup>). These figures were almost completely

reduced to  $0.3 \,\mu\text{mol g}^{-1}$  after fermentation of the germinated quinoa flour. At the same time a five- to eightfold increase in the amount of soluble iron was found.

### **Quinoa Leaves**

The leaves of quinoa are compared to spinach as regards flavor. Quinoa leaves are cooked as a green vegetable or used raw in salad. Leaves contain (on a dry basis) carbohydrates 4.8 g per 100 g; protein 3.3 g per 100 g; fat 1.8 g per 100 g; ash 3.3 g per 100 g; fiber 1.9 g per 100 g. The protein concentration of quinoa leaves is similar to spinach; however, it contains slightly more isoleucine (5.8 g per 100 g protein) and valine (7.5 g per 100 g protein). The amount of fatty acids such as palmitic (16:0; 16.7%) and stearic (18:0; 1.3%) is higher than in the grains. Quinoa leaves are a rich source of vitamin A: they contain 2085 µg RE (retinol equivalents) per 100 g (fresh wt), and vitamin E 2.9 mg  $\alpha$ -TE (alpha-tocopherol) per 100 g. Fresh quinoa leaves contain more magnesium (83 mg per 100 g fresh wt) and sodium (289 mg per 100 g fresh wt) than spinach leaves. Using a gas chromatography method, sapogenins were detected in the leaves of four sweet and bitter genotypes of quinoa. Sapogenins increased as the plant matured. After 120 days of sowing, the sapogenin content on the leaves of sweet genotypes varied between 0.013 and 0.017 g per 100 g (dry matter) and in bitter varieties varied between 0.02 and 0.16 g per 100 g (dry matter). Hederagenin was the major sapogenin present in the leaves.

#### **Uses**

Quinoa has a natural seed coating containing saponins, which encases the seed and confers the bitter taste which is characteristic of quinoa. Saponins must be removed before consuming. External coatings are removed using either a wet or dry process. The traditional wet process used in rural areas is hand scrubbing in alkaline water. This process is used on a commercial scale; it involves abrasive de-hulling to remove the external coverings, followed by a thorough washing. However, this method has economical and ecological restrictions: the water demand is high and waste water is contaminated with saponins, which are toxic to cold-blooded animals. Moreover, wet seeds must be dried immediately, or they may germinate after a few hours in wet conditions.

A dry method is also used. The seeds are scrubbed and polished in order to remove, as fine powder, external coatings. The equipment used to polish other grains has been adapted for use with quinoa seeds, with excellent results. This method presents several advantages; no water is needed, no heat treatment

to dry the seeds is required, and no environmental contamination is produced. This method is best suited to sweet varieties (low saponin content) of quinoa.

A combination of dry and wet processes is applied to bitter varieties (high saponin content) of quinoa. Saponin is first removed by polishing, when most of the saponin is removed. Then, saponins that remain in the seeds are washed with water, followed by a dry process. Any of the processes described above makes the quinoa ready for use by the consumer or further processing such as grinding.

After removing the saponins, quinoa seeds can be boiled in water (15-20 min) and served as a grain. Cooked seeds swell to about two or three times their original size. Seeds become transparent, with tiny white bands circling across the midsection.

In Chile, Ecuador, Peru, and Bolivia, the whole seeds are used in soups, salads, casseroles, chilli, and stew, as well as roasted and ground in several kinds of desserts.

Quinoa can be eaten as a rice replacement, as a hot breakfast cereal, or boiled in water to make an infant cereal. The seeds can even be popped like popcorn. Seeds can be ground and used as a flour, or sprouted. The sprouts need to turn green before they can be added to salads.

Quinoa flour can be mixed with maize or wheat flour. Several levels of substitution of quinoa flour have been reported, for instance, in bread (10–13% quinoa flour), noodles, and pasta (30–40% quinoa flour) and sweet biscuits (60% quinoa flour). All yield products of excellent quality. Quinoa flour can also be drum-dried and extruded, providing products with good physical, sensorial, and nutritional qualities. Solid-state fermentation of quinoa with Rhizopus oligosporus Saito was performed, giving a good-quality tempeh.

In Bolivia, in 1975, the government adopted a resolution mandating that 5% of quinoa flour must be added to all pastas, crackers, and breads.

Leaves, like the seed, can also be cooked, made into a spinach-like dish, or may be served raw in a salad. Tonics, puddings, and syrups can also be prepared from the leaves. The foaming qualities of saponin are sometimes used to produce a frothier "chicha."

In industry, saponins from quinoa have multiple purposes. They are used as soap for washing hair or clothes, in a compound for a fire extinguisher, or in photo processing. Dried stalks of the plant are used as fuel, or may be used in preparing bleach or dyes.

## **Future Perspectives**

The nutritional excellence of quinoa has been known since ancient times in the Inca empire. Nowadays,

quinoa has been recognized for its nutritional benefits all over the world, and for its protein, mineral, and vitamin content.

The importance that quinoa could play in nutritional behavior is being emphasized, not only in developing countries but also in the developed world. In the Andean countries, quinoa crops could play an important role in the future of their economies, giving a new export market, as well as in national subsistence. Moreover, quinoa could be a strategic crop used to complement the diet in rural/marginal regions where energy-protein malnutrition affects most of the population of the developing countries. Quinoa, as the "mother grain," represents an exotic and healthy rediscovery in the developed world.

Germplasm collection should continue in countries of the Andean region. Agronomic research, including plant density, potential cultivation, phenology, morphology, physiological maturity, yield, and weed control, should be performed. Further research is needed in order to study the adaptability of different cultivars to "new homes of quinoa" in the USA and Europe. Using mechanized agriculture may facilitate mechanical harvesting of the grain, reducing postharvest losses.

Improving methods for removing saponins without significant modification of nutritive value are encouraged. The selection of sweet genotypes with a very low saponin content in the seeds, large grain, and high yield are the main breeding goal. Sweet genotypes could be selected early in plant development in order to speed up the selection process. Further research is needed to find markers for indirect selection for sweet genotypes.

The need for intensive cultivation of quinoa should be emphasized; this could meet quality and quantity needs by the food industry. Besides, aggressive promotion campaigns should be carried out to encourage greater consumption of the grain. Finally, quinoa is being promoted as an extremely healthy food a supergrain – of the future (gluten free). It is a food of the twentyfirst century.

See also: Taxonomic Classification of Grain Species. Grain Production and Consumption: South America.

## **Further Reading**

Berti DM, Serri GH, Wilckens ER, Urbina PM, and Figueroa CI (1997) Determination of physiological maturity and the optimum maturity for harvesting quinoa (Chenopodium quinoa, Willd) in Chillan. Agro-ciencia (Chile) 13: 135-141.

Jacobsen S-E (1993) Quinoa (Chenopodium quinoal, Willd): A Novel Crop for European Agriculture. Ph.D.

- thesis. Denmark: Royal Veterinary and Agricultural University.
- Kent N (1983) Chemical composition of cereals. In: Technology of Cereals, 3rd edn., pp. 27–48. Oxford: Pergamon Press.
- Koziol MJ (1992) Chemical composition and nutritional evaluation of quinoa (*Chenopodium quinoa* Willd). *Journal of Food Composition Analysis* 5: 35–68.
- Kuhn M (1998) Pseudocereals: a challenge for further research and product development. In: *Increasing the Utilisation of Sorghum, Buckwheat, Grain Amaranth and Quinoa for Improved Nutrition*. International Association for Cereals Science and Technology, Cereals Conference Symposium Challenges in Speciality Crops. 16th ICC Cereals Conference. Vienna, Austria. London: Institute of Food Research.
- Mastebroek H, Limburg H, Gilles T, and Maevin H (2000) Occurrence of sapogenins in leaves and seeds of quinoa (*Chenopodium quinoa* Willd). *Journal of Science Agriculture*, 80: 152–156.
- Mujica A, Jacobsen SE, and Izquierdo J (1998) Libro de Campo: Prueba Americana y Europea de Quinua (Chenopodium quinoa Willd). Red de Cooperación Técnica en Produción de Cultivos Alimenticios de la Oficina Regional de la FAO para América Latina y el Caribe. Puno, Peru: Universidad Nacional del Altiplano.
- Nieto C and Fisher V (1993) La Quinua un Alimento Nuestro. Proyecto Desarrollo Comunitario, Bélgica Ecuador. Proyecto 3 P-90-160 INIAP-CIID. Santa Catalina, Ecuador: Programa Cultivos Andinos.
- Nieto C and Vimos C (1992) La Quinoa, Cosecha y Poscosecha. Algunas Experiencias en Ecuador. Boletín Divulgativo no. 224. Quito, Ecuador: Departmento de Comunicación Social del INIAP.
- Risi J and Galwey NW (1984) The chenopodium grains of the Andes. Inca crops for modern agriculture. *Advances in Applied Biology* 10: 145–216.
- Ruales J (1992) Development of an Infant Food from Quinoa (Chenopodium quinoa, Willd). Technological

- Aspects and Nutritional Consequences. Ph.D. thesis. University of Lund, Sweden.
- Tapia M, Gandarillas H, Alandia S, et al. (1979) La Quinua y la Kañiwa. Cultivos Andinos. Bogotá, Colombia: Centro Internacional de Investigaciones para el Desarrollo (CIID), Instituto Interamericano de Ciencias Agrícolas (IICA).
- Valencia S, Svanberg U, Sandberg A-S, and Ruales J (1999) Processing of quinoa (*Chenopodium quinoa* Willd): effect on the *in vitro* iron availability and phytate hydrolysis. *International Journal of Food Science Nutrition* 5: 203–221.
- Whali C (ed.) (1990) Quinoa: Hacia su Cultivo Comercial casila 17-110-6053, Quito, Ecuador: Latinreco.

## **Relevant Websites**

http://orton.catie.ac.cr — Website of the database INPERU of the National Agricultural Library (http://orton.catie.ac.cr/INPERUPE.htm) at Universidad Nacional Agraria La Molina, Lima, Peru, which can be searched for articles on quinoa.

http://www.ecoaldea.com — a medicinal plant website (in Spanish) with information on quinoa.

http://www.cipav.org.co – Website of Centro para la Investigación en Sistemas Sostenibles de Producción Agropecuaria (CIPAV Foundation) a Columbian NGO based in Cali, which can be searched for information on quinoa.

http://www.hort.purdue.edu — Website of the New Crop Online Program (http://www.hort.purdue.edu/newcrop) of the Center for New Crops and Plant Products at Purdue University. NewCROP provides windows to new and specialty crop profiles, including quinoa.

http://www.ecuarural.gov.ec.



Rape Seed see Canola: Genetics and Breeding; Agronomy; Harvest, Transport, and Storage; Processing.

## RESEARCH ORGANIZATIONS OF THE WORLD

Contents

Europe and North America
Asia/Pacific, Central/South America, and Africa/Middle East
CGIAR
Global Trends and the Commercial Sector

## **Europe and North America**

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#### Introduction

This article describes the activities of some of the major research institutes in Europe and North America in the area of grain science. There is a deliberate bias towards provision of more detailed information on institutions in English-speaking countries, reflecting the main readership of this article. Compared with the long history of cultivation of grains (many thousands of years), use of research principles to address constraints is a comparatively recent approach. Agricultural societies were established in the UK, France, and Germany in the mid-1800s (e.g., The Royal Agricultural Society of England (1839); La Société des Agriculteurs de France (1850); Deutsche Landwirtschafts-Gesellschaft (1885)) and by the midlate nineteenth century agriculture experiment stations were appearing in Europe and North America. The tradition of government-funded grains research is longest established in Europe and North America,

although similar initiatives were started in the late nineteenth century in a number of European colonies.

A number of trends are identified, including the move towards formation of networks between research institutes as well as the shift in focus of several grain R&D institutions from a largely production focus to an emphasis on environmental issues such as water-use efficiency and sustainability of farming systems. Other consistent trends include the greater importance of levies from grain-growers in supporting research in a number of countries (e.g., since 1959 in France), and the increase in commercial involvement in grain breeding and biotechnology. Governmentfunding bodies have also shown a greater interest in assessing the impacts of research that they fund, including carrying out more economic analysis of projects - research is increasingly viewed as an investment rather than as "grants." The most significant trend in terms of research intensity is the very significant growth over the last couple of decades in the level of grains R&D carried out by the private sector. Most publicly-funded agricultural research in North America and Europe is concentrated in the US, France, and Germany, with the UK and Canada having smaller roles. There is also a much greater proportion of agricultural (including grains) research

carried out in universities in Europe and North America compared with developing countries, where it is overwhelmingly within government-funded research institutes. Grains R&D carried out by commercial and industry organizations is reviewed in a separate article (*see* Research Organizations of the World: Global Trends and the Commercial Sector).

## **Taxonomy of Research Institutions**

## Major Public Sector (Government and University) Grain Science Organizations

USA The USA has very large private sector (seed companies, milling, baking, brewing, and oilseed processing companies, and biotechnology companies) and government investments in grains R&D. A wide range of temperate grain crops are targeted — especially wheat, barley, maize, oilseeds, and pulses (also subtropical rice), with most resources dedicated to maize and wheat research. Both state and federal governments invest in agricultural research, with the federal investment being slightly higher.

The Agricultural Research Service (ARS) is the principal in-house research agency of the (National) US Department of Agriculture (USDA), and together with the "land grant" universities form the main research centers for grains research. In the past, there has been comparatively limited interaction and coordination between different USDA centers carrying out grains research, although the development of a "national program" structure by USDA-ARS in recent years has increased the amount of coordination. Most of the grains research activities are within the "crop production, product value, and safety" cluster of programs. Some of the major objectives of USDA grains research include:

- crop improvement, integrated crop production systems;
- crop protection and quarantine;
- crop genetic resources, genomics, and genetic improvement;
- fundamental research on plant productivity and quality and plant-pathogen interactions; and
- postharvest quality-maintenance, environmentally friendly efficient-processing, and value-added products including the use of grain crops as biofuels.

Some USDA centers are free-standing multidisciplinary USDA research centers while others are colocated on university campuses. Many centers have the mandate to carry out research directed at the particular agro-ecological zone in which the institute is based. For example, wheat is grown in many parts of the US, but there are different quality types suited for different locations — Soft Red Winter Wheat in the Midwest, Virginia, and North Carolina; Hard White and some Hard Red Winter Wheat in Pacific Northwest, Hard Red Winter varieties in the Great Plains, from Texas to Montana on the Canadian border, and Spring varieties in northern central states such as Minnesota, North, and South Dakota and Montana — and appropriately located USDA centers develop germplasm and agronomy packages for the different types of varieties. Some of the major USDA centers carrying out grains research, shown in alphabetical order of location, are based in:

- Aberdeen, ID: small grains and potato germplasm research;
- Albany, CA (Western Regional Research Center): genomics and gene discovery; crop improvement/ utilization; plant mycotoxins;
- Ames, IA: corn insects and crop genetics;
- Beltsville, MD: (Beltsville Agricultural Research Center) Sustainable Agricultural Systems Laboratory; Instrumentation and Sensing Laboratory; National Germplasm Resources Laboratory; Soybean Genomics and Improvement Laboratory;
- College Station, TX (Southern Plains Agricultural Research Center): crop germplasm and insect pest research;
- Colombia, MO: plant genetics research;
- Corvalis, OR: forage seed and genetics research;
- Fargo, ND (Red River Valley Agricultural Research Center): cereal crops research, wheat quality;
- Fort Collins, CO: National Center for Genetic Resources Preservation;
- Ithaca, NY: plant genetic resources, plant protection research;
- Lane, OK (Southern Central Agricultural Research Laboratory): genetics and production research;
- Lincoln, NE: wheat, sorghum, and forage research;
- Lubbock, TX: Cropping Systems Research Laboratory:
- Madison, WI: cereal crops research;
- Manhattan, KS (Grain Marketing and Production Research Center): biochemical and structural aspects of grain quality, stored grains entomology, fungal diseases, plant physiology of cereals;
- Peoria, IL (National Center for Agricultural Utilization Research): cereal products and food science research, plant polymer research, mycotoxin research, new crops processing research;
- Raleigh, NC: soybean and nitrogen fixation research, basic plant science;

- Shafter, CA: western integrated cropping systems research;
- Stillwater, OK: plant science and water conservation research;
- Stoneville, MS: crop genetics and production research;
- Stuttgart, AR: Dale Bumpers National Rice Research Center;
- Tifton, GA: crop genetics and breeding research, crop protection and management research;
- Urbana, IL: soybean/maize germplasm, pathology, and genetics research;
- Wooster, OH: soft wheat quality research, corn, and soybean research; and
- Wyndmoor, PA: crop conversion science and engineering.

USDA also has a (smaller) Economic Research Service, which carries out research and communication activities in many areas relevant to grains, including farm-level risk management, commodity outlook research, and research on marketing and production sustainability. The USDA Federal Grain Inspection Service has a technical center in Kansas City, MO, which validates equipment and methods for grains analysis.

The "land grant" college system was established in 1862 under the Morrill Act, in the same year that USDA was established – the name is derived from the fact that the colleges were originally endowed by grants of public lands in the expanding western United States. The 1887 Hatch Act established the agricultural experiment station system to enable these colleges to undertake research, and the 1914 Smith—Lever Act established the cooperative extension system. This unique system has the advantage of linking state government R&D and extension activities to university research. At the federal level, land grant university programs are supported through the USDA Cooperative Research, Education, and Extension Service. Universities have anywhere from a few to over a dozen discrete experiment stations at different locations in their state, again often corresponding to different agro-ecological zones, but also allowing researchers to carry out a local outreach function. Usually a significant proportion of the researchers - maybe half - are located on the main university campus and the rest throughout the state. There is usually a balance of applied grains research on constraints to productivity in the particular state and a proportion directed towards more fundamental research. Cooperative extension systems from land grant universities have a state development mandate and often involve location of farm advisors in counties throughout the

state. Virtually all US states have one (and in some cases, two) land grant universities, and about half of them have strong grains research activities, usually across several departments rather than in a single program. A summary of some of the main US universities that carry out grains research is provided in Table 1. Land grant universities are usually predominantly state-funded, but with significant federal funding as well.

Canada The Canadian Grains Commission in Winnipeg, Manitoba regulates grain handling and establishes and maintains grain quality standards for Canada. Its Grain Research Laboratory has emphasis on understanding the genetic, environmental, structural, and biochemical basis on end-use quality of major Canadian grains, including bread and durum wheat, barley, oilseeds, and pulses (peas, lentils, chickpeas, and beans). There is a special focus on analytical methods and processing technologies. The Canadian International Grains Institute, also in Winnipeg has a technical training and information dissemination role.

Agriculture and Agri-Food Canada carries out grains research at several of its 19 research centers across the country. Cereal research is carried out mainly at the Winnipeg (Manitoba) Cereal Research Centre emphasizes the development of bread and durum wheat and oat varieties for the prairies, with research strengths in genetics, biotechnology, plant pathology, cereal chemistry, and quality evaluation. There are also active research groups on grain and oilseed storage and insect pests of stored commodities. The Eastern Cereal and Oilseed Research Centre (Ottawa, Ontario) develops new varieties of wheat, barley, corn, oats, and soybeans along with crop protection and management systems. Wheat breeding is also carried out at the Semi-arid Prairie Agricultural Research Centre (Swift Current, Saskatchewan) and the Lethbridge Research Centre (Kamloops, Alberta). There is an emphasis on disease and disease resistance as well as agronomy. The Brandon Research Centre (Brandon, Manitoba) is the main site of barley breeding, while the Lacombe Research Centre (Lacombe, undertakes oat-varietal improvement Alberta) research.

The Saskatoon Research Centre (Saskatoon, Saskatchewan) is the main center of breeding programs for canola and mustard and conserves many of the grains genetic resources of Canada. Some canola breeding also takes place at the Lacombe Research Centre, while the Greenhouse and Processing Crops Research Center also carries out soybean breeding. Agronomy research for grains production is undertaken at Soils and Crops Research and

 Table 1
 Some United States universities undertaking significant grains research

University (website)	Grains research emphasis
Colorado State University, Fort Collins, CO; College of Agr. Sci. (www.agsci.colostate.edu)	Genomics, biotechnology, dryland farming systems, wheat breeding, precision agriculture
Cornell University, Ithaca, NY; College of Agr. and Life Sci. (www.cals.cornell.edu)	Genetics, biotechnology, basic plant science, maize breeding, seed science, cereal pathology, cereal product rheology
Iowa State University, Ames, IA (www.ag.iastate.edu)	Corn and soybean breeding, nonfood crop products, corn quality, basic plant sciences, crop genomics, seed technology
Kansas State University, Manhattan, KS (www.oznet.ksu.edu)	Wheat, soybean, and sorghum breeding; cereal (wheat, corn, sorghum, millet) agronomy and physiology, entomology, cereal chemistry, milling and baking, livestock feeds
Michigan State University, East Lansing, MI (www.maes.msu.edu)	Grain crop breeding, cereal chemistry, genomics
Montana State University, Bozeman, MT; College of Agr. (www.montana.edu/agriculture)	Winter wheat, barley, durum, pulses, canola, and soybean breeding, precision agriculture, integrated pest management
North Carolina State University, Raleigh, NC; College of Agr. and Life Sci. (www.cals.ncsu.edu)	Bioinformatics, integrated pest management
North Dakota State University, Fargo, ND; College of Agr., Food Systems and Nat. Res. (www.ag.ndsu.nodak.edu)	Basic research on wheat quality and processing; milling; pasta production; brewing; farming systems, pathology, and entomology
Oklahoma State University, Stillwater; Div. of Agr. Sci. and Nat. Res. (www1.dasnr.okstate.edu)	Baking, grain protein chemistry, oilseed chemistry, plant diseases
Ohio State University, Columbus, OH; Ohio Agr. R&D Center (www.oardc.ohio-state.edu)	Maize production systems, cereal and oilseed quality, Soft Red Winter Wheat and soybean agronomy and breeding, soy protein functionality
Pennsylvania State University, Univ. Park, PA; College of Agr. Sci. (www.research.cas.pse.edu)	Starch chemistry, entomology
Purdue University, West Lafayette, IN (www.agriculture.purdue.edu/arp)	Plant stress physiology, biotechnology, integrated pest management, precision agriculture, new grain products, carbohydrate research
Rutgers University, New Brunswick, NJ; Center for Adv. Food Tech. (www.foodsci.rutgers.edu/caft) NJ Agr. Experiment Station (www.cook.rutgers.edu)	Cereal processing, plant biotechnology
Texas A&M University, College Station, TX (www.agresearch.tamu.edu; www.agrprogram.tamu.edu)	Precision agriculture, farming systems, plant genomics, grain food processing
University of Arkansas, Fayetteville, AR; Dale Bumpers College of Agr. Food and Life Sci. (www.uark.edu/admin/aes)	Rice, wheat, oat, and soybean breeding; agronomy and physiology, rice processing
University of California, Davis College of Agr. and Environ. Sci. (www.ucanr.org; www.caes.ucdavis.edu)	Genetic resources conservation, genetics of cereals and legumes, agronomy, wheat breeding, entomology, plant pathology, rice breeding (with California Rice Exp. Station and USDA)
University of Illinois, Urbana-Champaign, IL (www.web.aces.uiuc.edu)	Maize and soybean breeding and agronomy, cereal diseases, starch chemistry
University of Idaho, Aberdeen, Moscow College of Agr. and Life Sci. (www.ag.uidaho.edu)	Grains breeding and agronomy (wheat, oilseeds)
University of Minnesota, St. Paul, MN; Colleges of Agr., Food and Environ. Sci. (www.maes.umn.edu)	Cereal structure, wheat and barley breeding and pathology, soybean diseases, precision agriculture, cereal food processing
University of Missouri, Colombia, MO; College of Agr., Food and Nat. Res. (www.cafnr.missouri.edu)	Tillage, soybean, sorghum, and corn cropping systems and soybean breeding, basigrain science, maize genome mapping (Donald Danforth Center, St. Louis, MO)
University of Nebraska, Lincoln, NE Institute of Agr. Natural Res. (www.ard.unl.edu)	Processing of grain starches, cereal breeding, genomics
Washington State University, Pullman, WA; College of Agr. and Home Econ. (www.cahe.wsu.edu)	Grain crops breeding and genetics, pathology/entomology, soil science, processing

Development center (Sainte-Foy, Quebec), Crops and Livestock Research Center (Charlottetown, Prince Edward Island), the Brandon Research centre, and the Semi-arid Prairie Agricultural Research Centre.

Several universities carry out grains research in Canada, and in some cases individual departments and staff interact closely with Agriculture Canada scientists. These include:

- University of Manitoba, Winnipeg, through its Faculty of Agricultural and Food Sciences carries out research on canola and wheat breeding and postharvest technology, grain storage technology, cereal chemistry, baking technology, pulse food science, functional foods, and cereal agronomy including precision agriculture.
- University of Saskatchewan, Saskatoon, through its College of Agriculture has a research emphasis on wheat, barley, oat, canola, and legume (lentil, pea, dry bean, and chickpea) breeding and agronomy, plant genetic engineering, molecular marker development, and malting and brewing science.
- University of Alberta, Edmonton, through its Department of Agricultural, Food and Nutritional Science conducts studies on grain and oilseed crops for Western Canada – breeding, disease resistance, agronomy, and livestock feed utilization.
- University of Guelph, Ontario, through its Department of Plant Agriculture emphasizes grain crops breeding (soybean, barley, wheat, and canola), agronomy, pathology, and genome mapping.
- McGill University, Quebec, Ontario, through its Faculty of Agricultural and Environmental Sciences, is involved with basic plant science, biotechnology, and some grains food processing research.

The National Research Council (NRC) Plant Biotechnology Institute in Saskatoon carries out basic research in genomics and proteomics, and has a special interest in the modification of oil composition and of agronomic traits of oilseeds, as well as in cereal and legume molecular marker and transformation technology. Some individual provinces also have agencies that carry out applied agriculture and food research.

Mexico In Mexico, maize is the main grain crop but the national program played an important role in semi-dwarf wheat breeding ahead of the establishment of CIMMYT. Most federal government agricultural research is carried out by the Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias (INIFAP); some of the state governments have small grains research activities. There is also significant activity within the higher education and science and technology ministries Consejo Nacional de Cienia y Tecnologia (CONACYT), with grains research being carried out in several of their regional centers (Centro de Investigación en A, limentación Y Desarrollo AC (CIAD), Centro de Investigación Y Assistencia en Tecnologiia Y Diuseno del Estado de Jalisco (CIATEJ), Centro de Investigaciones Biologicas del Noreeste (CIBNOR), and El Colegio de la Frontera Sur (ECO SUR)). Postgraduate colleges, within the universities managed under the agriculture secretariat (SAGAR), along with the agronomy faculties in autonomous universities in different Mexican states are also active. Mexico is home to a number of national seed companies (maize, sorghum, wheat), along with subsidiaries of foreign (especially US) seed companies and these carry out variety trials and agronomy work.

Western Europe In Western Europe, yields of most major cereals are high, and it is clear that there has been a shift away from research on increasing production to an emphasis on environmentally friendly production. Other research areas include:

- quality and processing technologies, including value-addition and nonfood uses of grains;
- food safety, nutrition, and health with grains the main emphasis is on mycotoxins, nutraceuticals, dietary fiber, and allergies and intolerances;
- traceability e.g., of GM cereals; and
- breeding, genomics, and diagnostic technologies.

Among the United Kingdom government institutes, the Biotechnology and Biological Sciences Research Council (BBSRC) places some emphasis on research on grains, with several of its eight institutes involved in the research program. These institutes and their research areas include:

- John Innes Centre, Norwich starches, plant biotechnology, fundamental biology of cereals and brassica crops;
- Institute of Food Technology, Norwich basic food science, including research on grain foods ingredients stricture, protein functionality in breadmaking quality, and allergenicity;
- Silsoe Research Institute; and
- Institute for Arable Crops Research (recently renamed Rothamsted Research after consolidation of the Rothamsted and Long Ashton sites) grain crop performance and improvement, disease management, invertebrate pests, and weeds of grain crops and crop-environment interactions.

The former Ministry of Agriculture, Fisheries and Food (now known as the Department for Environment, Food and Rural Affairs) is directly responsible for the Central Science Laboratory in York. The Laboratory has an emphasis on food safety and quality, pest and disease management in crops and chemical residues. The Department also carries out some research on sustainability of grain-based farming systems in the UK. There is a separate Department of Agriculture for Scotland, with two relevant institutes, the Rowett Research institute (food processing, nutrition, biotechnology) and the Scottish Crop Research Institute (cereal quality and nutrition, cereal and brassica breeding, plant pathology).

Major British universities carrying out research in grain science include:

- University of Reading has research programs on agriculture-environment interactions, crop protection, crop breeding and agronomy, seed science, farming systems, and cereal chemistry;
- University of Nottingham, Sutton Bonington has programs in basic plant sciences, agriculture and food sciences, including agronomy of temperate and tropical cereals, and research on grain crop abiotic field stress;
- University of Bristol, Long Ashton;
- University of East Anglia, Norwich carries out research on cereal molecular biology;
- Heriott-Watt University, Edinburgh, Scotland;
- University of Plymouth, Devon;
- University of Leeds, Procter Department of Food Science;
- University of Manchester Institute of Science and Technology, Satake Centre for Grain Process Engineering;
- University of Wales, Centre for Arid Zone studies; and
- Queen's University Belfast, The School of Agriculture and Food Science plant pathology, grain crop physiology, aflatoxins, oat quality.

The Home-Grown Cereals Authority is an organization funded on grower levies to support research, provide market information and extension information for British grain farmers.

In France, the Institut National de la Recherche Agronomique (INRA) is the main government research organization in agriculture and grains research. INRA has a large center in Nantes with a research emphasis on agriculture and food processing. There is an additional emphasis on cereal food and nutritional quality and nonfood uses, with a strong emphasis on processing technologies and basic protein and starch molecular sciences related to cereals. At INRA Montpelier, there is also on-going

research on processing technology, grain crop production systems, plant protection, and genetic resource conservation as well as more basic studies on grain crop development. Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD) is a foreign-aid organization that carries out research both within France and in developing countries. CIRAD has well-founded programs covering production and postharvest technology of rice, maize, and tropical grain legumes, research on soil and water management for crop production, and strong molecular marker/biotechnology programs. Auvergne is a partnership involving private companies such as Groupe Limagrain and public organizations such as INRA, and have established a cereal genotyping laboratory to form a link between breeding and genomic research. Several French universities have Ecole Nationale Superieure Agronomique – for example in Paris-Grignon, (the Institut National Agronomique (INA-PG)), in Montpellier, Rennes, Nancy, and Toulouse. ARVALIS Institut du végétal is responsible for Applied Research on cereals, maize, pulses, potato, and forage crops. The activities of this technical institute, which is run and financed by farmers, include sustainable cropping systems, precision farming, remote sensing, satellite images, biotechnology, farm economics, quality assurance, safety quality, traceability, human food, animal feed, and technology transfer. CETIOM is responsible for similar topics on oil crops.

In Germany, the Federal Ministry of Food, Agriculture and Forestry has several relevant institutes – the federal institute for Grain, Potato, and Lipid Research (BAGKF, Detmold), the Federal Research Centre for Plant Breeding (BAZ), the Federal Research Institute for Nutrition (BFE), and the Institute of Agricultural Engineering (ATB, Potsdam-Bornim). There is also an institute for Plant Genetics and Crop Plant Research (IPK, Gatersleben). At least two of the Max Planck institutes undertake grains research – the Institute of Plant Breeding in Cologne and the Institute of Molecular Plant Physiology in Golm. Many of the German states have research agencies or centers in agriculture and several of these carry out grains research, sometimes in conjunction with universities. Universities carrying out grains research include Humboldt University, Technical University of Berlin, Technical University of Munich, and the Universities of Bonn, Goettingen, Hohemheim, Kassel, Kiel, Rostock, and Paderborn.

In Ireland, the Irish Agriculture and Food Development Authority (TEAGASC) has nine research centers. The Oak Park Research Centre emphasizes research on grain crops, especially aiming for more efficient production systems for cereals, grain lupins,

and rape oilseed. There is a strong emphasis on the management of fungal and viral diseases of cereal crops. The main university for production research on grain crops is University College Dublin, while University College Cork emphasizes grains processing research.

In Switzerland, the major government grains research institutes are the Federal Office of Agriculture Research Station for Plant Production and Research Station for Agro-ecology and Agriculture, while in the university sector the Swiss Federal University for Technology in Zurich carries out most of the applied grains sciences research.

The Cereal Institute, National Agricultural Research Foundation (NAGREF), Thessaloniki, Greece is the Greek government research institute responsible for cereal breeding and cereal food product development. However, the Ministry of Agriculture has a separate research service with a network of institutes in different parts of the country. At least three universities carry out some grains research work - Agricultural University of Athens, Aristotle University of Thessaloniki, and the Mediterranean Agronomic Institute at Chania. In Hungary, the Ministry of Agriculture and Rural Development manages the Central Food Research Institute (KEKI) and the Agricultural Biotechnology Center (MBK), as well as the Cereal Research Institute in Szeged. The Hungarian Academy of Science has several institutes relevant to grain science – the Agricultural Research Institute, the Research Institute for Soil Science and Agricultural Chemistry, and the Plant Protection Institute. Universities with research areas most relevant to grain science are the Technical University of Budapest (grains processing), the Universities of Agricultural Science at Godollo, Debrecen, and Pannon. In Austria, the Federal Ministry of Agriculture and Forestry has research institutes for general agriculture as well as an Institute of Cereal Processing, Applied Soil Science and Agricultural Engineering. There is also a University of Agriculture, Forestry and Natural Resources in Vienna. In the Czech Republic, relevant Ministry of Agriculture research institutes include the Food Research Institute, Research Institute of Crop Production, Research Institute for Soil and Water Conservation, and Research Institute of Agricultural Engineering. There are semicommercial institutes responsible for cereals (Kromeriz Ltd.), Oilseed crops (Oseva Ltd.), and brewing and malting. Two universities - Mendel University of Agriculture and Forestry and Czech University of Agriculture are relevant to grains research.

In the Netherlands, several institutes within the government Department of Agricultural research (DLO) carry out grains research – including the

Agrotechnological Research Institute (ATO – cereal chemistry and processing), Plant Research International, and Applied Plant Research Institute (PPO). Wageningen Agricultural University is the main university research center for grains research. In Belgium, there are relevant government agricultural research centers in Gent and Gembloux, while at least four universities carry out some aspects of grains production and processing research – Ghent, Leuven Catholic University, Gembloux, and Louvain-la-Neuve.

The Italian government Ministry of Agriculture and Forestry has several research institutes covering grains research, including the Research Institutes for Cereals (ISC), Industrial Crops, Plant Nutrition, Plant Pathology and Food and Nutrition (IMRAN). The National Research Council (CNR) has a separate system of research institutes, and some of these also carry out grains research. Many major states in Italy host universities which have agriculture faculties, and most of these carry out at least some grains research — Turin, Milan, Padua, Udine, Bologna, Piacenza, Florence, Pisa, Ascoli, Peruge, Viterbo, Naples, Bari, Potenza, Reggio Calabria, Catania, Palermo, and Sassari.

In Sweden, the Government Swedish Institute for Food Research and the Swedish Institute for Food and Biotechnology (SIK, a joint industry/government body) carry out grains processing research. Production research and basic scientific investigations within universities include the Swedish University of Agricultural Sciences at Svalov and Lund University. Three main institutes in Norway carry out grains research. Within the government Ministry of Agriculture, there is the State Agricultural Research Station (SFL) and the Agricultural University of Norway at Aas, while MATFORSK, the Norwegian Food Research Institute is a nonprofit organization.

In Finland, the Ministry of Agriculture and Forestry has the Agricultural Research Centre of Finland and Plant Breeding Institute, while VTT Biotechnology in Espoo carries out cereal technology research in close collaboration with industry. The University of Helsinki is the main higher education center dealing with grains research. In Denmark, the Ministry of Food, Agriculture and Fisheries has Institutes in Agricultural Sciences and Agricultural Engineering. The Royal Veterinary and Agricultural University also carries out grains research. Activities related to the Carlsberg laboratory are described in Research Organisations of the World: Global Trends and the Commercial Sector.

In Spain, the Ministry of Agriculture, Fisheries and Food runs the National Institute of Agricultural and Food Technology (INIA), while the Madrid Polytechnic University has a large faculty involved with research in agriculture. There is also an INIA in

Portugal, while agricultural research is carried out at the Technical University of Lisbon, the Universidade de Tras-os-Montes e Alto Douro, and Universidade dos Ascores.

In Russia, the Russian Academy of Agricultural Sciences comprises about 200 institutes. These include All-Russia Research Institutes (e.g., Plant Quarantine (Bykovo), Plant Protection (St. Petersburg), Biological Plant protection (Ekaterinburg) which carryout entomology research related to grains) and Regional Research Institutes in former republics. The latter often cover a range of agricultural disciplines and some have an almost 100 year history; many have significant grain breeding and seed distribution programs. Three of the All-Russian Research Institutes most relevant to grains are the Institute of Legumes and Groat Crops (Streletskove), Institute of Sorghum and Cereals (Zernograd), and The N.I. Vavilov Institute of Plant Industry. The latter institute, based in St. Petersburg, but with a number of experimental stations throughout Russia, is the main internationally renowned Russian institute emphasizing the conservation and study of plant genetic resources, including grain crops. Most states have State Agrarian Universities and there are a small number of specialist agrarian academies including Moscow Agricultural Academy.

After separation from Russia in 1991, National Agricultural Research Systems developed in most of the former Soviet Republics using the institutional assets left by the former USSR; several of these have a strong crops research emphasis although resources for research are very limited and it has been difficult (and often undesirable) to maintain the staff numbers and infrastructure left behind. They have had to develop their priority setting to match specific requirements of their agro-ecological zone and changed policy/economic environment including a greater emphasis on markets.

See also: Research Organizations of the World: Asia/Pacific, Central/South America, and Africa/Middle East; CGIAR; Global Trends and the Commercial Sector.

## **Further Reading**

Pardey RG and Beintema NM (2001) *Slow Magic:* Agricultural R&D a Century after Mendel. Washington DC: International Food Policy Research Institute.

## **Relevant Websites**

http://www.asti.cgiar.org — Agricultural Science and Technology Indicators (for various developed and developing countries).

http://www.nps.ars.usda.gov — Agricultural Research Service, US Department of Agriculture (USDA).

http://www.pw.usda.gov – Western Regional Research Center, Albany, CA, USA.

http://www.barc.usda.gov — Beltsville Agricultural Research Center, Beltsville, MD, USA.

http://www.sparc.usda.gov — Southern Plains Agricultural Research Center, College Station, TX, USA. http://www.usgmrl.ksu.edu — Grain Marketing and Production Research Center, Manhattan, KS, USA. http://www.ncaur.usda.gov — National Center for Agricultural Utilization Research, Peoria, IL, USA. http://www.ers.usda.gov — Economic Research Service, USDA.

http://www.usda.gov — USDA Federal Grain Inspection Service, technical service center, Kansas, MO, USA. http://www.reeusda.gov — USDA Cooperative Research, Education and Extension Service.

http://www.grainscanada.gc.ca — Canadian Grains Commission, Winnipeg, Manitoba, Canada.

http://www.cigi.ca - Canadian International Grains Institute, Winnipeg, Manitoba, Canada.

http://www.res2.agr.gc.ca - Agriculture and Agri-Food Canada.

http://www.res2.agr.ca - Cereal Research Center, Winnipeg, Manitoba, Canada.

http://www.res2.agr.ca — The Eastern Cereal and Oilseed Research Center, Ottawa, Ontario, Canada. http://www.umanitoba.ca — Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, Canada.

http://www.ag.usask.ca - College of Agriculture, University of Saskatchewan, Saskatoon, Canada.

http://www.afns.ualberta.ca — Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada.

http://www.plant.uoguelph.ca – Department of Plant Agriculture, University of Guelph, Ontario, Canada.

http://www.mcgill.ca – Faculty of Agricultural and Environmental Sciences, McGill University, Quebec, Ontario, Canada.

http://www.nrc.ca — The National Research Council (NRC), Plant Biotechnology Institute, Saskatoon, Canada.

http://www.bbsrc.ac.uk — Biotechnology and Biological Sciences Research Council, UK.

http://www.jic.bbsrc.ac.uk – John Innes Centre, Norwich, UK.

http://www.ifrn.bbsrc.ac.uk – Institute of Food Technology, Norwich, UK.

http://www.iacr.bbsrc.ac.uk — Institute for Arable Crops Research (recently renamed Rothamsted Research after consolidation of the Rothamsted and Long Ashton sites), UK.

http://www.csl.gov.uk - Central Science Laboratory, York, UK.

http://www.scri.sari.ac.uk - Scottish Crop Research Institute, UK.

http://www.apd.rdg.ac.uk - University of Reading, UK. http://www.nottingham.ac.uk - University of Nottingham, Sutton Bonington, UK.

http://www.uea.ac.uk - University of East Anglia, Norwich, UK.

http://www.ce.umist.ac.uk - Satake Centre for Grain Process Engineering, University of Manchester Institute of Science and Technology, UK.

http://www.qub.ac.uk - The School of Agriculture and Food Science, Queen's University Belfast, UK. http://www.hgca.co.uk - The Home-Grown Cereals Authority, UK.

http://www.nantes.inra.fr - Institut National de la Recherche Agronomique, Nantes, France.

http://www.cirad.fr - Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement, France.

http://www.arvalisinstitutduvegetal.fr - ARVALIS Institut du végétal, France.

http://www.cetiom.fr - CETIOM, France.

http://www.ucd.ie - University College Dublin, Ireland. http://www.ucc.ie – University College Cork, Ireland. http://www.nagref.gr - National Agricultural Research Foundation, Greece.

http://www.cragx.fgov.bex - Government Agricultural Research Centers, Gent and Gembloux, Belgium. http://www.vtt.fi - VTT Biotechnology, Espoo, Finland.

http://www.vir.nw.ru - The N.I. Vavilov Institute of Plant Industry, St. Petersburg, Russia.

## Asia/Pacific, Central/ South America, and Africa/ Middle East

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#### Introduction

This article describes the activities of some of the major research institutes active in grain science in regions other than North America and Europe. These countries vary significantly in their state of development, and correspondingly in the development of their grains research capacity. Furthermore, grains

research in some countries such as China has advanced so much since 1980s that in some areas, such as biotechnology and the development of hybrid rice, pioneering work in the area of R&D is carried out. The national agricultural research systems (NARS) of countries such as China, India, and Brazil are now among the largest institutions internationally and thus make an increasing input to international grain science efforts. On the other hand, capacity for grains research (and agricultural research in general) has fallen in many African countries. There is a special effort to review relevant international agricultural research centers. While attempts are made to review organizations that carry out grains research worldwide, there is a deliberate bias toward provision of more detailed information on institutions in English-speaking countries. This article focuses on government and public sector research institutes; private sector research is reviewed in the next article. Research Organizations of the World: Global Trends and the Commercial Sector.

Many of the trends in North America and Europe are reflected in both the developed and developing countries in these regions. In Australia especially, there has been a move towards formation of networks between research institutes. Other trends in Australia include the greater importance of levies from graingrowers in supporting research in a number of countries, and the increase in commercial involvement in grain breeding and biotechnology. More broadly, across each of the regions reviewed, is a shift in focus of many of the grain R&D institutions from a largely production focus to an emphasis on environmental issues such as water-use efficiency and sustainability of farming systems. For the developing countries that benefited from the higher-yielding varieties of the "Green Revolution," sustaining the gains through more efficient use of water and prevention of erosion, waterlogging, salinity, and acidification is now critical. Also critical is research on grain quality and addressing market requirements, as grain production in several of these countries struggles to compete with subsidized European and US agriculture.

## The International Agricultural **Research Centers**

The CGIAR system (Consultative Group for International Agricultural Research) is a nonprofit network of 16 semiautonomous R&D centers, supported by the World Bank and a range of national Governments, usually through their overseas development assistance programs. Several of the centers have a grain science

focus, including the first two centers that were established in the early 1960s, International Rice Research Institute (IRRI) and Centro Internacional para Mejoramiento del Mais y Trigo (The International Maize and Wheat Centre – CIMMYT). CIMMYT is based in Mexico, but has programs in Latin America, Africa, Central Asia and West Asia, and North Africa. Other CGIAR centers with a strong grains focus include International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Patancheru, India (focusing on sorghum, pearl millet, chickpea, pigeon pea, and peanut) and International Center for Agricultural Research in the Dry Areas (ICARDA) in Aleppo, Syria (focusing on barley, wheat, lentil, faba bean, chickpea, forage legumes).

Three centers carry out studies on rice improvement. IRRI, centered at Los Banos, Philippines is the main one, and its work aims to help poor farmers in developing countries grow increased yields of rice using less water, less labor, and fewer chemical inputs. The semidwarf rice varieties and management packages developed by IRRI were central to the successes of the Green Revolution in the 1960s and 1970s. IRRI has a focus on Asia while two other centers undertake rice breeding and management in Latin America and Carribean (International Center for Tropical Agriculture – CIAT, in Cali, Colombia) and West Africa (Western Africa Rice Development Association – WARDA, in Bouake, Cote de Ivore).

The CGIAR centers have traditional strengths in germ plasm development, providing much of the germ plasm for development of the modern varieties now utilized throughout the developing world (as well as in some developed countries such as Australia). To support germ plasm development, centers have strengths in agronomy, quality assessment, biotechnology, pathology, natural resource management, and economics. Conservation of genetic resources is an important part of the centers' mandates. At CIM-MYT, wheat and maize breeding (agronomy and quality evaluation programs) are backed up by programs in economics, natural resource management, and biotechnology. ICRISAT has a geographical emphasis on south Asia and sub-Saharan Africa, and its research addresses water scarcity through natural resource management (rainwater conservation and utilization) and plant breeding to improve wateruse efficiency and drought tolerance. ICARDA has a geographical emphasis on central west Asia and northern Africa, but its germ plasm is utilized worldwide. ICARDA focuses on agriculture in tropical dry areas, while ICRISAT emphasizes temperate and subtropical dry areas. It also has an emphasis on water-soil management in integrated farming systems for low-rainfall areas. The International Institute of Tropical Agriculture (IITA) with head-quarters in Ibadan, Nigeria focuses on germ plasm (including several grains — cowpea, maize, and soybean), pest management, and crop-based farming systems for smallholder farmers of sub-Saharan Africa.

Some work on processing quality is carried out by the centers, but very little work on grain storage and other postharvest technologies. While germ plasm development is still the main focus, there has been an increased involvement in recent years in natural resource management, farming systems research, and in understanding social, policy, and economic constraints. There is a level of tension within the CGIAR system as a whole on the appropriate balance of such activities; given that there have been few additional funding resources in real terms over the last decade.

# Major Public Sector (Government and University) Grain Science Organizations

#### **Asia Pacific**

There are three main public sector providers in grains research in Australia – state departments, Commonwealth Scientific and Industrial Research Organization (CSIRO - national government organization), and universities. State departments are the main research providers. The national government department - Agriculture, Fisheries, and Forestry Australia (AFFA) – does not conduct laboratory or field research in agriculture. In the last 10–15 years, there has been a shift in emphasis of many state departments from an overwhelming emphasis on production agriculture toward sustainable production. Nonetheless, the states remain particularly strong in crop improvement and agronomy, and in soil science and farming systems research. A summary is provided in Table 1. An increasing proportion of extension services, formerly provided by the states, are now provided to farmers on a commercial basis by these departments or by the private sector.

Some of the divisions of the CSIRO conduct research in grains production and processing, including:

- Plant industry genomics of grains including wheat and rice; barley genetic engineering; wheat starch composition; breeding of winter wheats and oilseeds; physiology, agronomy, and adaptation of cereal crops, soybean, mung bean; sustainable croppasture systems; Mediterranean cropping systems; cereal chemistry and rapid testing methods.
- Entomology Stored Grain Research Laboratory.

Table 1 Some Australian state government departments undertaking significant grains research

Department (website)	Grains research emphasis
NSW Agriculture (www.agric.nsw.gov.au)	Bread, biscuit, and durum wheat breeding; malting barley breeding; oat breeding; rice breeding, nutrition, pests, and quality assessment
(	Cereal, canola, and pulse agronomy and disease management; crop-pasture rotations
	Chickpea, faba bean, field peas, canola, lupins with enhanced yield, and disease resistance
Dept. of Primary Industries, Agriculture,	Dryland cropping; sustainable cereal/pasture-based farming systems
Victoria (www.nre.vic.gov.au)	Cereal molecular marker development
	Milling wheat, malting barley, pulse and oilseed brassica breeding
Overage and Double of Drive and Individual	Pulse agronomy, pathology, and processing
Queensland Dept. of Primary Industries	Wheat and oat breeding and quality assessment
(www.dpi.qld.gov.au)	Malting barley breeding and barley biochemistry
	Tropical maize improvement; sorghum breeding and pest management
	Soybean and peanut breeding and agronomy Subtropical grain farming systems; new grain crops (pearl millet, guar, and adzuki beans)
	Integration of cropping systems and climate models
	Grain storage technologies
South Australian R&D Institute (SARDI) (www.sardi.sa.gov.au)	Bread and durum wheat, triticale, oat and malting barley improvement Chickpea and lentil improvement
,	Biotechnology in cereal crop improvement
	Cereal chemistry and quality assessment
	Cereal, pulse, and oilseed pathology and disease diagnostics
	Soil-borne root diseases and abiotic constraints to productivity
	Agronomy, tillage practices, climate risk-management
Dept. of Agriculture, Western Australia	Wheat and barley breeding and agronomy for Mediterranean environments
(www.agric.wa.gov.au)	Cereal-pasture farming systems
	Canola and field pea
	Grain storage and protection
	Cereal chemistry and Asian products
	Pulse pathology
Dept. of Primary Ind., Water and Environment,	Cool temperate cereal breeding and selection
Tasmania (www.dpiwe.tas.gov.au)	Brassica oilseed variety assessment
	Assessment of soybean and broadbean varieties
Dept. of Business, Indy and Resource Development, Northern Territory (www.nt.gov.au)	Agronomy of irrigated maize, peanuts, sesame, and rice production

- Food Science Australia extrusion cooking; mycotoxin contaminants.
- Land and water sustainable use of soil and water resources in grain cropping.
- Sustainable ecosystems rodent pests of cereal crops; predictive understanding of biophysical and ecological processes.

BRI Australia Ltd. is an independent nongovernment grains research organization. Areas of research expertise include milling, near infrared reflectance technology, baking science and technology, and Asian wheat foods (noodles and steamed breads). AWB Ltd.'s (formerly The Australian Wheat Board) R&D laboratory, Agrifood Ltd., has an emphasis on testing services, although some research on nutrition, milling, and wheat products is carried out.

There are  $\sim$ 20 tertiary institutions that teach and research aspects of agriculture, horticulture, forestry,

fisheries, and natural resource management. Relevant grains research is carried out at several universities. This includes:

- University of Adelaide bread and durum wheat and barley breeding, molecular markers, cereal root diseases, cereal pathology, biotechnology, weed management, malting, and brewing biochemistry;
- University of Sydney breeding for rust resistance in barley and wheat; wheat, oat, rye breeding for processing quality; cereal diseases; cereal genomics; precision agriculture; soil science; crop modeling;
- University of Melbourne, through its Joint Centre for Crop Innovation associated with the School of Land and Food emphasizes research to underpin the sustainability of temperate grain crop production, with emphasis on variety development,

- agronomy and farming systems, and protection of natural resources;
- University of New England agronomy and soil science, plant nutrition, crop protection, weed science, integrated pest management;
- University of Queensland production and postharvest handling of cereals, grain legumes and oilseeds; weed management; plant protection; molecular biology; sustainable grains production systems; and
- University of Western Australia grain crop science and molecular genetics.

Smaller university research efforts are based at:

- Charles Sturt University agronomy, irrigation, fertilizer management of grain crops, plant pathology;
- Curtin University Muresk Institute agronomy, precision agriculture;
- Murdoch University molecular markers for barley and wheat, grain legume genome mapping, transgenics diseases and viruses;
- Southern Cross University cereal genotyping technology: cereal processing and health benefits;
- University of New South Wales –grain storage engineering;
- University of South Australia agricultural machinery research and design;
- University of Southern Queensland plant-pathogen interactions, cereal genetic engineering, marker-assisted breeding, canola breeding; and
- University of Tasmania —cereals, grain legumes and oilseeds as break and rotation crops.

Over the last 10–15 years, there has been a significantly increased emphasis on establishment of more formal links between research organizations. This has been driven to a significant extent by the advent of the Cooperative Research Centres (CRCs) program and encouragement by funders such as the Grains R&D Corporations (RDCs). A special feature of the Australian research landscape are the CRCs. This program, funded by the government, was launched in 1990 to strengthen collaborative linkages between industry, research organizations, universities, and government agencies. CRCs cover all areas of science and technology, but several of the centers relate to grain production and processing. As of January 2003, these include:

 CRC for Value-Added Wheat, following on from the Quality Wheat CRC, focuses on the development of wheat varieties, agronomic management, new approaches for the assessment of wheat quality, and improved wheat processing quality;

- CRC for Innovative Grain Food Products emphasizes high-value functional grain foods;
- The CRC for Sustainable Rice Production emphasizes increased production efficiency, development of value-added products, and improved management of soil and water resources;
- The CRC for Molecular Plant Breeding emphasizes the identification and utilization of molecular markers in cereal and pasture grass breeding;
- The CRC for Weed Management Systems emphasizes nonchemical management of weeds, including in cereal-based farming systems;
- Centre for Legumes in Mediterranean Agriculture (CLIMA) emphasizes research on the role of grain and annual pasture legumes in the Mediterranean climate of Western Australia. Research strengths include breeding, germ plasm assessment, pests and diseases, molecular biology, and crop physiology of legumes.

The Rural RDCs, of which the Grains RDC is the relevant one for grains research, facilitate and manage funding for research and assist in communicating results. They are generally funded on the basis of the government, matching the industry R&D levies dollar-for-dollar up to a maximum of 0.5% of the industry's gross value of production (GVP). Until the early to mid-1990s, most RDCs saw themselves as "reactive" funding bodies that selected a limited number of projects for funding from a larger number of competitive proposals. Managers from most RDCs now play a much greater role in determining research priorities in consultation with particular industries, and are being involved in project design and negotiating research partnerships. In grains, funding mechanisms have provided strong encouragement for different laboratories working on particular diseases and breeding targets within a given agro-ecological zone to collaborate more closely and formally.

Much of Australia's plant breeding is still done in the public sector, although, in many cases private sector seed companies now license the varieties developed from the research and distribute and market the seed to farmers. Increased commercialization of plant breeding and development of national breeding networks for major grains are recent developments and have reduced research duplication.

The main New Zealand organization undertaking grains research is the NZ Institute of Crop and Food Research. It is one of nine government-owned research institutes. It has a strong emphasis on grain crop improvement as well as basic cereal science and baking technology. Universities with research

interests in grains include Massey University (Palmerston North) with a research emphasis on food science and engineering. Lincoln University (Christchurch) carried out research on grain foods composition, crop agronomy, seed science and technology, and some biotechnology. Although, wheat is one of the major cereals, the growing environment is very different from that in Australia, requiring rather different varietal characteristics.

In Japan, much of the grains research is carried out in institutes managed under the Agriculture, Forestry, and Fisheries Research Council of the Ministry. Crops (including grains) research is carried out in a series of regional institutes in Hokkaido, Tohoku, Hokuriku, Chugoku, Shikoku, and Kyushu, and grains nutrition at the National Food Research Institute in Tsukuba. Major universities with agriculture faculties include Tokyo University of Agriculture and Technology, Tohoku, Kyoto, Kyushu, Hokkaido, Kagoshima, Yamaguchi, Tottori, and Nagoya. Several universities carry out research in grains processing.

The Korean Rural Development Administration of the Ministry of Agriculture and Forestry has a series of national research institutes, including the National Crops Research Institute, National Institute of Agricultural Science and Technology (with relevant work on crop protection and agro-ecology), National Institute of Agricultural Biotechnology, National Rural Nutrition Institute, and nine provincial agricultural and extension institutes. Korea Food Research Institute and the Korea Research Institute of Bioscience and Biotechnology function under the Ministry of Science and Technology. Grains research is strongly rice-focused, but with an increasing environmental protection and biotechnology emphasis.

China has one of the largest NARS in the world, and in recent years the government has placed a high priority on developing research capacity and infrastructure. Because of the large range of production environments, research on both temperate and tropical grains is important, and some institutes undertake pioneering grains research, for example in the development of high-yielding rice varieties, rice functional genomics, and transgenic crops. In recent years, the Chinese government has been making investments in R&D that are huge by any standards; the physical infrastructure and equipment have significantly improved, and there has been a move to retain or attract back eminent scientists. There is an increasing shift in emphasis in China from research aimed at increasing grain production to increasing quality and ensuring that production is environmentally sustainable, especially in view of the increasing pressure on water resources for agriculture in China.

Grains research is carried out in five main types of organizations. The Chinese Academy of Agricultural Sciences (CAAS) governed by the Ministry of Agriculture is the national academy with the largest number of grains research projects. It can also confer postgraduate degrees. Of its 38 institutes, 16 carry out grains science research, including the Institute of Crop Germplasm Resources, Beijing; Institute of Crop Breeding and Cultivation, Beijing; Institute of Plant Protection, Beijing; Institute of Biological Control, Beijing; Biotechnology Research Center, Beijing; Institute of Feed Research, Beijing; Institute of Soils and Fertilizers, Beijing; Institute of Agricultural Mechanisation, Nanjing; China National Rice Research Institute, Hangzhou; Agro-Environment Protection Institute, Tianjing; Institute of Oil Crops, Wuhan. Main thrusts are crop breeding, germ plasm conservation, development of biotechnology tools, natural resource management and protection (with a recent emphasis on water-saving agriculture for arid and semiarid regions), integrated pest management and agricultural mechanization to improve efficiency.

The Chinese Academy of Sciences (CAS) also carries out some research relating to grains, at the Institute of Botany and Institute of Genetics (Beijing, basic plant biology and biotechnology), Institute of Zoology (Beijing, pest management), Institute for Soil Research (Nanjing), National Centre for Genetic Research, Shanghai (rice genomics), Chengdu Institute of Biology (cereal breeding); Institute of Soil and Water Conservation, Yangling (agronomy), NW Plateau Institute of Biology, Qinghai (crop assessment), and Shijiazhuang Institute of Agricultural Modernization, Hebei (farming systems for the North China plain) among others.

Provincial academies of agricultural science are increasing in resources. Those with strengths in strategic or applied grains include Jiangsu, Sichuan, Guangdong, Fujian, Henan (for wheat), Shandong, Liaoning, and Gansu. The Beijing Food Research Institute carries out some cereal-processing research, while the State Administration of Grain Reserves also carries out grain-storage research, including through the Grain Storage Research Institute in Chengdu. The four strongest universities in terms of agricultural science are China Agricultural University (Beijing), Nanjing Agricultural University, Zhejiang University, and Huazhong Agricultural University. Other agricultural universities carrying out grains research are South China Agricultural University (Guangzhou), Shenyang Agricultural University, Central China Agricultural University (Wuhan), South-West Agricultural University (Chongqing) and North-West Agricultural University (Yangling). The

botany department at the University of Hong Kong carries out basic research on rice grain structure, maize breeding and processing research on wheat, oats, and soybeans.

India has one of the largest NARS in the world, and a traditional strength in grains sciences. ICAR has almost 30 000 staff, including 7000 research scientists. Most grains research is done within the Indian Council for Agricultural Research Institutes. Of its 46 Central Research Institutes, most of the grains research institutes are within the Crop Science Division. Major thrusts include development of grain varieties resistant/tolerant to a range of abiotic and biotic stresses, hybrid technology, application of molecular techniques, plant genetic resources conservation, and farming systems research. The main ICAR institutes carrying out grains research include:

- Indian Agricultural Research Institute, New Delhi (emphasis on more strategic research);
- Central Rice Research Institute, Cuttack, Orissa;
- Indian Institute of Pulses Research, Kanpur, Uttar Pradesh;
- National Bureau of Plant Genetic Resources, New Delhi;
- National Research Centre for Groundnut, Junagarh, Gujarat;
- National Research Centre for Rapeseed Mustard, Bharatpur, Rajasthan;
- National Research Centre on Sorghum, Hyderabad, Andra Pradesh;
- National Research Centre on Soybean, Indore, Madhya Pradesh;
- National Centre for Integrated Pest Management, New Delhi;
- National Research Centre for DNA fingerprinting, New Delhi; and
- National Research Centre on Plant Biotechnology, New Delhi.

Several smaller project directorates covering biological control, oilseeds, rice, wheat, and maize. In addition, All-India Coordinated Research projects attempt to link institutes; current coordinated projects cover all major (and most minor) cereal, oilseed, and pulse crops, as well as specific pests such as white grubs and rodents. An even bigger resource is the 29 State Agricultural Universities (SAUs), which fall under the ICAR. They employ almost 26 000 scientists in teaching, research, and extension roles. Some of the SAUs with strong grains research programs include Acharya NG Ranga AU (Andhra Pradesh), CCS Haryana AU, GB Pant AU (Uttar Pradesh), Punjab Agricultural University, and Tamil Nadu AU. The Council for Scientific and Industrial

Research (CSIR) has a set of national institutes in different areas of science and technology. The Central Food Technological Research Institute (CFTRI, Mysore) has a department which researches cereal (especially wheat) milling, baking, and biochemistry.

The major Indonesian government agricultural research and development agency is Indonesian Agency for Agricultural Research and Development (IAARD). Research institutes working on grains within the Central Research Institute for Food Crops are the Rice Research Institute, Sukamandi, Java; Research Institute for Maize and Other Cereals, Makassar, Sulawesi; Research Institute for Legume and Tuber Crops, Malang, Java and a research station for (rice) tungro disease. New research institutes for biotechnology and genetic resources and for postharvest technology have recently been established. Adaptive research is carried out by Assessment Institutes for Agricultural technology (under IAARD, but located in each province). Major agricultural universities include Bogor Agricultural University (IPB), Gadjah Mada University (Yogyakarta), Hassanuddin University (Makassar), University of Mataram (Lombok), and Universitas Sam Ratulangi (Manado).

Under the Philippines Department of Agriculture (DA), there is a national rice research center (PhilRice in Munoz, Luzon), addressing both research and technology dissemination, and the Bureaus of Plant Industry, Soil and Water Management and Postharvest Research and Extension. The last of these is based at Munoz (on the Central Luzon State University campus) and has two main research emphasis - grain storage and processing of high-value crops such as potato, cashew, coffee, and beans. There are also Regional Integrated Agricultural Research Centres there is one organization in each of the 15 Regions, but some have 2-3 research stations, and several focus on grain crops. The strongest university in grains research is the University of Philippines at Los Banos, which has specialized institutes of plant breeding and of farming systems research.

There are also a number of universities in regions with strengths in agriculture, and the universities have a far greater share of research capacity than DA. These include Benguet, Central Luzon State, Visayas State College of Agriculture, Don Mariano Marcos State University, Central Mindanao, and Southern Mindanao. There are also several others with some strengths in agriculture (Mindanao State, Bicol, Central Visayas State). Some of the universities have regional research and development centers involving the National departments of Agriculture and of Environment and Natural Resources, and some of

the state universities also have quite good extension/ outreach systems.

The Thailand Department of Agriculture (DOA) of the Ministry of Agricultural and Cooperatives is responsible for crop research while the Department of Agricultural Extension (DOAE) is responsible for transfer of technology to farmers. While there are experiment stations in every region of the country, most research is carried out in Bangkok. DOA has technical divisions covering agricultural chemistry, agricultural engineering, plant pathology and microbiology, entomology and zoology, soil science, botany, and weed science, and agricultural toxic substances (pesticides), and specialist rice and field crops institutes (there is reasonable research capacity in maize and legumes research). The Land Development Department conducts research in relation to land improvement and soil and water conservation. Grains postharvest technology is carried out within the Thailand Institute of Scientific and Technological Research (TISTR) in Bangkok. One of the three research centers with National Science and Technology Development Agency is Genetic Engineering and Biotechnology (BIOTECH). Kasetsart in Bangkok, Khon Kaen, Chiang Mai University, Prince of Songkla University (Hat Yai), and King Mongkut's Institute of Technology in Bangkok are all involved in grains research, the latter having a special focus on grains postharvest technology.

In Malaysia, the Rice and Industrial Crops Research Centre of the Malaysian Agricultural Research and Development Institute (MARDI) researches rice, maize, and groundnuts as well as several nongrain crops. The most relevant activity is development of high-quality rice varieties with disease resistance and accompanying agronomy. The food technology research centre does work on the development of rice-based snacks and other food products. The major university carrying out grains science research is University Putra Malaysia.

The Pakistan Agricultural Research Council, PARC coordinates much of the government agricultural R&D effort. The main institute, the National Agricultural Research Centre at Islamabad has several sections relevant to grains research, covering soil fertility, sorghum and millet, farm machinery, water resources, agricultural biotechnology, vertebrate pests, crop diseases, crop production, land resources, plant genetic resources, and seed sciences. There is a separate tropical agricultural research center at Karachi, four arid zone research centers in different regions of Pakistan. There are several agricultural universities in Pakistan which carry out grains research, the largest being the Pakistan Agricultural University at Faisalabad. Sindh Agricultural

University, the University of Arid Agriculture (Rawalpindi), the Northwest Frontier Agricultural University (Peshawar) are all smaller, but with a similar range of activities. The Ministry of Science and Technology manages the Pakistan Council of Scientific and Industrial Research, which carries out grains postharvest research. There is also a separate National Institute for Biotechnology and Genetic Engineering (Faisalabad).

Most grains research in Vietnam is conducted under the Ministry of Agriculture and Rural Development (MARD). Other relevant ministries are Education and Training (university sector); Science, Technology and Environment (MOSTE), which has some excellent crop biotechnology and plant protection research facilities and the Ministry of Industry (which has an Oil Plant Research Institute — with the mandate for peanut, soybean, sunflower, and sesame). While most institutes are located in either Hanoi or Ho Chi Minh city, many maintain "research centers" in more remote locations.

The key MARD institutes in grains science are the National Institute for Plant Protection, National Institute for Soils and Fertilizers, Post-Harvest Technology Institute, Vietnam Agricultural Science Institute, Institute of Agricultural Genetics, and the Cu Long Delta Rice Research Institute. The Department of Plant Protection of MARD is also becoming an important applied research organization. The main universities with research interests in grain science include Hanoi Agricultural University, Hanoi University of Science (also known as Hanoi College of Sciences), University of Agriculture and Forestry (in Ho Chi Minh city, the university is now known as Nong lam University), and Can Tho University.

### **Central and South America**

Brazil has a well-developed NARS, with many centers falling under EMBRAPA (Empresa Brasilera de Pesquisa Agropecuaria - Brazil Agricultural Research Corporation), a specialized science agency of the Agriculture Ministry. Brazil has the largest NARS of any Central or South American country, followed by Mexico; Brazil also has an advanced university system. There are separate EMBRAPA institutes relating to genetic resources and biotechnology (CENAR-GEN), rice and beans (CNPAF), corn and sorghum (CNPMS), soils (CNPS), soybean (CNPSO), wheat (CNPT), temperate climate agricultural research (CPACT, Temperate rice), and agro-industrial food technology (CTAA). Many of the Brazilian states have state "research corporations" that carry out grain crop assessment. There are also nonprofit centers – wheat experimentation and research (FUNDACEP)

Pelotas, Rio Grande do Sul, Rural do Rio de Janeiro, Santa Catarina, Sao Carlos, Santa Maria, Vicosa, and Brasilia as well as several state universities — Santa Catarina State, Paulista, Campinas, Sul de Santa, and Sao Paulo. Some Brazilian companies, such as Agroceres, Duraflora, Mogiana, and Braskalb carry out breeding and agronomy research to support seed distribution businesses.

The Argentine Government Instituto Nacional de Technologia Agropecuaria (INTA) is under the Agriculture Ministry, but there are a large number of programs and centers relevant to grains research under CONICET, which is under the Culture and Education Ministry. This includes centers for maize (CIG), plant protection (CIPEN), rice and biotechnology (IMBIV), postharvest technology (INSIBIO and INTECH), and semiarid crops (CERZOS). Several national universities have agronomy science faculties — Buenos Aires, Buenos Aires Province, Catamarca, Comahue, Cordoba, Cuyo, Entre Rios, Jujuy, La Pampa, La Plata, Litoral, Lomas de Zamora, Mar del Plata, Noredeste, Rio Cuarto, Rosario, Santiago del Estero, de Sur, de Salta, Tucuman, and de Formosa.

The Chilean Ministry of Agriculture has an Instituto de Investigaciones Agropecuarias (INIA) which undertakes some grains research. There are also agronomy departments at several universities – Austral, Conception, Frontera, Serena, Magallanes, Talca, Tarapaca and Universidad de Chile, Universidada Iberomerica de Ciencas as well as the Catholic universities - Temuco, Valparaiso, and Maule. In Colombia, the main government crops research agency is CORPOICA; under the Asociacion Nacional de Acuicultores (ACUANAL), there are research groups working on wheat, barley, maize, sorghum, and rice. Apart from the campuses of the national university, other universities with agronomy faculties include Cordoba and Tolima. The main research agency in the Ecuador agriculture ministry is INIAP (Instituto Nacional Autonomo de Investigaciones Agropecuarias); universities in most regional centers have small agronomy departments.

In addition, Roseboom and co-workers have provided information on grains research in the Carribean.

#### Africa and the Middle East

South Africa has the best-developed NARS of the African countries, and reasonable research capacity in maize (the main grain crop), sorghum, and some experience in wheat and barley. The Agricultural

Research Council is the main agricultural research structure, and has a series of research institutes, several carrying out grains research – these include the Institutes for Agricultural Engineering, Range and Forage, Industrial Crops, Plant Protection, Grain Crops Institute and Soil, Climate, and Water. The nine provincial departments also carry out selection and agronomy research, including those in Eastern Cape (Dohne), Free State (Glen), Natal (Cedara), and Elsenburg. There are some well-resourced grains research programs at the University of Pretoria, Natal, Free State, and Stellenbosch. Traditionally Black universities are increasing in strength, and several emphasize agriculture, including University of Fort Hare, University of the North and Venda. In the 1990s, there has been a significant shift in research emphasis in South Africa to accommodate the needs of the wider population, rather than just white commercial agriculture, to include emerging farmers and subsistence farmers in former homelands. In Zimbabwe, most crops research is carried out by the Department of Research and Specialist services in the Ministry of Lands, Agriculture and Water Development, with the University of Zimbabwe maintaining reasonable strength. Some of the grains IARCs, such as CIM-MYT and ICRISAT have maintained successful incountry programs in Zimbabwe, too.

The Kenya Agricultural Research Institute (KARI) is comparatively strong in crops and livestock; overall the Kenyan government agricultural research system is the second strongest in Africa, next to that of the Republic of South Africa. In the grains area, mandate crops include cereals (wheat, maize, sorghum, millets, and rice) and grain legumes (dry beans, cowpeas, mung beans, pigeon pea, chickpeas, and Dolichos) with efforts ranging from plant breeding for adaptation to different agro-ecological zones and stress resistances, agronomy, postharvest technology, and cooking quality. Almost all Kenyan grain production is rain-fed. The maize breeding and seed distribution program has probably been the most extensive. The breeding and postharvest research is backed up by a soil and water management research program. Some grains research is carried out at various universities (Egerton, Moi, University of Eastern Africa, and University of Nairobi). The Ethiopian Agricultural Research Organization (EARO) has national centers emphasizing maize, plant protection, soils, and agricultural mechanization as well as a number of centers in regional areas. Crops research is carried out at Alemaya University and Mekele University. In Nigeria, the Ministry of Agriculture has a series of research institutes - National Cereals Research Institute, Lake Chad Research Institute, and the Institute for Agricultural research. Postharvest technology

research is carried out under the Ministry of Industry at the National Stored Products Research Institute. Universities with relevant research programs include the University of Ibadan, University of Nigeria, Obafemi Awolowo, and Maiduguri. In Zambia, the Soils and Crops Research Branch of the Ministry of Agriculture, Food and Fisheries and the University of Zambia are the main grains research providers. In Tanzania, it is the Directorate of Research and Development of the Ministry of Agriculture and Food Security, while in Ghana, the Crops Research Institute of the Council for Scientific and Industrial Research, along with the University of Ghana and the University of Science and Technology are the crops research providers. In Sudan, the major grains research organizations are the Agricultural Research Corporation (within the Ministry of Agriculture and Forestry) and the Universities of Khartoum and Gezira.

The Israeli NARS is reasonably well resourced. Within the Agricultural Research Organization several institutes are relevant to grains research, including the Institutes for Field and Garden Crops (IFGC); Plant Protection; and Soil, Water and Environmental Services. The IFGC has a particular emphasis on wheat breeding to overcome drought, disease stress and heat tolerance in wheat and sorghum. There are also peanut breeding and rhizobiology programs. Universities carrying out grains research include the Technicon, Israel Institute of Technology (Haifa), and the Ben-Gurion University of Negev. Egypt also has a very large government agricultural research system, within the Agricultural Research Center (ARC), within the Ministry of Agriculture and Land Reclamation. In ARC, there are separate institutes for field crops, plant protection, plant pathology, food technology (bread and pasta research), agricultural genetic engineering, and soil, water, and environment research. There are programs on wheat, barley, maize, rice, sorghum, legumes, and oil crops. Universities with agriculture faculties include Alexandria, Cairo, Ain Shams, Al Azhar, Suez Canal, Zagagig, Assiut, Mansoura, Minia, Menoufa, Tanta, and South Valley. Further information on grains research in other West Asia and North African countries is provided in Casas et al. (1999).

See also: Research Organizations of the World: Europe and North America; CGIAR; Global Trends and the Commercial Sector.

## **Further Reading**

Cassas J, Sohl M, and Hafaz H (eds.) (1999) The National Agricultural Research Systems in the West Asia and North Africa Region, 278 pp. Allepo, Syria: ICARDA.

Roseboom J, Cremers M, and Lauckner B (2001) Agricultural R&D in the Carribean: An Institutional and Statistical Profile. ISNAR Research Report No. 19. The Hague: International Service for National Agricultural Research.

## **Relevant Websites**

http://www.asti.cgiar.org — Agricultural Science and Technology Indicators (for various developed and developing countries).

http://www.irri.org – International Rice Research Institute.

http://www.cimmyt.org — Centro Interncional para Mejoramiento del Mais y Trigo (The International Maize and Wheat Center).

http://www.ciat.cgiar.org — International Center for Tropical Agriculture.

http://www.warda.cgiar.org — Western Africa Rice Development Association.

http://www.icrisat.org — International Crops Research Institute for the Semi-Arid Tropics.

http://www.icarda.cgiar.org — International Center for Agricultural Research in the Dry Areas.

http://www.iita.org – International Institute of Tropical Agriculture.

http://www.sgrl.csiro.au — Stored Grain Research Laboratory, Canberra, Australia.

http://www.bri.com.au - BRI Australia Ltd.

http://www.agrifood.com.au — Agrifood Ltd., AWB Ltd., Australia.

http://www.sciences.adelaide.edu.au — University of Adelaide, Australia.

http://www.agric.usyd.edu.au - University of Sydney, Australia.

http://www.jcci.unimelb.edu.au — Joint Centre for Crop Innovation, University of Melbourne, Australia. http://www.landfood.unimelb.edu.au — School of Land and Food, University of Melbourne, Australia. http://www.sciences.une.edu.au — University of New England, Australia.

http://www.aghort.uq.edu.au – University of Queensland, Australia.

http://www.fnas.uwa.edu.au — University of Western Australia, Australia.

http://www.csu.edu.au - Charles Sturt University, Australia.

http://www.curtin.edu.au – Curtin University Muresk Institute, Australia.

http://www.sabc.murdoch.edu.au - Murdoch University, Australia.

http://www.scu.edu.au – Southern Cross University, Australia.

http://www.chem.unsw.edu.au - University of New South Wales, Australia.

http://www.unisa.edu.au – University of South Australia, Australia.

http://www.usq.edu.au – University of Southern Queensland, Australia.

http://www.scieng.utas.edu.au - University of Tasmania, Australia.

http://www.qwcrc.webbit.com.au - CRC for Value-Added Wheat, Australia.

http://www.ricecrc.org - CRC for Sustainable Rice Production, Australia.

http://www.molecularplantbreeding.com - CRC for Molecular Plant Breeding, Australia.

http://www.weeds.crc.org.au - CRC for Weed Management Systems, Australia.

http://www.clima.uwa.edu.au - Center for Legumes in Mediterranean Agriculture, Australia.

http://www.crop.cri.nz - New Zealand Institute of Crop and Food Research.

http://www.sciences.massey.ac.nz - Massey University (Palmerston North), New Zealand.

http://www.lincoln.ac.nz – Lincoln University (Christchurch), New Zealand.

http://www.home.rda.go.kr/emg – Korean Rural Development Administration.

http://www.kri.re.kr — Korea Food Research Institute. http://www.web.kribb.re.kr — Korea Research Institute of Bioscience and Biotechnology.

http://www.caas.net.cn — Chinese Academy of Agricultural Sciences.

http://www.english.cas.ac.cn - Chinese Academy of Sciences.

http://www.hku.hk – Botany department, University of Hong Kong.

http://www.icar.org.in - Indian Council for Agricultural Research Institutes, India.

http://www.philrice.gov.ph — PhilRice, national rice research center, Philippines Department of Agriculture. http://www.uplb.edu.ph — University of Philippines, Los Banos.

http://www.doa.go.th - Thailand Department of Agriculture.

http://www.ldd.go.th - Land Development Department, Thailand.

http://www.tistr.or.th - Thailand Institute of Scientific and Technological Research.

http://www.nstda.or.th - National Science and Technology Development Agency, Thailand.

http://www.ku.ac.th - Kasetsart University, Bangkok, Thailand.

http://www.kku.ac.th - Khon Kaen University, Thailand.

http://www.cmu.ac.th - Chiang Mai University, Thailand.

http://www.psu.ac.th - Prince of Songkla University (Hat Yai).

http://www.kmutt.ac.th - King Mongkut's Institute of Technology, Bangkok.

http://www.mardi.my — Malaysian Agricultural Research and Development Institute.

http://www.agri.upm.edu.my – University Putra Malaysia.

http://www.parc.gov.pk – Pakistan Agricultural Research Council.

http://www.nibge.org — National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan. http://www.agroviet.gov.vn — Ministry of Agriculture and Rural Development, Vietnam.

http://www.arc.agric.za – Agricultural Research Council, South Africa.

http://www.kari.org — Kenya Agricultural Research Institute.

http://www.agri.gov.il – Agricultural Research Organization, Israel.

## **CGIAR**

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### Introduction

Agriculture has played a central role in human affairs since the Neolithic revolution, when humans first made the transition from food gathering to a food-producing society.

At the dawn of the twenty-first century, agriculture's important role in human affairs has not diminished. (In this section, the term "agriculture" is used sensu latu, i.e., comprising crop and animal husbandry, fisheries, agro-forestry, and forestry.) There is growing international consensus that agricultural development remains central to economic advance, environmental protection, and social well-being. Agriculture is closely linked to the development agenda, including the Millennium Development Goals (MDGs) and goals of the 2002 "World Food Summit: Five Years Later" and the "World Summit on Sustainable Development."

## **Current and Future Challenges**

Agriculture's links to the broad development agenda are evident from the following:

• poverty affects three billion people, of which 1.2 billion live in deep deprivation earning less than \$1 per day;

- most of the world's poor people depend directly or indirectly on agriculture for their livelihoods;
- 815 million people in the world are undernourished;
- earth's ecosystems are under stress: 40-50% of land has already been irreversibly transformed or degraded by human actions and agricultural activities;
- biodiversity is being lost at unprecedented rates: onethird of terrestrial biodiversity, accounting for 1.4% of the Earth's surface, is in vulnerable "hot spots" and threatened with complete loss in the event of natural disasters or further human encroachment;
- most of the world's water is used for irrigation, and agriculture frequently accounts for 70% of freshwater withdrawals; and
- agricultural activities are one of the leading causes of climate change, contributing ~20% of all greenhouse gases.

Globally, the agricultural sector is set to face pressing challenges in the future.

- World population is expected to reach nine billion by 2050.
- Over the same time frame, demand for food is set to more than double, with most of the increased demand coming from developing countries.
- Erosion, salinization, compaction, and other forms of soil degradation affect 30% of the world's irrigated lands, 40% of rainfed agricultural lands, and 70% of rangelands.
- Most of the world's freshwater is used for irrigation; it takes 3000-5000 l of water to produce 1 kg of rice; water use is expected to increase by 50% by the 2030s.
- Arable land per person in developing countries has shrunk from 0.32 ha in 1961–63 to 0.21 ha in 1997–99 and is expected to drop to 0.16 ha by 2030.
- Cereal productivity growth increases (i.e., rises in cereal yields per ha) are decelerating, and in developing countries these declined from 2.2% per year in 1967–82 to 1.5% per year during the 1980s and early 1990s
- Forty percent of plant productivity in Africa and Asia is lost to insect pests and pathogens, and
- Earth's average surface temperature could rise by as much as 1.4–5.8°C over the next 100 years, increasing thermal stress, negatively affecting agricultural production in developing countries.

Given these challenges, science-based agricultural development will continue to be an important method for promoting economic growth and addressing current and future food, poverty, and environmental challenges.

## Plants, Food, and People: History of Concern

Hunger has been a constant companion of the human family, and concern about hunger and the world's ability to feed growing populations has been a recurring theme throughout history.

In the modern epoch, the Irish famine, and the famines of the twentieth century in Bengal (1943–44), Bangladesh (1974–75), China (1959–61), Holland (1944–45), and during the 1970s–80s in large parts of Africa (Ethiopia, Sudan, Mozambique, Nigeria, Niger, Somalia, and Zaire) caused millions of deaths. Famines, the most severe form of deprivation, can result from breakdowns in food grain production and distribution, and from a restricted flow of income with which people buy food. Shocks in the agricultural sector (plant disease as in the case of Irish famine, extreme weather events such as droughts and floods) and human-induced conflicts and civil wars can all trigger famines.

In the continuing debate on food adequacy, one of the early alarms was sounded by Thomas Robert Malthus in 1798, with the publication of *An Essay* on the Principle of Population as it Affects the Future Improvement of Society. His hypothesis was simple: left unchecked, the world would exhaust its food supplies because population grows geometrically while agricultural production grows arithmetically.

The 1960s marked a period when deep-rooted pessimism about food availability surfaced anew. In the 1990s, the Worldwatch Institute warned that due to population pressures and food grain shortages, China would resort to massive grain imports, triggering unprecedented increases in world food prices and causing starvation in the rest of the world.

In a fortunate coming together of human ingenuity and grain science, food and environmental scientists working with farmers successfully averted the worst Malthusian specter by ensuring abundant agricultural yield. The story of wheat improvement in England is compelling. It took nearly 1000 years for wheat yields to increase from 0.5 to 2 metric tons per hectare (mt ha<sup>-1</sup>), but only 40 years to climb from 2 to 6 mt ha<sup>-1</sup>, illustrating the ability of modern grain science to increase agricultural productivity.

## Mobilizing Grain Science for Development

The Consultative Group on International Agricultural Research (CGIAR) was established in 1971.

A group led by The Rockefeller Foundation, Ford Foundation, Food and Agriculture Organization, The World Bank, and others created the CGIAR.

The founding objective was based on a profound belief in the ability of science, especially grain science, to increase the world's "pile of rice," i.e., food in low-income countries facing severe food scarcity. The central idea of mobilizing grain science internationally to meet food and development needs, within a public goods framework, was novel.

The CGIAR is best known for the "Green Revolution," a term coined by William S. Gaud and used to refer to the set of agricultural innovations (new high-yielding varieties (HYVs) of wheat, rice, and maize, increased investments in irrigation, easy availability of farm inputs such as fertilizers and pesticides, improved grain distribution, and food policies in developing countries) that helped boost grain yields and incomes of farmers in poor, agrarian economies.

The Green Revolution transformed agriculture in developing countries especially in India, Mexico, Pakistan, and Philippines.

Cereal crop yields and food production more than doubled between 1960 and 1985. The new HYVs of rice, wheat, and maize increased daily calorie supply in developing countries by 25%, from under 2000 calories per person in the early 1960s to 2500 calories per person in 1990s. The evidence is incontrovertible: without the productivity growth in basic food crops, the numbers of poor and hungry would have been far greater.

Critics of the Green Revolution have argued that the new crop technologies favored farmers in wellendowed areas, worsened pollution through excessive use of fertilizers and pesticides, threatened biodiversity, promoted monocultures, and required higher input costs from impoverished farmers.

While a comprehensive discussion of these issues is beyond the scope of this article, subsequent studies and analyses have increased understanding about the effects of the new technologies. A range of subsequent policy interventions (e.g., increased credit, improved marketing and seed distribution facilities, land reforms, and strengthened emphasis on pesticide safety, and others) have helped poor farmers to benefit from Green Revolution technologies.

However, the benefits of the Green Revolution were spread unevenly and bypassed Africa. Gordon Conway has called for a "Doubly Green Revolution" focused on the research and development needs of Sub-Saharan Africa. On an average, crop yields in Africa are one-third of those achieved by Asian farmers. Less than 4% of Africa's arable land is irrigated. There is enormous potential for growth-led productivity in Sub-Saharan Africa where agriculture provides 70% of employment, 40% of exports, and one-third of gross domestic product.

## Evolving Research Agenda, Steadfast Focus on Fundamentals

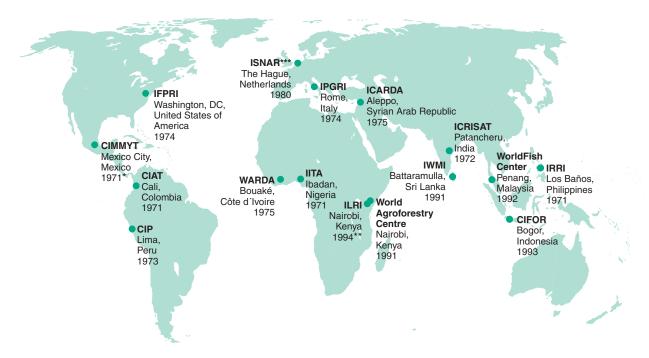
CGIAR's mission is "to achieve sustainable food security and reduce poverty in developing countries through scientific research and research-related activities in the fields of agriculture, forestry, fisheries, policy, and natural resources management." CGIAR achieves its mission through the work of 16 International Agricultural Research Centers (IARCs) that work with national agricultural research systems, the private sector, and civil society (see Figure 1). The IARCs are autonomous institutions, linked by a common commitment to promoting agricultural development in developing countries; 13 IARCs are headquartered in developing countries.

CGIAR's mission has evolved over time (see Figure 2), and this is reflected in its structure. When the CGIAR was founded, four IARCs (International Center for Tropical Agriculture (CIAT), International Maize and Wheat Improvement Center (CIMMYT), International Institute of Tropical Agriculture (IITA), and International Rice Research Institute (IRRI)) were brought under its umbrella. Subsequently, new IARCs were established to pioneer improvements in key food crops (legumes, roots, tubers, and other cereals), livestock health, ecological regions (dry, semiarid, and tropical regions), and to conduct research on pressing issues involving agroforestry, forestry, water management, aquatic resources, food policies, and strengthening of national agricultural research systems.

The research programs undertaken at the 16 IARCs are organized around five focus areas:

- increasing productivity (of crops, livestock, fisheries, forestry, and the natural resource base);
- strengthening national systems (through joint research, policy support, training, and knowledgesharing);
- protecting the environment (by developing new technologies that make more prudent use of land, water, and nutrients and help reduce agriculture's adverse impacts on ecosystems);
- saving biodiversity (collecting, characterizing, and conserving genetic resources – the CGIAR holds in public trust one of the world's largest seed collections available to all); and
- improving policies (with a major impact on agriculture, food, health, the spread of new technologies and the management and conservation of natural resources).

CGIAR's research portfolio has evolved from the original focus on increasing productivity in individual critical food crops. The current approach



**Figure 1** The CGIAR-supported network of International Agricultural Research Centers (IARCs). \*Year when IARC joined CGIAR, \*\*The International Livestock Center for Africa (ILCA) and International Laboratory for Research on Animal Diseases (ILRAD) were merged to form ILRI, and \*\*\* In March 2004, ISNAR ceased operations and key elements of its mandate were brought under IFPRI governance in a program located in Addis Ababa.

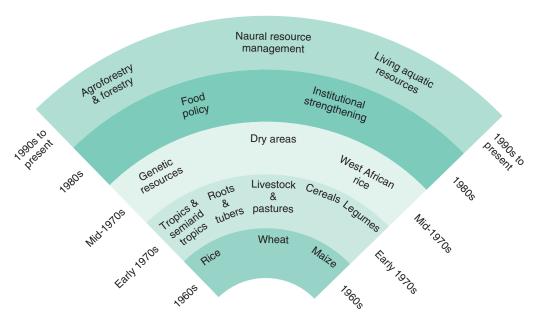


Figure 2 CGIAR's evolving research agenda.

recognizes that saving biodiversity, protecting the environment, improving food policies, and strengthening scientific capacities of developing countries through training and knowledge-sharing are all key components in the drive to enhance sustainable

agricultural productivity. CGIAR's focus on the fundamentals remains as strong as ever: agricultural growth and increased farm productivity in developing countries creates wealth, reduces poverty, hunger, and protects the environment. In 2002, the CGIAR's

research investment of \$337 million represented the single-largest effort in mobilizing science for agricultural development.

## **Ensuring the Quality of Science**

CGIAR's founding fathers recognized the importance of independent scientific advice for ensuring the effectiveness and efficiency of research programs undertaken by the IARCs. This task was entrusted to a Technical Advisory Committee (TAC), an independent body charged with identifying research priorities and monitoring the relevance and quality of science. In 2004, as part of the ongoing reforms a new "Science Council" was established to strengthen the quality of CGIAR science. By arrangement, the Science Council operates from the Rome-based of the Food headquarters and Agriculture Organization (FAO).

### **Grain Science and the CGIAR**

Improving the sustainable productivity of food grains is a major objective of CGIAR research. Grains are broadly defined as cereals belonging to the monocot family (barley, maize, millets, rice, sorghum, wheat, and other coarse grains) excluding legumes and pulses that are included in the dicot family. Nine of the 16 IARCs have specific crop improvement mandates (see Table 1). For the purposes of this paper, only eight IARCs have crop improvement mandates that correspond to the definition of grains cited above, the exception being root and tuber crops, including potato. An additional qualification is in order. Even though the International Plant Genetic Resources

Institute (IPGRI) does not have a plant breeding program, its mandate to serve as a world center for conserving plant genetic resources is germane to the focus of this article.

The following section provides a synopsis of CGIAR research impacts. Results are reported for the major food grains, ranked by importance. Space constraints preclude a more detailed overview, including the role and contributions of partners who have participated in and supported the research. The examples of impact are restricted to cereal grain crops as per the definition outlined above.

# Impacts of CGIAR Grain Research — An Overview

### Wheat

Wheat is a primary grain consumed by humans. The two most common kinds of wheat are bread wheat and durum wheat. World demand for wheat is surging, and global wheat consumption has doubled in the last 30 years to reach nearly 600 million ton (Mt) per year. World wheat production has remained steady at under 600 (Mt), with Australia, Canada, China, the European Union, India, Pakistan, Russia, Turkey, Ukraine, and the United States accounting for 80% of world wheat production.

Domesticated wheat and humans help each other in a relationship known as "mutualism" where humans first domesticated wheat but dependence on the grain also led to their domestication. The uniqueness of wheat in contrast to other cereals is that its kernel

Center	Year founded	Host country	Mandate crops
International Center for Tropical Agriculture (CIAT)	1967	Colombia	Cassava, beans, rice, tropical forages
International Maize and Wheat	1966	Mexico	Maize, wheat (bread and durum wheat, triticale)
Improvement Center (CIMMYT)	.000		maze, meat (ereat and daram meat, initially)
International Center for Agricultural	1975	Syria	Barley, wheat (durum species), lentil,
Research in the Dry Areas (ICARDA)			chickpea, faba bean, forage legumes
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)	1972	India	Sorghum, pearl millet, groundnut, chickpea, pigeonpea
International Institute of Tropical Agriculture (IITA)	1967	Nigeria	Cassava, yams, maize, soybean, cowpea
International Plant Genetic Resources Institute (IPGRI) <sup>a</sup>	1974	Italy	Genetic resources, cocoa, coconut/ <i>Musa</i> species
International Rice Research Institute (IRRI)	1960	Philippines	Rice
International Potato Center (CIP)	1970	Peru	Potato, sweet potato
West Africa Rice Development Association (WARDA)	1975	Cote d'Ivoire	Rice

<sup>&</sup>lt;sup>a</sup>The definition used to describe "grains" excludes potato and sweet potato, the mandate crops of the International Potato Center (CIP, founded in 1970), Lima, Peru. The work of IPGRI is included because of its global mandate to conserve plant genetic resources.

contains a gluten protein that makes it possible to produce a wide array of end products.

Global impacts CIMMYT has a global mandate for wheat improvement (bread and durum species).

In the late 1960s, about one-third of all wheat varieties in developing countries were CIMMYT crosses. By the 1990s, these numbers rose to about half from CIMMYT crosses and another quarter from varieties that had a CIMMYT parent. Worldwide, around 90% of all spring bread wheat releases had at least one CIMMYT ancestor; the percentage for durum wheat was even higher with nearly all spring durum wheat releases having a CIMMYT ancestor. In 1970, semi-dwarf wheat varieties were important only in Asia. By the 1990s, semi-dwarf wheat varieties covered 80% of world wheat area, with adoption rates of 90% and higher in Asia and Latin America. Wheat research has generated a total economic surplus of about \$2.5 billion annually in developing countries, for total research costs that never exceeded \$70 million annually.

Wheat improvement in West Asia and North Africa (WANA) ICARDA's wheat improvement research focuses on spring bread and durum wheats in the WANA region. Per capita bread wheat consumption is highest in the WANA region (185 kg per year), and rising. Wheat is generally consumed as flat (Arabic) or leavened (French) bread.

ICARDA grain scientists have made major advances in identifying new sources of resistance to abiotic stresses (drought, heat, and cold) and biotic stresses (rusts, Septoria diseases, and Hessian fly), in broadening genetic diversity, and in selecting for grain quality. In 2001, 15 international durum wheat nurseries representing over 800 lines and 10 000 segregating populations suitable for three WANA environments (continental, temperate, and highland) were distributed to national programs for testing. Efforts to broaden the genetic base of durum wheat (that is notably poor in genetic diversity) involved crossing improved dryland genotypes with WANA landraces, wild relatives, and bread wheat. Wild relatives of durum (Triticum diccoides and T. monococcum) were used to improve grain quality and resistance to leaf rust, leaf blotch, and yellow rust.

#### Rice

Rice is a member of the grass family (Gramineae) and belongs to the genus *Oryza*. *Oryza* includes 20 wild species and 2 cultivated species (cultigens): Asian rice (*Oryza sativa* L.) and African rice (*Oryza glaberrima*).

Rice farming dates back to the origins of agriculture. Rice cultivation is the world's most important economic activity, and rice is the staple food for the largest number of people on earth. Rice is a water-intensive crop, using two to three times more water than other cereals such as maize or wheat.

CGIAR's rice improvement mandate is fulfilled by IRRI, WARDA, and CIAT.

Global impacts IRRI began rice improvement in the 1960s, and the institute became the first IARC of the CGIAR. Expanding on the successes in wheat improvement, IRRI scientists successfully introduced semi-dwarfism into two *Indica* rice varieties (IR5 and IR8). The latter variety would lay the foundations of the Green Revolution in Asia.

IRRI has made substantial contributions towards improvement of rice varieties. Of the estimated 2040 rice varieties released over 40 years by national rice research systems of South and Southeast Asia, 219 were direct IRRI lines. Of the 2021 varieties, 31% had originated from one or more parents developed at IRRI. This number rises to 46% when the IRRI-developed varieties are included. In the 1970s, the release of IRRI-related varieties peaked at 60%, subsequently stabilizing at 40% of all varieties released.

The widespread diffusion of rice varieties signals a success story. The HYVs, in 1990s accounted for 75% in Asia and 40% in Latin America of the total area under rice cultivation. The increase in rice production has been enormous. Starting in the 1960s, world rice cultivation area expanded from 139–197 million hectares (Mha). Rice production more than doubled to 540 (Mt) in 2000, up from 199 (Mt) in 1961. A recent review shows that the Green Revolution continues to spread in favorable growing environments.

Rice improvement in Africa WARDA conducts rice improvement research in West and Central Africa, focusing on three distinct rice ecologies: upland, rainfed lowland, and irrigated land. Rice is a unique, and highly political commodity in Africa. The volume of rice consumed in West Africa exceeds \$2.75 billion annually, of which \$1 billion is spent on imports. Major rice producers in the region are Nigeria, Guinea, Côte d'Ivoire, Sierra Leone, Mali, Ghana, and Senegal which together account for 90% of rice cultivation and production .

Despite limited investments in agricultural research in the region, more than 197 improved varieties have been released since the 1980s, and 122 more are targeted for release in the next five years. 54 of the 197 varieties (27%) are related to CGIAR germplasm

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enhancement, while an additional 31 varieties (16%) have parents or ancestors developed by CGIAR.

Using inter-specific hybridization techniques, WARDA has successfully combined the ruggedness of local African (O. glaberrima) rice species with the phenomenally high productivity traits of Asian rice (O. sativa) that was the mainstay of the Green Revolution. The new rice varieties, NERICAs or New Rices for Africa, resist droughts and pests and are able to thrive in poor soils. In Guinea alone, NERICAs are planted on 90 000 ha saving an estimated \$10 million in rice import bills. Efforts are underway to accelerate diffusion of NERICAs throughout Sub-Saharan Africa through The Africa Rice Initiative. WARDA's research earned the CGIAR King Baudouin Award in 2000.

Rice improvement in Latin America CIAT has a mandate to promote sustainable increases in rice production and productivity in Latin America and the Caribbean region. Of the 299 rice varieties released from 23 national agricultural systems in the region, 95 varieties were released in Brazil. Of all the released varieties, 40% were crossed at CIAT. However, only 13 varieties in the region were developed from CIAT parents or ancestors. IRRI's International Network for Germplasm Evaluation and Research (INGER) has been instrumental in fostering rice collaboration in a region where only 19% of rice varieties released came from national agricultural systems.

## Maize

Maize (*Zea mays* L.), a member of grass family Poaceae, is one of the most important dietary cereal grains, and the second-most abundant cultivated crop in the world. Maize (an open-pollinated crop unlike wheat and rice) is grown in a wide range of geographical areas, and its growing season ranges from 3 to 13 months.

The United States is the leading producer of maize. Developing countries account for the bulk of maize production (64% of area under maize cultivation and 43% of world harvests). In Sub-Saharan Africa, maize is the principal cereal crop. Of the 23 countries in the world with the highest per capita consumption of maize as food, 16 are in Sub-Saharan Africa. Maize provides 50% of the calories in diets in southern Africa, 30% in eastern Africa, and about 15% in West Africa. Despite its importance, maize yields average 1.4 t ha<sup>-1</sup>, more than 1 t ha<sup>-1</sup> below the average for all developing countries.

Global impacts CIMMYT has a global mandate for maize improvement, and it targets lowland

tropical, subtropical, mid-altitude, and tropical highland environments throughout the developing world. Mexico, where CIMMYT headquarters are located, is a center of origin for maize.

A recent study found that of all government-funded maize varieties released from 1966 to 1998, ~52% contained CIMMYT germplasm. The use of CIMMYT germplasm has increased over time, with 64% of all public-sector varietal releases containing CIMMYT germplasm.

Estimates of economic benefits from CIMMYT's maize research vary but based on the above results (21.2 Mha planted to CIMMYT-related varieties) and a conservative estimate of farm-level maize prices (US\$120 per ton, on import parity basis) gross benefits range from US\$1.3 billion to US\$4 billion per year. These benefits take into account both germplasm improvements and better crop management practices.

A promising area of research is to develop a better understanding of apomixis, a plant's ability to reproduce asexually. Apomictic modes of reproduction are found most commonly in dicots Rosaceae and Asteraceae, and in the monocot Poaceae to which maize belongs. Apomictic gene technology is relevant to the problems of developing country agriculture because it will allow farmers to plant high-yielding maize year after year without having to buy costly hybrid seeds. Moreover, even with successive plantings, there is no risk of losing desirable traits in the progenies (e.g., high yields and resistance to stresses). A breakthrough in this area has the potential to boost agricultural production significantly, vastly improving food availability and farmer incomes.

CIMMYT maize breeder Surinder K. Vasal and biochemist Evangelina Villegas shared the 2000 Millennium World Food Prize for developing quality protein maize (QPM), a product of 30 years of research. QPM looks and tastes like normal maize but has nearly twice the amount of lysine and tryptophan, two amino acids essential for protein synthesis in humans. QPM is now being planted on 1 Mha in 20 countries, boosting food, nutrition, and income security.

Regional impact IITA, based in Ibadan, Nigeria, has a regional maize improvement mandate mainly targeting humid tropical and moist savannah zones of western and central Africa (WCA).

Maize accounts for more than 20% of domestic food production in Africa. Maize production has been growing steadily at an annual growth rate of 4% in West and over 2.35% in Central Africa. A survey of 12 countries (9 in West Africa and 3 in Central Africa), representing over 95% of maize

area showed that public breeding programs released 186 maize varieties, while the private sector released 81 maize varieties. There are three sources of germplasm for the released varieties: IITA, CIMMYT, and landraces. In the 1970s, IITA and CIMMYT supplied nearly 95% of the germplasm, a number that has continued to remain as high as 60% in the 1990s.

In 2000, two new maize varieties (Grace and Zm521) specifically adapted for southern Africa were released.

## **Sorghum**

Grain sorghum (Sorghum bicolor [Linn.] Moench) is a native African cereal also widely grown in India, China, and the Americas. Sorghum is grown in 85 countries, and the crop ranks fifth in world cereal grain production and fourth in value (after rice, wheat, and maize). Sorghum is a hardy plant, tolerant to drought and well adapted to cultivation in saline soils; given its adaptability, sorghum holds promise for feeding growing populations in Africa and Asia, as feed grain in the Americas and Australia, for brewing purposes, and as fodder for livestock.

ICRISAT's research on sorghum improvement began in the 1970s, with India as the hub, and regional centers in west, eastern, and southern Africa, and in Latin America. A total of 405 improved sorghum cultivars are available in 43 countries in Africa, Asia, and the Americas. Of these, 146 were released from materials classified as ICRISAT-bred (64), ICRISAT parent (29), or ICRISAT network (53). In addition, average yields increased (0.74 t ha<sup>-1</sup> during 1981–94, up from 0.58 t ha<sup>-1</sup> in 1966–81).

### **Pearl Millet**

Pearl millet (*Pennisetum glaucum*) is a native African cereal cultivated in the arid and semiarid tropics of Africa and Asia. The species is morphologically complex, and 13 cultivated, 15 weed and 6 wild species have been recorded. Pearl millet accounts for only 3.5% of land under cereal cultivation globally and about 1% of total cereal production. Nevertheless, pearl millet and finger millet (*Eleusine coracana*) are important crops for smallholder farmers because they provide grain and fodder in harsh growing conditions (e.g., shallow, sandy, infertile soils having low water-retention capacity that are common in hot, dry environments).

ICRISAT has a global mandate for improving pearl millet and finger millet. During the 1980s, of the 49 varieties released worldwide, 23 varieties were of ICRISAT origin. During the 1990s, 52 of the 59 releases originated from ICRISAT materials. More importantly, many of the varieties released in 13 African

countries were developed by ICRISAT. In India, where the national millet program was stronger, ICRISAT contributed parental material (rather than finished varieties).

ICRISAT's contribution in terms of adoption of improved cultivars has been sizeable. In Namibia, about 50% of total area pearl millet cultivation is under Okashana 1, a variety developed by ICRISAT. In Mali, adoption of improved cultivars increased from 12% in 1990 to 23% in 1995.

ICRISAT's work on pearl millet earned the CGIAR King Baudouin Award in 1996.

#### **Barley**

Barley belongs to the grass tribe Triticeae, to which wheats and ryes also belong. Barley is used mainly for animal feed, as malt for brewing beer, and is only marginally important as a human food. Barley is a hardy crop that can survive in harsh growing conditions (e.g., in nitrogen-depleted soils) and is therefore important for subsistence farmers.

Since 1980, ICARDA and CIMMYT have operated a joint Barley Breeding Program to develop HYVs with resistance to a broad range of diseases (stripe and leaf rusts, scald, fusariam head blight, and barley yellow dwarf).

During 1980–99, a total of 111 barley varieties were released in 23 developing countries. Using pedigree analysis, 78% of all barley varieties released were ICARDA-related material, 52% were ICARDA crosses (38% selected by ICARDA scientists and 14% by scientists partnering with national agricultural research systems). This collaborative breeding program has had significant impacts in Asia, West Asia and North Africa, and Latin America.

## **Conserving Biodiversity**

Biological diversity — or biodiversity — refers collectively to the variety of life on earth. Nature's cornucopia of plants are an abundant source of food and fiber for the human family. According to a recent estimate, about 272 000 species of flowering plants have been described worldwide, with the true number being closer to 300 000. Each year about 2000 new species are added to *Index Kewensis*, botany's standard reference work.

For more than 30 years, CGIAR scientists have been collecting, characterizing, and conserving biodiversity. Currently, the CGIAR holds 532 508 samples of crop, forage, and agroforestry genetic resources in 11 gene banks around the world (Table 2). In 1994, CGIAR signed an agreement with FAO, placing the collections in public trust, available to researchers

Table 2 The CGIAR collections

Center	Crop(s)	Number of accessions
International Center for Tropical Agriculture (CIAT), Cali, Colombia	Bean	31 718
	Cassava	5 728
	Forages	18 138
International Maize and Wheat Improvement Center (CIMMYT), Mexico	Maize	20 411
	Wheat	95 113
International Potato Center (CIP), Lima, Peru	Andean roots and tubers	1 112
	Sweet potato	6 413
	Potato	5 057
International Center for Agriculture in the Dry Areas (ICARDA), Aleppo, Syria	Barley	24 218
	Chickpea	9 116
	Faba bean	9 074
	Forages	24 581
	Lentil	7 827
World Agroforestry Center, Nairobi, Kenya	Sesbania	25
	Wheat	30 270
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT),	Chickpea	16 961
Patancheru, India	Groundnut	14 357
	Pearl millet	21 250
	Pigeonpea	12 698
	Sorghum	35 780
	Minor millets	9 050
International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria	Bambara groundnut	2 029
	Cassava	2 158
	Cowpea	15 001
	Soybean	1 909
	Wild <i>Vigna</i>	1 634
	Yam	2 878
International Livestock Research Institute (ILRI), Nairobi, Kenya	Forages	11 537
International Plant Genetic Resources Institute (IPGRI), Maccarese, Italy	Musa	931
International Rice Research Institute (IRRI), Los Baños, Philippines	Rice	80 617
West Africa Rice Development Association (WARDA) – The Africa Rice Center, Bouaké, Côte d'Ivoire	Rice	14 917
Total		532 508

worldwide. In fulfilling its stewardship obligations, CGIAR invests \$6 million every year to maintain these valuable resources for the benefit of humanity.

Since the 1980s, CGIAR gene banks have distributed over one million germplasm samples worldwide, and the vast majority of requests, 80% and more, came from developing countries.

Recognizing the challenge of creating a sustainable funding base for conservation activities, the Global Crop Diversity Trust (GCDT) was established to support global conservation of biodiversity.

## **Back to the Future**

International cooperation in grain science has a brief but successful history, dating back to less than 50 years. The Green Revolution, called a "paradigm of knowledge for development," was a success. Its achievements have been foreshadowed by newer challenges, especially in Sub-Saharan Africa. Agricultural research, conducted within a public goods framework, has achieved a track record of success. Rates of return to investment in crop research range from 16% to more than 100% per annum. Throughout history, agricultural research has been publicly driven and funded, with the benefits shared as well. But new realities mean that the successes of the past cannot easily be repeated in the future.

International interest in agricultural issues has waned. Record yields and low commodity prices have led to complacency. World Bank lending for agriculture has declined dramatically, from an average 31% of its total lending in 1979–81 to less than 10% in 1999–2000. A new rural development strategy "Reaching the Rural Poor" is helping to increase support for the agricultural and rural sectors in developing countries.

Deepening intellectual property rights (IPRs) have created a revolution in agricultural science and a race for exclusive property rights for agricultural and biological knowledge. The US Patent Office received 4000 genetic patent requests in 1991, which rose sharply to 22 000 in 1995. One year later, patent requests increased to an unmanageable 500 000 year. Patenting activity is one indicator of innovation. The top 20 patenting countries in the world, with less than 15% of the world's population and 77% of gross national product account for 99% of all current patenting in the US. The challenges posed by these inexorable trends are twofold: to channel research to benefit poor people in developing countries and to achieve this during a period of declining public financial support for public agricultural research.

In 2002, the global area cultivated with transgenic crops was nearly 59 Mha, and the bulk of area was sown to soybean (62%), corn (21%), cotton (12%), and canola (5%). These crops are only marginally relevant to the agricultural development needs of developing countries.

The Commission on Intellectual Property Rights has warned that "too often, the interests of the 'producer' dominate in the evolution of intellectual property policy, and those of the ultimate consumer are either not heard or heeded." Public goods research where both knowledge and technologies are kept in the public domain offers a meaningful way forward to redress these imbalances.

The prospects for a new era in mobilizing grain science for development have never been better. The ongoing biological and information revolutions are transforming the science and science-for-development landscapes, creating unprecedented new opportunities for scientific cooperation. More than four decades of scientific cooperation has strengthened national research capacities in developing countries. The Internet is already the single-largest repository of biological information. Rapid advances in molecular biology are helping decode genomes of model plant species (*Arabidopsis thaliana*) and vital food crops such as rice.

Plant comparative genetics is predicted to unlock the secrets of crop plants. Knowledge of few major crops is being pooled, and extrapolation of information from well-studied species to orphan crops (which include many tropical species) is providing a solid-base for their improvement."

Grasping these opportunities will require new science-based partnerships — international, regional, national, and local — that are anchored in the common good. Consumers, farmers, policymakers, producers, and scientists all have a role to play. Public policies and institutions will need to address their concerns and protect their interests, while building an architecture of innovation that can continue to

effectively marshal grain science for promoting sustainable agriculture.

See also: Research Organizations of the World: Europe and North America. Asia/Pacific, Central/South America, and Africa/Middle East; Global Trends and the Commercial Sector.

## **Further Reading**

Asker S and Jerling L (1992) *Apomixis in Plants*. Boca Raton, Florida: CRC Press.

Alston JM and Pardey PG (1996) Making Science Pay – The Economics of Agricultural R&D Policy, 203pp. Washington, DC: American Enterprise Institute.

Barton J, Alexander D, Correa C, Mashelkar R, Samuels G, and Thomas S (2003) *Integrating Intellectual Property Rights and Development Policy – Report of the Commission on Intellectual Property Rights*, 10pp. London: Department for International Development (DFID).

Baum WC (1986) Partners against Hunger: Consultative Group on International Agricultural Research. Washington, DC: The World Bank.

Bouman BAM, Hengsdijk H, Hardy B, Bindraban PS, Tuong TP, and Ladha JK (2002) *Water-Wise Rice Production*. Proceedings of the International Workshop on Water-wise Rice Production, 8–11 April 2002, Los Baños, Philippines: International Rice Research Institute.

Brown LR (1995) Who Will Feed China? Wake-up Call for a Small Planet. Washington, DC: Worldwatch Institute.

Conway G (1999) The Doubly Green Revolution – Food for All in the 21st Century. London: Penguin Books.

Enriquez J (2000) As The Future Catches You – How Genomics and Other Forces are Changing Your Life, Work, Health and Wealth, pp. 95–96. New York: Crown Business, A Member of the Crown Publishing Group.

Evenson RE and Gollin D (eds.) Crop Variety Improvement and its Effect on productivity — The Impact of International Agricultural Research, pp. 71–108. Wallingford: CABI Publishing.

Gale MD and Devos KM (1998) Plant comparative genetics after 10 years. *Science* 282: 656.

Hazell P (2003) Green revolution. In: Mokyr J (ed.) *The Oxford Encyclopedia of Economic History*. UK: Oxford University Press.

Hazell P and Ramasamy C (1991) The Green Revolution Reconsidered: The Impact of High-yielding Rice Varieties in South India. Baltimore, MD: Johns Hopkins University Press.

James C (2002) Global Status of Commercialized Transgenic Crops. Manila, Philippines: The International Service for the Acquisition of Agri-biotech Applications (ISAAA).

Karanja DD and McNeil MR (2003) African agriculture – a crucial lifeline. In: *Agriculture in the Global Economy*, pp. 73–89. Washington, DC: Bread for the World Institute.

- Kiple KF and Ornelas KC (2002) The Cambridge World History of Food. Cambridge: Cambridge University
- Lipton M and Longhurst R (1989) New Seeds and Poor People. Baltimore, MD: Johns Hopkins University
- Maclean JL, Dawe DC, Hardy B, and Hettel GP (2002) Rice Almanac. Manila, Philippines: International Rice Research Institute.
- Newman LF (1990) Hunger in History: Food Shortage, Poverty, and Deprivation. Cambridge: Basil Blackwell.
- Ravallion M (1997) Famines and economics. Journal of Economic Literature XXXV: 1205-1242.
- Rosenzweig C and Hillel D (1998) Climate Change and the Global Harvest: Potential Impacts of the Greenhouse Effect on Agriculture 70pp. New York: Oxford University Press.
- Sachs JD and McArthur JW (2002) Technological advancement and long-term economic growth in Asia. In: Chong-En Bai and Chi-Wa Yuen (eds.) Technology and the New Economy, pp. 157-185. Cambridge, Massachusetts: The MIT Press.
- Sen A (1981) Poverty and Famines: An Essay on Entitlement and Deprivation. New York: Oxford University Press.
- Singh I (1990) The Great Ascent: The Rural Poor in South Asia. Baltimore, MD: Johns Hopkins University
- The World Bank (2003) Sustainable Development in a Dynamic World: Transforming Institutions, Growth and Quality of Life. World Development Report (WDR), pp.3, 83.
- The World Bank (1999) Knowledge for Development. World Development Report 1998/1999. Washington, DC: The World Bank.
- Timothy JD and Guei RG (2003) Productivity gains from rice genetic enhancements in West Africa: countries and ecologies. World Development 31(2): 359-374.
- Wilson EO (2002) The Future of Life. New York: Alfred A. Knopf.
- WEHAB Working Group (2002) A Framework for Action on Agriculture. WEHAB Working Group, World Summit on Sustainable Agriculture (WSSD), Johannesburg, South Africa, August 2002, United Nations.

#### **Relevant Websites**

http://www.un.org. http://www.icrisat.org. http://www.fao.org. http://www.iprcommission.org. http://www.johannesburgsummit.org. http://www.un.org. http://www.cimmyt.org. http://www.worldfoodprize.org. http://www.singer.cgiar.org. http://www.cgiar.org. http://www.startwithaseed.org.

## Global Trends and The Commercial Sector

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### Introduction

This article intends to synthesize some of the recent trends in the subject matter and institutional organizations of grains research internationally. Among these trends include a greater emphasis on demanddriven rather than science-driven research. Accompanying this is greater involvement of industry and farmer groups in setting research priorities, and in some cases, farmer groups are setting up and managing their own research trials. While ex ante economic impact assessment studies have shown that very significant returns on research investments have come from germ plasm development, there is an increasing emphasis in grains research on topics such as production sustainability, crop-livestock systems, systems modeling, and use of techniques from molecular and information sciences. Priority-setting processes and funding agencies have also encouraged greater collaboration between disciplines and between research institutes. It is now the norm for grains researchers to be part of large networks. The private sector has greater involvement in agricultural R&D than ever before particularly in developed countries. In the case of grains research, this sector is especially involved in linkages between seed business and biotechnology/life science concerns. Pardey and Beintema have calculated that since the mid-1990s, in developed countries, expenditures from the private sector in agricultural research have exceeded public expenditure.

## **Grains Research in** the Commercial Sector

## **Grain Processing Companies**

Most of the research carried out by these companies is concerned with practical applications, aimed to support their flour milling and bakery products businesses (including breakfast cereals), but some carry out more basic research and their staff contribute to industry research conferences and the scientific literature. Major examples in the US include the ConAgra Grain Processing Company in Omaha, NE, General Mills, Kellogg Company in Battle Creek, MI, and

Nabisco, New Jersey. Nabisco Research carries out rather fundamental studies on dough rheology and physical properties. Riceland Foods (Stuttgart, AR, USA) and Riviana Foods (Houston, TX, USA) are two very large manufacturers of rice- and soy-based food ingredients, and hence undertake applied research to support their markets. The National Starch and Chemical Company, Bridgewater, NJ focuses on cereal starch-based polymers and supports a significant research program including work on new adhesives, specialty food starches, and biodegradable packaging materials. Cargill has wheat flour milling and maize wet-milling divisions and provides grainsbased feeds for livestock. Through Cargill Health and Food Technology, it focuses on nutraceuticals research, especially on developing products derived from soy. Other companies with an interest in research in soy products as nutraceuticals include DuPont Protein Technologies, St. Louis, MO; Central Soya Company, Fort Wayne, IN; and Schouten USA, Minneapolis, MN.

In Japan, two large milling companies, Nisshin Flour Milling and Nippon Flour, have played an important role over the last few decades in increasing the consumption of wheat in the Japanese diet. They also carry out a significant amount of research. In the UK, Rank Hovis and ADM Milling, Associated British Foods, Allied Bakeries and British bakeries are among the largest milling and baking companies, and they conduct some in-house research and technology development. Barilla, has a large cereal products research center in Italy, while in Australia, Goodman Fielder, Weston Foods, and Penford starches have active technology development programs. Other companies within the oilseed crushing and animal feeds industry also have applied research programs.

## **Baking Industry Associations**

Industry associations are also an important provider of research and technical services in the grains industry. In the UK, the Campden and Chorleywood Food Research Association, Chipping Campden, is a membership-based organization that provides research and consultancy services for cereal-processing companies. The RHM Technology Centre in High Wycombe, Bucks, UK, is part of the RHM (Rank Hovis McDougall) group. The center conducts theoretical and experimental studies in cereal (especially wheat) food science and technology. It also undertakes R&D projects for other commercial and government clients. Leatherhead Food International started as a UK food-industry association, but has evolved into an independent provider of food research

and market information. In France, ARVALIS Institut du végétal, a farmers' organization, carries out research on grains to satisfy the demands of markets and consumers. The American Institute of Baking, in Manhattan, KS, USA was founded by the North American baking industry to apply research findings to the industry in the US and internationally. Emphasis of the research includes cereal science, baking technology, nutrition, and food safety.

BRI Australia Ltd. (formerly the Bread Research Institute) is an independent Australian organization supported by the industry through membership fees and by competitive government and industry research grants. It carries out applied research in flour milling, baking, and Asian foods. The Japan Institute of Baking Technology in Tokyo, also plays a similar role.

## **Seed and Grain Biotechnology Companies**

In a large number of cases, these two sectors have merged since the 1990s. A brief description of some of the largest multinational companies is in Table 1. Many of the companies were initially agrochemical suppliers, but the development of transgenic, herbicide-resistant grain crops provided them with an opportunity to market the seed and agrochemical as a package. Several companies have an obvious leadership role in the development of agrochemicals and transgenic crops. However, the merger of many of these enterprises with seed companies increased their importance in breeding and agronomy and, more significantly, resulted in the consumer benefiting from research on transgenic crops. Genetic engineering has been carried out mostly on maize, cotton, soybean, and canola. To achieve resistance to herbicides and insecticidal activity, genes for Bacillus thuringiensis toxin were incorporated. For example, Monsanto Company's products (St. Louis, MO) include herbicide-resistant soybeans, canola, and corn and insect-protected corn. A wider range of traits and crops such as wheat, as well as output traits relating to processing quality is now being targeted. For example, Syngenta is currently developing a transgenic *Fusarium* head blight-resistant wheat. The companies also have broader genomics and gene-discovery programs. Syngenta and Basel Switzerland through its Torrey Mesa Research Institute in California, have published a rice genome analysis and made the information publicly available.

Research and development budgets of numerous companies run into several hundreds of millions of US dollars, comparable with government agricultural R&D budgets in some medium-sized developed countries.

Table 1 Major international life sciences and seed companies carrying out grain research

Company/locations	Product/technology R&D emphasis	Website
Monsanto Company St. Louis, MO, USA	Agrochemicals, seeds, genomics	www.monsanto.com
Agracetus (part of Monsanto) Middelton, WI, USA	Crop plant transformation, therapeutic protein expression in plants	www.agracetus.com
Pioneer Hi-Bred (a Du Pont Company) Des Moines, IA, USA	Seeds, grain additives Crop management, precision farming Grain seeds, especially hybrid maize (also millet, rice, sorghum, wheat)	www.pioneer.com
BASF Limbergerhof, Germany Raleigh, NC, USA	Crop protection products Transgenic crops with stress tolerance, high oil content and pathogen tolerance	www.basf.de/en; www.basf.com
Bayer Crop Science Akeno and Yuki, Japan Frankfurt, Germany Kansas City, MO, USA Lyon, France	Crop protection	www.bayercropscience.com
Syngenta Basel and Stein, Switzerland Jealott's Hill, UK Research Triangle Park, USA Toulouse, France	Crop protection, seeds, crop genetics Genomics, marker-assisted breeding BT corn, transgenic fungal disease resistance in wheat, glyphosate-tolerant soybeans, transgenic maize with improved starch quality	www.syngenta.com
Dow Agrosciences	Plant genetics and biotechnology; agrochemicals Engineered canola with different oil composition BT corn Maize hybrids, sorghum, soybeans, sunflowers	www.dowagro.com www.mycogen.com
Groupe Limagrain (Biogemma)	Cereal seeds; plant genome analysis Maize, wheat, rapeseed, sunflower, soybean, barley, peas Baking technology	www.limagrain.com

### **Brewing Companies**

These form the second largest group of private sector companies carrying out grains research. The largest brewing industry research organization is BRI (Brewing Research International), in Nutfield, UK. They undertake research on behalf of their members worldwide on aspects of food safety, barley and malt quality, fermentation, and beer quality. In addition, a number of the major brewery companies have significant in-house R&D activities, such as the giant US brewer, Anheuser-Busch, the European Brewer, Interbrew, and Tepral (Strasbourg, France) as well as smaller brewing companies worldwide. Applied research on barley and malt composition and quality is carried out.

The research activities of Carlsberg Brewers spread much further than malting and brewing. Since the establishment of research laboratories in 1876, Carlsberg has made major contributions to fundamental chemistry and such as the development of the Kjeldahl protein analytical method. There are

now three affiliated research centers located near the Carlsberg brewery site in Copenhagen, Denmark. The Carlsberg Research Center, emphasizes both basic and applied research on grain protein and carbohydrates and their reactions during brewing, and biochemical studies on amino acid transport, protein-folding seed germination, and yeast physiology. The second, Carlsberg Research Laboratory, emphasizes barley breeding, genetics, microbiology, and plant biotechnology. Recently, a third laboratory, Carlsberg Biosektor, has been established, covering a range of biotechnologies designed for commercialization.

## **Major Trends among Grains Research Organizations**

## **Increased Planning and Collaboration**

Since the 1980s, there have been significant changes in the organization and management of grains

research. There has been a greater degree of planning and an increased degree of focus in the programs of many research organizations. Much of the increased focus has been on market needs – it is hard to criticize the important influence this has had on breeding programs, grains chemistry, and processing research. Greater linkages between research on grain production and marketing is important so that the research facilitates farmers who engage in market-specified production, as opposed to research to underpin increased production of grain as a bulk commodity. A less ad hoc approach in the activities carried by government research organizations has to an extent been forced on them by funding agencies – research centers have often moved from being provided with guaranteed government allocations to being dependent on competitive funding. Research planning exercises have been in favor in a number of commercial and government organizations across the globe. It is unclear to what extent individual creativity has been affected by these trends – certainly there may be less scope for curiosity-driven grains research.

An overarching trend has been the greater level of cooperation between research organizations; funding networks such as those established by the EC and Australian Government CRC system have facilitated this trend, as well as organizational changes in major government departments such as US Department of Agriculture. However, it is probably still true to state that US grain research appears less well coordinated at a national level – although locally, the close interactions between a number of USDA-ARS facilities and land grant universities is very productive. The increased importance of networks in many areas of science is a response to the increased cost of carrying out research plus an increasing realization that problems are multidimensional and are best addressed through multidisciplinary approaches. Certainly there has also been pressure from governments and funding bodies for research providers to network more, in part to avoid duplication of research and also to ensure that particular subprojects are carried out by the most qualified group.

In the developing world, development banks such as the World Bank and Asian Development Bank have funded reform programs that encourage rationalization in the number of research providers as well as greater coordination. Scientist-to-scientist collaboration across institutional and national boundaries has been greatly facilitated by the communications revolution — the ability to email large amounts of data and draft manuscripts for immediate receipt has totally changed the possibilities for collaboration

compared with only a decade ago. Globalization in industry — both through development of alliances between commercial research providers in different countries, and increased emphasis on grains breeding for export markets have also become more important forces. Markets are becoming more differentiated so there is an increased emphasis on grain quality — both as a target for breeding efforts, additional to yield, and disease resistance — and on research aimed at improving the understanding of the genetic, chemical, and structural factors underpinning grain quality.

## **Changes in Research Subject Matter**

Grains agronomy programs have had to develop a stronger research emphasis on sustainability of production in recent years, responding to a shift in community values as well as serious scientific concerns about the long-term impact of some grain farming practices. Concerns about effects of irrigation, salinity, acidification (especially with certain legume crops), loss of soil, and of soil structure have led to an increase in research on reduced tillage, water conservation, and weed control, and maintenance of soil fertility. Indeed, the major factor underpinning sustainable agriculture has been the ability to feed the world's increasing population through enhanced yields, and with the exception of a few communities, grains, especially cereals are the central element of almost diets. Since the 1940s, there has not been a significant increase in the area of land available for agriculture. However, it is true that with the expansion of irrigation systems and with new varieties and increased fertilizer use in the middle of the twentieth century, there was an expansion of grain-growing areas. Since the 1980s, the expansion of irrigated areas has largely ceased and so production increases must come from increases in yield. Therefore the key contributor to sustainability in grains production will continue to be productivity; increases are important for both developing and developed country farmers, as the terms of trade for farmers continue to become poorer. An even greater challenge to developing countries is how North American and European governments shield their grain farmers from market realities through subsidies.

Livestock are becoming a very significant consumer of grains worldwide; the trend is especially noticeable in developing countries, where meat consumption has increased significantly through the 1980s and 1990s, from a low base. Many of these animals are now grain-fed, so research has had to underpin

the increased importance of grains for feed rather than food applications. This research is also driven by increased intensification of monogastric animal production, including in developing countries. In many countries, farmers are not solely grain growers or cattle or sheep farmers, but manage a mixed enterprise. There has been a more active development of systems-based research to address their needs.

#### Institutional Trends

Another major change in the nature of agricultural research since the 1980s, particularly in the US and Europe, has been the shift in balance of research to a greatly increased role of the private sector. In some parts of the world, such as Europe, there has been a long history of significant plant breeding efforts being carried out by industry, in others, such as Australia (with the exception of hybrid cereals), almost all plant breeding was formerly carried out by government and universities. Different countries also have different patterns of involvement of universities in breeding, although in the process of training breeders some universities have developed significant breeding programs or in other cases have been the mainstay of breeding efforts for a region or country. Several factors, some of which are interdependent, have given rise to greater commercial involvement in grains breeding, including increased importance of plant breeders' rights, development, and application of biotechnology in grain science, shrinkage of government support for agricultural research, and the increased importance of intellectual property protection. In some cases, privatization of former public sector plant breeding institutes has taken place, such as PBI in the UK (once Plant Breeding Institute, now Plant Breeding International).

The other industry that has become more involved in grains research are farmers themselves. Farmerdriven groups now often have a key role in identifying research priorities as well as being a vehicle for dissemination of research results – farmers groups directly manage on-farm adaptive research trials on technologies such as rotations, weed control, and reduced tillage. Realizing that both small and large farms are often diversified enterprises, grains research has a much greater emphasis on farming systems than in previous year. The influence of farmers is important in fitting grains research into a wider farming systems context. Organizations that use farmers' levies to fund research such as the Home-Grown Cereals Authority in the UK or the

Australian Grains Research and Development Corporation have also been instrumental in encouraging greater farmer involvement.

Most grains science is done within institutional structures, which have evolved from a range of historical situations, rather than having been developed with a consideration of whether the institutional structures fit the needs of the twenty-first century. In many state or provincial-based departments, there is a linkage of cereal breeding and cereal chemistry, and the scientists involved carry out a service function as well as independent research – often creating a tension between the time spent on the two, but with the advantage that the research objectives are informed by genuine industry problems. There are few grain science institutions in the world that cover all of cereals, legumes, and oilseeds. Instead, there may be research clusters within university agronomy or agriculture faculties or grains researchers within plant science or crops research organizations. It is more usual for postharvest-technology institutions or grain-storage laboratories to consider various issues relating to cereals and other grains on the basis that storage and handling of grains as durable commodities has common research challenges. Some institutes cover several cereals, although usually with a focus on those relevant for their country or targeted group. For example, wheat and barley are sometimes studied together. Some of the equipment and techniques are common, while others specialized – for example, while Falling number is used as a measure of postharvest sprouting for both wheat and barley, dough rheology testing is specific to wheat. Specialized grain science departments are found in a small number of US universities. Arguments for this model are the critical mass of researchers and ability of students to train in aspects from grains breeding to postharvest technology. On the other hand, such departments can lack linkages to discovery science and to a broader range of disciplines in agriculture and science.

One of the lessons of modern molecular biology is that techniques such as those in functional genomics are often readily transferable between grains, other crops, microorganisms, and animals. The high degree of synteny tells us that gene structure and function is conserved across cereals, and the great similarity of their physiological and biochemical processes often makes it simple to utilize research ideas and techniques between different grain crops. There are no definitive answers to the issue of an optimal institutional structure for grains research other than to assume that earlier structures need not necessarily be

more important than ever for breeders, agronomists, crop protection scientists, and researchers of processing quality to interact.

See also: Research Organizations of the World: Europe and North America; Asia/Pacific, Central/South America, and Africa/Middle East; CGIAR.

## **Further Reading**

Alston JM, Pardey PG, and Smith V (1999) *Paying for International Productivity*. Baltimore and London: Johns Hopkins University Press.

Pardey RG and Beintema NM (2001) *Slow Magic: Agricultural R&D a Century after Mendel.* Washington, DC: International Food Policy Research Institute.

## RICE

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## **Overview**

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# Global Distribution, Varieties, and Commercial Importance

Rice is the most important crop in the world in terms of total developing world production (480  $\times$   $10^6$  tons (t) of rough rice) and the number of consumers (2.5 billion) dependent on it as their staple food. Rice is widely grown in over 100 countries, in every continent (except Antarctica), from 53°N to 40°S, and from sea level to an altitude of 3 km. Total production in 1999 was 596  $\times$   $10^6$  t of rough rice. Asia accounts for  $134 \times 10^6$  ha out of the  $149 \times 10^6$  ha world area that is under for rice cultivation. Mean rough rice yield was  $3.84\,\mathrm{t\,ha^{-1}}$  in

1999. About 90% of the world's rice is grown and consumed in Asia. Major rice producers in 1999 were China, India, Indonesia, Bangladesh, Vietnam, and Thailand. In terms of water regime, 54% of the total area under rice cultivation was irrigated in 1991, 25% rainfed lowland, 13% upland, and 9% as flood-prone wetland, corresponding to total rough rice production of 76% from irrigated, 16% from rainfed lowland, 4% from upland, and 4% from flood-prone wetland. Since the rice-growing area is shrinking, rice production must keep up with the 1.7% per year increase in population in tropical Asia through increased yield.

There are estimated to be  $\sim 100\,000$  rice varieties; only a small proportion is actually widely cultivated. They vary in grain weight, size and shape, degree of dormancy, longevity, and seedling vigor, and some have red to purple-black pigments. About 75% of area under rice cultivation in Asia is planted with varieties of improved semidwarf plant type with erect leaves. The newer, improved varieties have

similar yield potential as the first variety (IR8) but have better resistance or tolerance to biotic and abiotic stresses. "Super" rice being bred has fewer tillers but all of them are productive.

#### Structure of the Grain

The rice grain (rough rice or paddy) consists of an outer protective covering, the hull (husk), and the edible rice caryopsis or fruit (brown, cargo, de-hulled or de-husked rice) (Figure 1). Brown rice consists of the outer layers of pericarp, seedcoat and nucellus, the germ or embryo, which are maternal tissues, and the endosperm. The endosperm consists of the aleurone layer and the starchy or inner endosperm. Pigmentation is confined to the pericarp, but there is varietal difference in the extent of retention of pigment with the degree of milling. The aleurone layer encloses the embryo.

The inedible hull constitutes 16–28% (mean 20%) of rough rice weight. Brown rice consists of 1-2%pericarp, 4-6% aleurone plus nucellus and seedcoat, 1% embryo, 2% scutellum, and 90–91% endosperm. The aleurone and embryo cells are rich in lipid bodies (spherosomes,  $0.2-1.5 \,\mu m$ ) and in protein bodies (aleurone grains) containing inclusions of phytic acid bodies or globoids  $(1-3 \mu m)$ .

The endosperm cells are thin-walled and packed with amyloplasts containing polyhedral compound

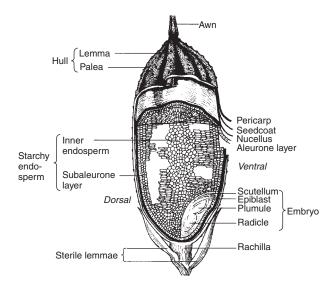


Figure 1 Longitudinal section of the rice grain. (Reproduced with permission from Juliano BO (1984) Rice starch: production, properties and uses. In: Whistler RL, BeMiller JN, and Paschall EF (eds.) Starch Chemistry and Technology, 2nd edn., 509p. Orlando, FL: Academic Press.)

starch granules  $\sim 3-9 \, \mu m$  in size. Protein occurs mainly in the form of large (1-2 µm) and small  $(0.5-0.8 \,\mu\text{m})$  spherical protein bodies and crystalline protein bodies (2–4 µm). Spherical protein bodies (PB I) are rich in prolamin (alcohol-soluble protein) and crystalline protein bodies (PB II) are rich in glutelin (alkali-soluble protein). Spherosomes are present in the subaleurone or the two outermost cell layers of the endosperm.

## Handling, Grading, and Storage

Tropical rice is usually harvested at 20% or more moisture content,  $\sim 30$  days after 50% flowering, when grains will provide optimum total and head rice yields. Moisture content at harvest is lower during the dry season than in the wet season because of more sun-drying of grain even before harvest. The actual period of dry-matter production is no more than 14-18 days, after which the grain undergoes drying. The ripening period is longer in japonica rice.

Rice is still, in most cases, harvested by cutting the panicle with enough stem to allow threshing by hand. The panicles are sun-dried on the bund prior to threshing by hand, treading by people or animals, or processing by mechanical threshers. When threshing is delayed while the cut crop is stored in heaps, "stack burning" often results as a consequence of the anaerobic respiration of microorganisms on the straw (70-80% moisture) and grain. Yellow or tan grains are formed when the panicle temperature reaches ~60°C for a few days. The discolored grains have a better head rice yield and are more translucent than control grains. The mechanism seems to be nonenzymic browning, which results in decreases in the lysine content of the protein ( $\sim 0.5\%$ ) and in the true digestibility to 92% and NPU to 61%.

Delayed harvest in rainy weather frequently leads to grain sprouting on the panicle, particularly for nondormant japonica rice. Lodging may also cause sprouting for nondormant rice. The incidence of the heavy rains (cyclones) during the harvesting season in India correlates with aflatoxin contamination of the crop.

Solar radiation is normally used, particularly in the dry season, to dry rough rice. Drying capacity is limited in the wet season, when more rice is grown because of water availability. Flash dryers are ideal for the first round of drying of harvested rough rice, to decrease the moisture content to 18-20% and the grain may be safely stored for 4-5 weeks before final drying. Grain cracking is minimal above 18% moisture. But no mechanical dryer has been adopted widely by Asian farmers.

An important property of harvested grain is its moisture content: 14% on a wet-weight basis is considered a safe storage value; grains become susceptible to fissuring from moisture adsorption stress below critical moisture contents of 12-16%, depending on variety. The glass transition temperature ( $T_{\rm g}$ ) concept has been used to explain grain fissuring during drying: below the  $T_{\rm g}$ , e.g., at  $40^{\circ}{\rm C}$ , there is no risk of fissuring, but above the  $T_{\rm g}$ , in the rubbery state, e.g., at  $60^{\circ}{\rm C}$ , tempering is required to prevent fissuring.

Rice is stored as rough rice in most of the tropics in sacks to maintain variety identity, whereas in Japan it is stored as brown rice. Storage space is reduced, but brown rice is more sensitive to environmental stress in the absence of the insulating enclosing hull and readily fissures in transit. Rubber rollers minimize bruising of the brown rice surface and improve the shelf life of the de-hulled grain. Bulk or bin storage is practiced in some countries. They are transported in 50 kg sacks or in bulk.

Storage changes or aging of sun- or oven-dried rough rice for up to 2 months after harvest, at ambient temperature >15°C, improve milling yields. This ensures that the milled rice expands more during cooking and is hence flakier. Heating during grain drying accelerates aging. Aged rice is cream colored. Aging improves the texture of tropical rice, but not in japonica rice, wherein stickiness is prized.

## **Processing**

The per capita consumption of milled rice was 86 kg per year in 1999 in Asia in comparison to 4-31 kg in other continents. About 20% of rice is consumed as parboiled rice. Parboiling consists of boiling or steaming steeped rough rice until the hull starts to open and then cooling and drying the gelatinized grain. Diffusion of bran B-vitamins into the endosperm occurs during parboiling. Mycotoxins are a potential problem in parboiled rice. Milling involves de-hulling, followed by removal of the pericarp, seedcoat, nucellus, aleurone layer, and the germ, i.e., the outer 7-10% of the brown rice, either by friction or abrasion. In the Engelberg mills, hull and bran are removed together in one step with high-grain breakage. Milling is done in several steps in modern cone mills, with tempering carried out in between to minimize breakage. Many modern mills have shifted to milling at >14% moisture to minimize grain breakage and to moisture-mist treatment (through hollow shaft) during milling to soften the bran and improve surface gloss.

There is no international standard for milled rice size and shape. International Rice Research Institute (IRRI) uses the following scale for size: extra long, >7.50 mm; long, 6.61-7.50 mm; medium, 5.51-6.60 mm; and short, <5.50 mm; whereas Food and Agriculture Organization of the United Nations (FAO) uses: extra long,  $\geq 7.00$  mm; long, 6.00-6.99 mm; medium, 5.00-5.99 mm; and short, <5.00 mm. For grain shape based on length: width ratio, the following scale is used by IRRI: slender, >3.0; medium, 2.1-3.0; bold, 1.1-2.0; and round,  $\leq 1.0$ ; and by FAO: slender, >3.0; bold, 2.0-3.0; and round, <2.0.

Grades are based on grain size and shape, degree of milling, percentage head or whole-grain milled rice, immature grains, damaged (discolored) and heat-damaged grains (chalky grains, red grains, and red-streaked grains), aroma, and organic and inorganic extraneous matter.

Only  $\sim 4-5\%$  of the world's rice production enters the international trade. The major exporters in 1997 were Thailand, Vietnam, India, USA, and Pakistan. Major importers in 1997 were Iran, Brazil, Nigeria, Philippines, Iraq, Saudi Arabia, Malaysia, South Africa, and Cote d'Ivoire.

## **Chemical and Nutritional Composition**

Rice has one of the lowest protein contents (7%) among the cereals. The bran layers and embryo are richer in nonstarch constituents than the milled rice (Table 1). The major nutritional advantage of brown rice over milled rice is its higher content of B-vitamins. Although higher in minerals, bran phytic acid and probably dietary fiber in the aleurone form complexes with minerals and proteins, reducing their bioavailability. Recent zinc bioavailability data in rats showed that the amount of zinc absorbed from brown rice was even higher than that absorbed from milled rice. Confirmatory human studies on iron and zinc biovailability in Filipino rice-fish vegetable diet based on brown and milled rice are underway to resolve this question and to determine the required precautions. The energy content of brown rice and bran is higher than that of milled rice due to the higher fat content. Rice has no vitamin A, C, or D.

Although cereal proteins are deficient in lysine, rice protein has one of the highest lysine contents among them, corresponding to an amino acid score of 59% in milled rice (based on the amino acid pattern of 5.8 g lysine per 16 g N as 100%, proposed by Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) (Table 2)). The solubility fractions of protein are

Table 1 Comparison of nutrient composition of brown rice, milled rice, and rice bran

Property		Amounts (per 100 g)	
	Brown rice	Milled rice	Rice bran
Moisture (g)	14.0	14.0	14.0
Energy content (J)	1520-1610	1460-1560	1670-1990
Energy content (kcal)	363-385	349-373	399-476
Crude protein (g)	7.1-8.3	6.3-7.1	11.3-14.9
Crude fat (g)	1.6-2.8	0.3-0.5	15.0-19.7
Crude fiber (g)	0.6-1.0	0.2-0.5	7.0-11.4
Crude ash (g)	1.0-1.5	0.3-0.8	6.6 - 9.9
Available carbohydrates (g)	73–87	77-89	34-62
Total dietary fiber (g)	2.9-4.0	0.7-2.3	17-29
Water-insoluble fiber (g)	2.0	0.5	15-27
Sugars (g)	1.9	0.2-0.5	6.4
Thiamin (mg)	0.3-0.6	0.02-0.11	1.2-2.5
Riboflavin (mg)	0.04-0.14	0.02-0.06	0.18-0.43
Niacin (mg)	3.5-5.3	1.3-2.4	26.7-49.9
Pantothenic acid (mg)	1.4	1.0	6.8
Vitamin B <sub>6</sub> (mg)	0.5	0.2	3.7
Folate (µg)	19	8	58
Vitamin E,	0.8-2.5	< 0.01-0.30	3-15
α-tocopherol (mg)			
Calcium (mg)	10-50	10-30	30-120
Phosphorus (g)	0.17-0.43	0.08-0.15	1.1-2.5
Phytic acid P (g)	0.13-0.27	0.02-0.07	0.9-2.2
Iron (mg)	0.2-5.2	0.2-2.8	8.6-43.0
Zinc (mg)	0.6-2.8	0.6-2.3	4.3-25.8
γ-Oryzanol (mg)	45.6		340-474

Sources: Juliano BO (ed.) (1985) Rice: Chemistry and Technology, 2nd edn., 774p. St. Paul, MN: American Association of Cereal Chemists; US Department of Agriculture (1998) Nutrient Database for Standard Reference, Release 12. Riverdale, MD: USDA.

 $\sim$ 15% albumin–globulin (water and salt soluble), 20% prolamin (PB I), and 65% glutelin (PB II) in milled rice. Bran proteins are 66–98% albumins. Prolamin is poorest in lysine but rich in sulfur amino acids. The high lysine content of rice protein is due to low prolamin content.

Energy digestibility is higher in milled rice than in brown rice due to lower dietary fiber and phytic acid levels as verified by poor energy digestibility of rice bran (Table 2). Protein true digestibility (TD) of milled rice is also higher than that of brown rice, but the biological value (BV) is lower, resulting in similar net protein utilization (NPU). Bran protein has lower TD but higher BV than brown and milled rice proteins. Amino acid score, corrected for TD in rats, proposed by FAO as a protein quality index, showed similar values to NPU for the rice proteins. Black or purple rice has lower NPU (72%) than brown rice and higher tannin level (0.6%) than red (NPU 83% and 0.2% tannin) and nonpigmented rices (NPU 97% and  $\leq 0.02\%$  tannin). However, their milled rices have identical NPUs. Rice complements legumes in amino acid composition for human diets. Cooking and parboiling reduce TD in growing rats by 5-15%, with a corresponding increase in BV but little change in NPU. However, lysine digestibility remains close to 100%, whereas cysteine digestibility drops to  $\sim$ 82%. The fraction that remains in the feces as fecal protein particles represents the lipid-rich core of large PB I, with less than 1% lysine in its protein and a high cystine content. PB II is readily digested.

Starch varies in apparent amylose content (by iodine colorimetry): waxy, 1-2%; very low amylose, 2-12%; low, 12-20%; intermediate, 20-25%; and high, 25-33%, all on milled rice dry weight basis.

Glycemic index of cooked brown rice tends to be lower than that of cooked milled rice due to higher phytic acid and fiber in brown rice. Among cooked milled rice, glycemic index decreases with increasing amylose content regardless of cooking method (Table 3). Processing, including parboiling, tends to reduce glycemic index. Resistant starch is <5% and may not be as important as glycemic index in humans.

Breeding efforts to improve the nutritional value of rice grain include higher micronutrient density;

Table 2 Essential amino acid profile and energy and nitrogen balance in growing rats of raw brown rice, milled rice, and rice bran

Property	Brown rice	Milled rice	Rice brai
Arginine (g per 16 g N)	7.2	7.9	7.6
Histidine (g per 16 g N)	2.4	2.2	2.5
Isoleucine (g per 16 g N)	4.0	4.1	4.0
Leucine (g per 16 g N)	7.9	7.9	7.3
Lysine (g per 16 g N)	3.6	3.4	4.6
Methionine (g per 16 g N)	2.2	2.2	2.2
Methionine + cystine (g per 16 g N)	3.3	4.2	4.4
Phenylalanine (g per 16 g N)	4.9	5.1	4.5
Phenylalanine + tyrosine (g per 16 g N)	8.5	8.3	7.5
Threonine (g per 16 g N)	3.5	3.4	4.0
Tryptophan (g per 16 g N)	1.2	1.1	0.8
Valine (g per 16 g N)	5.6	5.8	6.3
Amino acid score <sup>a</sup> (%)	63	59	80
Digestible energy <sup>b</sup> (% of total)	94.3b	96.6a	67.4c
True digestibility (TD) <sup>b</sup> (% of diet N)	96.9b	98.4a	78.8c
Biological value <sup>b</sup> (% of digested N)	68.9b	67.5b	86.6a
Net protein utilization <sup>b</sup> (% of diet N)	66.7b	66.4b	68.3a
Amino acid score × TD (%)	61	58	63

<sup>&</sup>lt;sup>a</sup>Based on 5.8 g lysine per 16 g N as 100%.

higher iron, zinc, and vitamin A contents; low phytic acid (high phosphate) and prolamin content; and absence of lipoxygenase-3 activity and of allergenic globulin, and transgenic rice endosperm with vitamin A, with soybean glycinin gene, and with soybean ferritin gene.

## **Food Uses**

There are various ways of cooking milled rice. In tropical Asia, they are prewashed to remove dirt, but this results in losses of B-vitamins and fat. Presoaking for 30 min reduces cooking time (particularly of parboiled rice). During soaking, grain cracking is minimized by adjusting milled-rice moisture content to 15% by high-pressure hydration. Rice may be cooked in the water it absorbs, or boiled in excess water and the cooking liquor discarded. A steaming process is used in Indonesia for waxy rice. Oil or ghee may be added in the Middle East, to reduce surface stickiness. Enriched rice premixes containing iron and B-vitamins resistant to washing have been developed for rice, but have not been popular due to additional expense; the enriched rice can be readily distinguished from ordinary grain.

Apparent amylose content (AC) correlates positively with water absorption and volume expansion

during cooking, and with the hardness of boiled rice. South Asian consumers prefer high-AC, intermediate-gelatinization temperature (GT  $> 70^{\circ}$ C) rice; Southeast Asians prefer intermediate-AC, intermediate-GT rice. Japonica rice preferred in Japan, Korea, Taiwan, and northern China are mainly low AC, low GT ( $\leq 70^{\circ}$ C), with some varieties having intermediate AC being preferred in Europe. Steamed waxy rice is consumed as a staple only in Laos, and north and northeastern Thailand. Amylopectin of low-GT, high-AC rice has longer mean chain length than amylopectin of intermediate-GT, high-AC rice. Among waxy and low-AC rice, low-GT rice has softer cooked rice than high-GT rice, but among intermediate- and high-AC rice, intermediate-GT rice has softer cooked rice than low-GT rice. Longer-chain amylopectin contributes to the flaky cooked rice of low-GT, high-AC rices. Amylopectin staling is less in low GT cooked rice than in higher-GT rice within each amylose type and is reversed by

Specialty rice include Italian Arborio rice for making risotto, waxy rice (sweet rice, with opaque grain), Thai Jasmine rice (aromatic low AC long grain), and Punjab Basmati (aromatic intermediate AC long grain that elongate when presoaked rice is cooked). 2-Acetyl-1-pyrroline was the first major aroma principle identified in raw and cooked aromatic rice and bis-(2-methyl-3-furyl)-disulfide,

<sup>&</sup>lt;sup>b</sup> In each line, mean values followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test. Sources: (1) US Department of Agriculture (1998) Nutrient Database for Standard Reference, Release 12. Riverdale, MD: USDA; (2) Eggum BO, Juliano BO, and Maniñgat CC (1982) Protein and energy utilization of rice milling fractions by rats. Qualitas Plantarum Plant Foods for Human Nutrition 31: 371–376.

Table 3 Comparison of glycemic index and in vivo resistant starch of various rice and rice products

Rice food <sup>a</sup>	Studies (no.)	Glycemic	c index <sup>b</sup>	In vivo resistant starch (%)
		(% of glucose)	(% of bread)	
Brown rice				
Waxy purple	1	$78~\pm~8$	112 $\pm$ 11	
Low amylose	3	$81 \pm 7$	116 $\pm$ 10	
Intermediate amylose	3	$55 \pm 5$	$79 \pm 6$	
High amylose	1	$66 \pm 7$	94 $\pm$ 10	
Milled rice				
Waxy/low amylose	3	88 ± 3	126 $\pm$ 4	
Long grain (intermediate/high)	13	56 ± 2	81 ± 3	3.9
Intermediate/high amylose	3	59 ± 3	83 ± 5	
Instant rice (intermediate/high)	2	91 ± 4	$128 \pm 4$	5.1
Milled parboiled rice				
Low/intermediate/high amylose	13	47 ± 3	68 ± 4	$3.6~\pm~0.9$
Specialty rices (intermediate)	4	54 ± 1	78 ± 1	0.0 ± 0.0
Rice pasta, brown (low)	1	92 ± 8	131 ± 11	
Rice noodles, Chinese (high)	1	58 ± 4	83 ± 5	
Rice noodles, Thai (high)	1	54 ± 5	77 + 7	
Brown-rice cakes, molded (low)	2	86 ± 9	123 ± 6	
Brown-rice cakes, molded (high)	1	61 ± 5	87 ± 7	
Puffed rice (low)	1	86 ± 7	123 ± 11	
Rice Bubbles (low)	2	88 ± 7	126 ± 10	
Rice Krispies (low)	1	82 ± 4	117 ± 5	
Rice Chex cereal (low)	1	89 ± 4	$127~\pm~5$	2.9
Rice bran (low)	1	19 ± 3	$27 \pm 4$	
Wheat white bread + 20% rice bran (high)	1	55 ± 8	$79 \pm 12$	
Wheat white bread (high)	12	70 ± 0	$101 \pm 0$	$5.4 \pm 0.8$
Wheat spaghetti (high)	10	$41 \pm 3$	$59 \pm 4$	4.6
Bulgur (parboiled wheat) (high)	4	48 ± 2	$68 \pm 13$	6.4 ± 1.1

<sup>&</sup>lt;sup>a</sup> Apparent amylose content: waxy 1-2%, low 12-20%, intermediate 20-25%, and high 25-33%. Amylose content type in parentheses for some products. <sup>b</sup> Mean  $\pm$  standard deviation. Based on blood plasma glucose value for the glucose diet as 100%, and that for the white bread diet as 100%, respectively (Glycemic index of glucose is 0.70 of that of white bread).

Sources: (1) Jenkins DJA, Cuff D, Wolever TMS, Knowland D, Thompson L, Cohen Z, and Prokipchuk E (1987) Digestibility of carbohydrate foods in an ileostomate: relationship to dietary fiber, in vitro digestibility, and glycemic index. American Journal of Gastroenterology 82: 709-717; (2) Panlasigui LN (1989) Glycemic Response to Rice. PhD dissertation, University of Toronto; (3) Food and Agriculture Organization (1998) Carbohydrates in Human Nutrition, 153p. Rome: FAO; (4) Foster-Powell K and Brand Miller J (1995) International tables of glycemic index. American Journal of Clinical Nutrition 62: 869S-893S.

2-aminoacetophenone, and an unknown compound have been recently identified.

Various rice products are prepared for which specific AC types are preferred (Table 4). Freshly and well-milled rice is preferred for rice products to prolong shelf life by minimizing fat rancidity in the stored products. Parboiled rice are preferably high and intermediate AC, while extruded and flat noodles use mainly aged, high-AC low-GT rice. Rice with a low starch GT is preferred in rice puddings, breads and cakes, and beer adjuncts. Waxy and low-AC rice are preferred for rice wines (for higher ethanol yield) and in frozen sauces, desserts, snacks, and sweets because of their slow staling rate. Rice crackers are prepared from waxy and nonwaxy rice. Parboiled rice is preferred over raw rice for "idli" (pudding) and "dosai" (cake) with rice: black gram usually fermented at 3:1 weight ratio. Thermophysical properties of starch such as  $T_{\rm g}$  and GT, enthalpy of raw starch, staled amylopectin melting (45-60°C), amylose-lipid complex I (<100°C) and II (>100°C) melting, and staled amylose melting (>130°C) of gelatinized starch affect the properties of rice products in addition to amyloseamylopectin ratio and protein content.

Use of rice bran in cereal products increased in recent years due to the hypocholesterolemic effect (in humans) of the factor(s) in the high (up to 7%) unsaponifiable fraction of its oil, such as  $\gamma$ -oryzanol (ferulate ester of cycloartenol, 24-methylene-cycloartanol, and campesterol) and tocotrienols, an analogue of tocopherol (vitamin E). Defatted bran has no hypocholesterolemic activity, unlike in oat where the active principle is soluble  $\beta$ -glucan. Inactivation of antinutrition factors – trypsin inhibitor, oryzacystatin and hemaglutinin-lectin, and lipase and lipoxygenase that are concentrated in the rice bran by heat treatment and extrusion cooking – improves

Table 4 Apparent AC type and other properties reported as preferred for various processed rice products<sup>a</sup>

Rice-based product	AC type				Other properties
	Waxy	Low	Intermediate	High	
Parboiled rice	+	+	++	++	
Precooked/quick cooking	+	+	+	+	Based on table rice AC
Expanded rice	+	+	+	+	AC not a major factor
Expanded rice, molded		+	++	+	Waxy, does not expand
Rice cereals/snacks		+	+	+	Low fat, texture, affected by AC
Extruded rice food		+	+	+	Low fat
Rice-based infant food			+	+	Low fat
Rice flour and starch	+	+	+	+	Wet milled, freshly milled rice
Rice crackers/biscuits	++	++	+	+	Japanese "arare," "senbei"
Rice puddings	+	+			Japanese "uiro"
Rice breads		+	+	+	Low GT
Unleavened rice bread				++	Pakistani "roti"
Rice cakes, steamed	+	+	+		Fermented/nonfermented
Rice cakes, baked	+	+	+	+	Low GT, for celiac disease patients
Flat noodles/rice paper		+	+	++	Low shear process
Extruded rice noodles			+	++	Hard gel consistency
Rice pasta		+	+	+	Raw and parboiled rices
Rice frozen sauces	+	+			Gel stability
Rice desserts/sweets	+	+			Gel stability
Fermented rice foods		+	+	++	Parboiled idli, dosa
Rice wines	+	++			Low protein, low fat
Beer adjunct		+			Low GT, low fat
Rice in batters and fried foods	+	+	++	+	Crunchy texture
Rice in thickeners	+	+			Gel stability

<sup>&</sup>lt;sup>a</sup>GT = gelatinization temperature. Reported preferred AC type as raw material for rice products: ++ preferred more often than +. Source: Juliano BO (1998) Varietal impact on rice quality. *Cereal Foods World* 43: 207–211, 214–216, 218–222.

the shelf life of the bran and its nutritional value poultry. Bvcontrast, the antinutrition factor, phytic acid, is heat stable. Phytic acid in rice bran and brown rice has been recently reported to have antioxidant and medicinal activity in preventing some types of cancer. Phytic acid content of rice bran is highest among cereal brans (3-8% phytic acid, Table 1). Brown rice consumption is being popularized in and outside Asia. Recent studies on rats showed that zinc bioavailability is even higher in brown rice than in milled rice. Confirmatory human studies consuming local rice-based diets are needed to insure that brown rice will not aggravate the anemia problem in the rural population by further reducing iron bioavailability. Brown rice is a better source of vitamins and antioxidants than bran due to breakdown of antioxidants during processing and storage of rice bran. "Organic" rice is also in the market with lower pesticide residues, but higher price.

Total rice bran oil production in 1986–88 was 600 000 t year<sup>-1</sup>, mainly in India, China, Japan, and Vietnam, according to FAO. It was estimated to be less than 800 000 t in 1997. This represented only 11% of potential bran oil production requiring immediate oil extraction or stabilization due

to bran lipase. The high hull-contamination (70%) of rice bran produced by Engelberg mills makes oil extraction from their "bran" uneconomical. Essential fatty acid content of rice oil is 34.2% of 18:2, and 1.5% of 18:3. Levels of oryzanol and tocotrienols and unsaponifiable matter differ among crude oils and oryzanol and tocotrienols may be reduced up to 90% by conventional refining and deodorizing.

See also: Cultural Differences in Processing and Consumption. Grain Production and Consumption: Asia. Nutraceuticals from Grains. Nutrition: Beriberi, A Deficiency Related to Grains. Oil from Rice and Maize. Organic Growing of Grains; Rice: Genetics; Breeding; Chinese Food Uses. Whole-Grain versus Refined Products.

## **Further Reading**

Chaudhary RC, Tran DV, and Duffy R (eds.) (2001) Speciality Rices of the World. Breeding, Production and Marketing, 358p. Rome: Food and Agriculture Organization of the United Nations System and Enfield: Science Publ. Inc.

- Chikubu S, Ishitani T, and Ohtsubo K (eds.) (1996) *Science of Rice (in Japanese)*, 199p. Tokyo: Asakura Publishing Co.
- Eggum BO, Juliano BO, and Maniñgat CC (1982) Protein and energy utilization of rice milling fractions by rats. *Qualitas Plantarum Plant Foods for Human Nutrition* 31: 371–376.
- Food and Agriculture Organization (1998) Carbohydrates in Human Nutrition, 153p. Rome: FAO.
- Foster-Powell K and Brand Miller J (1995) International tables of glycemic index. *American Journal of Clinical Nutrition* 62: 8698–893S.
- Jenkins DJA, Cuff D, and Wolever TMS, et al. (1987) Digestibility of carbohydrate foods in an ileostomate: relationship to dietary fiber, in vitro digestibility, and glycemic index. American Journal of Gastroenterology 82: 709–717.
- Juliano BO (1984) Rice starch: production, properties and uses. In: Whistler RL, BeMiller JN, and Paschall EF (eds.) Starch Chemistry and Technology, 2nd edn., 509p. Orlando, Florida: Academic Press.
- Juliano BO (ed.) (1985) Rice: Chemistry and Technology, 2nd edn, 774p. St. Paul, MN: American Association of Cereal Chemists.
- Juliano BO (1998) Varietal impact on rice quality. *Cereal Foods World* 43: 207–211, 214–216, 218–222.
- Juliano BO (1993) Rice in Human Nutrition, FAO Food Nutr. Ser. No. 26. 162p. Rome: Food and Agriculture Organization and Manila: International Rice Research Institute.
- Juliano BO (2003) Rice Chemistry and Quality, 480p. Muñoz, Ecija, Philippines: Philippines Rice Research Institute.
- Juliano BO and Villareal CP (1993) *Grain Quality Evaluation of World Rices*, 205p. Manila: International Rice Research Institute.
- Matsuo T and Hoshikawa K (eds.) (1993) *Science of the Rice Plant*, vol. 1, Morphology, 686p. Tokyo: Food Agricultural Policy Research Center.
- Panlasigui LN (1989) *Glycemic Response to Rice*. PhD dissertation, University of Toronto.
- Satake T (1990) Modern Rice Milling Technology, 295p. Tokyo: University of Tokyo Press.
- Shamsuddin AM (1998) *IP*<sub>6</sub> *Nature's Revolutionary Cancer-Fighter*, 114p. New York: Kensington Publ.
- Singh RK, Singh US, and Khush GS (eds.) (2000) Aromatic Rices, 292p. Oxford and New Delhi: IBH Publ. Co.
- US Department of Agriculture (1998) Nutrient Database for Standard Reference, Release 12. Riverdale, MD: USDA.
- Whistler RL, BeMiller JN, and Paschall EF (eds.) Starch Chemistry and Technology, 2nd edn., 509p. Orlando, FL: Academic Press.

## **Relevant Websites**

http://www.riceweb.org — Includes the history of rice, where and how it is grown, rice, etc. Produced by International Rice Research Institute (IRRI),

Philippines, in association with WARDA (West Africa Rice Development Association), Cote d'Ivoire and Centro Internacional Agricultura Tropical (CIAT), Colombia.

http://www.irri.org — Website of the International Rice Research Institute (IRRI), Philippines.

## **Genetics**

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## Introduction

Rice is arguably the world's most important staple food crop and a primary food source for more than half of the world's population. More than 90% of the world's rice is produced and consumed in Asia where about three-fifths of the Earth's people live. A 70% increase in rice supply by the year 2025 will be required to maintain the food-population balance. Breeding new rice varieties with high yield potential, highly resistant to biotic and abiotic stress, and with high eating and nutritional quality is one of the best ways to help satisfy this demand.

Rice genetics is the science of studying heredity in rice and related plant species, and is the basis for all sound, effective, and efficient breeding programs (*see Rice*: Breeding). It provides both basic principles and applied selection methods for rice breeding. Better understanding of the inheritance of rice traits will stimulate significant progress either in selection efficiency or in accuracy for rice breeding. Greater insight on the molecular genetic basis of the detailed functioning of metabolic pathways will better explain how the plants inherit traits from their parents and how phenotypes are developed and regulated.

Cultivated rice (*Oryza sativa* L.) is a diploid species with 24 chromosomes (12 pairs). Besides O. *sativa*, 23 additional species have been identified in the *Oryza* genus, most of which are diploid, but a few are tetraploid (*see* Rice: Wildrice, Zizania). Rice has the smallest genome (~430 Mb) among the major cereals (Table 1). Rice is a monocot and a grass, and it has been regarded as a counterpart of the dicot model system, i.e., *Arabidopsis thaliana*, in genomic studies.

Table 1 Genome size and number of genes of cereal crops

Species	Genome size (Mb)	No. of genes	Predicted kb/gene
Rice	430	30-50 000	15
Sorghum	750	30-50 000	15-25
Maize	2500	50 000	50
Barley	4800	30 000	160
Diploid wheat	5300	30 000	175

Therefore, rice has become the best-characterized cereal at the molecular level of any crop species. The entire genome of the two subspecies of cultivated rice, *indica* and *japonica*, was sequenced in 2002. Rice is hence the first crop and the second plant to have had its genome sequenced.

The development of DNA molecular markers has helped to explore the information stored in the rice genome, and to understand the genetic behavior for many of the traits of agronomic importance at the molecular level by using genetic linkage maps. This article summarizes recent advances in rice genetics especially at the molecular level.

# From Quantitative Genetics to Molecular Quantitative Genetics

### The Basis of the Classical Quantitative Genetics

Rice traits under genetic study can be assigned to two broad categories according to their complexity in inheritance: qualitative traits and quantitative traits. Qualitative traits can be classified into discrete classes and are determined by either one or a few genes which show Mendelian inheritance. To study their inheritance requires differences in the expression of alleles at individual loci (genes), usually designated as the dominant allele (wild-type allele) and the recessive allele (mutant allele). The inheritance of qualitative traits is predictable, and the genotype (G) of individuals is determined by their phenotype (P). Quantitative traits cannot be classified into discrete classes, and the variants show normal distribution in a segregating population. Quantitative traits are genetically controlled by polygenes or minor genes (each of which may also show Mendelian inheritance), but are greatly modified by environment. To study their inheritance requires large-scale experiments and statistical analysis to estimate genetic parameters.

The phenotypes (P) of individuals and families in replicated traits include genetic effects (G) and

environmental effects (E), which can be expressed in a linear model as P = G + E. If the genetic effects are not consistent across environments, there are interactions between genotypes and environments. In such a case, the linear model becomes P = G + E + GE. The genetic effects (G) can be further partitioned into additive genetic effects (A), dominance effects (D), and epistatic effects (I), and expressed as G = A + D + I. The additive effects indicate the total effects for all the loci that include the genes affecting the trait; the dominance effects are the interactions of alleles at a locus; and the epistatic effects are the interactions between alleles of different loci. Additive genetic effects can be selected directly, whereas the interaction effects (D and I) are not transmitted directly from parents to their offspring. Estimation of A, D, and I requires different types of progenies in a well-designed experiment. The total variation among phenotypes  $(\sigma_P^2)$  is attributable to the genetic and environmental effects and the interaction of genetic and environmental effects. The broad-sense heritability of a trait can be expressed as  $h_b^2 = \sigma_G^2 / \sigma_P^2$ . If the total genetic variation can be partitioned into A, D, and/or I, the narrow-sense heritability can be calculated as  $h_n^2 = \sigma_A^2/\sigma_P^2$ .

#### **Molecular Dissection of Quantitative Traits**

Classical genetic analysis can only interpret the effect of all polygenes as a whole; it cannot effectively determine the number of genes expressed for the trait, the position of genes on chromosomes, and the effect of each gene. However, these problems can be resolved with the application of genetic markers together with the help of genetic linkage maps, by dissecting quantitative traits into quantitative trait loci (QTL).

Genetic markers include morphological markers, biochemical markers (e.g., isozymes), and DNA markers. The number of morphological and biochemical markers that can be identified is very limited. DNA markers denote variations in the pattern of DNA fragments or sequences in different individuals. The differences are referred to as DNA polymorphisms, which arise as a result of insertions, deletions, duplications, and substitutions of nucleotides. Restriction fragment length polymorphisms (RFLPs) is the first class of characterized DNA markers first reported in 1980. Since then, many other types of molecular markers have been developed, such as random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) or microsatellite, inter-SSR (ISSR), sequence tagged site (STS), amplified fragment length polymorphism (AFLP), and single nucleotide polymorphism (SNP).

The abundance of DNA markers allows genetic linkage map development. Construction of a genetic linkage map involves: (1) establishment of genetic populations with a series of progenies derived from two parents, such as F2 population, backcross population, doubled haploid (DH) population, and recombinant inbred line (RIL) population; (2) analysis of DNA markers with each progeny, and scoring of each allele; and (3) genetic linkage of the markers and the distance between every two markers is calculated. The first molecular genetic map of rice containing 135 markers based on RFLPs was developed at Cornell University in collaboration with the International Rice Research Institute. The map was generated from an *indica*  $\times$  *japonica*  $F_2$  population. Primary trisomics were used to assign linkage groups to each of the 12 chromosomes. Since then, many genetic maps have been developed based on either temporary populations (e.g., F<sub>2</sub> and backcross populations) or permanent populations (e.g., DH and RIL populations).

The availability of comprehensive molecular maps in rice has opened a new avenue to tag major genes governing simply inherited agronomic traits and to dissect complex quantitative traits into QTL with molecular markers. Many methods have been developed to dissect complex traits. The simplest method is one marker analysis, that is to analyze the trait value differences between groups of individuals differing for a particular marker. No linkage map is needed in this case. Another commonly used method is interval mapping method in which a maximum likelihood method is used to test the effect of a genomic position within a chromosome interval (two adjacent markers). The disadvantage of interval mapping is its inability to take into account the effects on a trait of multiple QTLs. The other method, composite interval mapping, can resolve this problem and is robust in detecting linked QTLs on the same chromosome (see Genome Mapping).

For QTL mapping, permanent populations such as DH and RI populations are generally used, because the homozygous nature of each line in these kinds of populations allows carrying out experiments with replication and in different environments. For an F2 population, the additive effects (A) and dominance effects (D), and percent of total variance can be estimated for each QTL. For a permanent population, dominance effects cannot be estimated because of the homozygosity of each locus. As in the traditional quantitative genetics, if the phenotypic traits are measured across different environments, the QTL × environment interaction can be dissected. Also, marker and marker interaction, referred to as epistasis (or epistatic effects), will be useful in

interpreting the variation in some of the measured phenotypes.

## From QTL to Gene

Markers are not genes in the classical sense; they often do not have biological function, but they are better thought of as constant landmarks in the genome. However, it is possible to clone the functional gene at a specific QTL without knowledge of its product. The approach to clone the QTL which has been mapped on a specific location of a chromosome is map-based cloning or positional cloning. This strategy is also applicable in cloning major genes whose map positions are known.

Three steps are necessary to carry out this strategy (Figure 1). First, finely mapping the target gene or QTL to as small as possible marker intervals using high-density genetic linkage map. Second, isolating the target chromosomal region containing the gene/ QTL of interest by using the flank molecular marker to select the bacterial artificial chromosome (BAC) and yeast artificial chromosome (YAC) libraries. Third, identify the candidate functional gene by subcloning the target region and proof of its identity by mutant complementation with the target gene. High-density genetic linkage maps, good development of BAC and YAC libraries, well-established transformation techniques, and availability of the whole genomic sequence of rice has highly facilitated the isolation of rice genes.

For cloning the QTLs, the construction of special genetic stocks such as near isogenic lines or substitution lines can be used to finely map the putative QTLs as Mendelian factors with high reliability. There are about eight rice genes and QTLs cloned at present based on the map-based cloning strategy (Table 2).

## **Comparative Genomic Mapping**

The development of molecular genetic maps of different cereals using a common set of DNA clones has facilitated comparative mapping among several cereal crops. The implication that knowledge gained from rice will aid in the improvement of other grass species gives an impetus to the studies in comparative mapping. Comparative mapping indicates that there are homoeologous relationships among the genomes of various crop plants. Comparative genome mapping in rice, maize, wheat, barley, sorghum, foxtail millet, and sugarcane demonstrates that gene content and order are highly conserved at both the map and megabase level between different species within the grass family, but the amount and organization of repetitive sequences have diverged

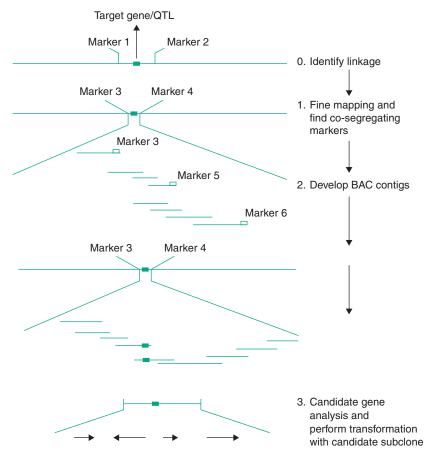


Figure 1 Principal steps for map-based cloning.

Table 2 Genes and QTLs cloned by map-based strategy in rice

Trait	Gene	Biochemical function
Resistance to blight	Xa21	Leucine-rich repeat (LRR) motif and a serine-threonine kinase
Resistance to blight	Xa1	LRR-nucleotide binding site (NBS) type of protein
Resistance to blast	Pi-b	LRR-NBS type of protein
Resistance to blast	Pi-ta	LRR-NBS type of protein
Tiller number	moc1	Plant-specific GRAS family protein
Flowering time	Hd1 (QTL) Se1	Transcription factor, Constans-like
Flowering time	Hd6 (QTL) CK2	Protein kinase
Flowering time	Hd3a (QTL)	Unknown

considerably. Microsynteny analysis using the rice YAC library clones of several hundred kilobases has also revealed remarkable similarities in marker order between rice and barley or wheat.

Comparative mapping allows prediction of the presence and location of orthologous loci in other species according to gene location in rice, which accelerates map-based cloning of orthologous genes. The availability of the whole genome sequence of rice has been of great help in other cereals for the discovery and cloning of the orthologous genes or QTLs. The progress in comparative mapping allows

comparison of the QTL intervals for similar traits in different crop species. Similarly, the results indicate that the QTL locations are convergent in different cereal species for the same traits. Comparative mapping for domestication-related traits has revealed co-evolution among different cereal crops.

## **Progress in Rice Genetics**

Recent studies show that some important characters in rice are controlled by loci having a major effect on phenotype, but most agronomically important traits such as yield, quality, and tolerance to abiotic and biotic stresses are quantitative in nature. Here, the current understanding of the genetic basis for the traits of agronomic importance is introduced and major gene tagging (Table 3), QTL analysis (Table 4), and gene cloning and expression (Table 2) underlying the traits of importance are also presented.

### **Agronomic Traits**

Yield and yield-related traits Yield is the most important trait for any rice variety, and thus becomes a major objective in rice breeding programs. Breeding rice with high yield potential is always a dream of rice breeders. Yield and yield-related traits - such as tillers per plant, grains per panicle, and grain weight – are very complex traits with low heritabilities. These traits are controlled by a set of QTLs, each QTL explaining only a few percent of the total variance. However, it seems that some interrelated traits are clustered together on chromosomes 1, 2, 4, and 5 (Table 4). The most useful QTL alleles are distributed among different rice materials. The most significant advance in QTL mapping for rice yield comes from the finding that QTLs from unadapted germ plasm or wild species can enhance the grain yield of cultivated rice. Two yield-enhancing loci, yld1 (RM5) and yld2 (RG256), located on chromosomes 1 and 2 of Oryza. rufipogon (common wild rice) have been identified, which are capable of improving the yield of modern rice cultivars. This finding is also applicable for identifying valuable QTLs for other traits from unadapted germ plasm so as to widen genetic diversity. Tillering in rice is an important agronomic trait for grain production. A major gene-controlling tiller number, moc1, has been cloned by map-based strategy and candidate gene analysis from a spontaneous monoculm 1 mutant that has only one main culm without any tillers (Table 2).

Plant height Plant height is important in breeding short-strawed, lodging-resistant cultivars in rice. The successful development of a high-yielding semidwarf variety of rice, IR8, based on the recessive gene, *sd1*, led to the so-called rice "Green Revolution" in the 1960s. Both qualitative and quantitative inheritance of plant height have been documented. More than 60 major genes for plant height have been identified by the analysis of naturally occurring variation and the semidwarfing and dwarfing mutants. Two major genes for semidwarfism, *sd1* and *sdg*, have been mapped on chromosmes 1 and 5, respectively (Table 3). Eleven major genes for dwarfism have

also been identified and mapped on rice chromosomes (Table 3). Similarly, results from QTL mapping indicate that QTLs controlling plant height distribute on all 12 chromosomes (Table 4). One major finding is that for each previously identified dwarfing or semi-dwarfing gene, at least one QTL has been mapped to its close proximity. This result supports the hypothesis that QTLs and major genes are different alleles of the same loci. The concept is useful in developing map-based cloning of QTLs. The green revolution gene, the semidwarf gene sd1, has been cloned with the candidate gene approach, and found to encode gibberellin 20 oxidase-2 (GA20ox-2), catalyzing the conversion of GA53 to GA20.

**Heading date** Heading date (HD) is a major determinant of the regional and seasonal adaptation of rice varieties, so the control of HD is an important objective in rice breeding. HD of rice is basically determined by three factors: duration of the basic vegetative growth (BVG), photoperiod sensitivity (PS), and temperature sensitivity (TS). Several genes are involved in controlling the first two factors. BVG is controlled by two or three genes. A major gene, EF-1, controlling the duration of BVG, is located on chromosome 10. PS is also controlled by several genes including Se1, Se3, Se4, Se5, Se6, Se7, E1, E2, E3, and PS (Table 3). Attempts to identify QTLs conferring HD have been carried out by many researchers. The QTLs distribute among all the chromosomes except 5 (Table 4). It seems that different rice genotype possesses different major genes for HD, so that the OTLs detected with large effects also differ in different genetic populations. That the populations derived from Lemont/Teqing, 9024/LH422, and Zhaiyeqing 8/Jingxi 17 have the same QTL on chromosome 8 explains more than 35% of the total variation. The population derived from Lemont/Teqing has anther QTL with large effects (44.7%) on chromosome 3. While in the  $F_2$  population derived from the cross between the japonica variety, Nipponbare, and the indica variety, Kasalath, the major QTL (Hd1) with large effects is on chromosome 6. Using several types of progeny derived from Nipponbare/Kasalath, a total of 15 QTLs has been identified for HD (Table 4). Among these 15 QTLs, Hd1, Hd2, Hd3a, Hd3b, Hd4, Hd5, and Hd9 have been mapped as single Mendelian factors. Three QTLs – *Hd1*, *Hd6*, and *Hd3a* – have been recently cloned by using positional cloning strategy (Table 2). The QTL Hd1 on chromosome 6 is identical with Se1 gene which is known as major photosensitive gene, and is closely related to the Arabidopsis flowering-time gene, CONSTANS. A minor QTL, Hd6, involved in PS can be dissected as a Mendelian factor using the advanced backcross

**Table 3** List of some major genes tagged using molecular markers

Traits	Gene	Chromosome	Linked marker
Plant he	ight		
	Sd1	1	RZ730-RG690
	sdg	5	RZ182
	d5	2	RG256
	d9	6	RG648-RG424
	d10	1	RG462
	d11	4	RG463
	d18	1	RG472
	d27 d30	11 2	RG103 RG171
	d32	2	RZ38-RG157
	d33	12	RG457
	d56	3	RG104-RG348
Photosei	nsitivity		
	Se1	6	RZ612
	Se3	5	A19
	PS	6	RG648-RG424
Bacterial	blight resistar		0000
	Xa-1 Xa-2	4 4	C600 Bpb235-Npb197
	ха-2 Ха-3	11	XNpb181
	ха-4	11	G181-L1044
	Xa-5	5	RS7-RM611
	Xa-7	6	G1091
	Xa-10	11	O072000-CDO365
	Xa-13	8	RZ28-RG136
	Xa-21	11	RG103
	Xa-22	11	R1506
	Xa-23	11	R1506
	Xa-25	12	S1269-S1327
	Xa-26(t)	11	R1506
Blast res			D0000 D7500
	Pi-1	11	RG303-RZ536
	pi-2(t)	6	RG64
	Pi-4(t) Pi-5(t)	12	RG869
	Pi-5(t) Pi-6(t)	4 12	RG498-RG788 RG869
	Pi-7(t)	11	RG103A-RG16
	Pi-8(t)	6	Amp3-Pgi
	Pi-9(t)	6	RG64-R2123
	Pi-10(t)	5	OPF6
	Pi-12(t)	12	RG869
	Pi-13(t)	6	Amp3
	Pi-14(t)	2	Amp1
	Pi-15(t)	9	Pi-I
	Pi20	12	XNbp88
	Pi-21(t)	4	G271-G317
	Pi-24(t)	1	K5
	Pi-25(t)	2	RG520
	Pi-26(t)	5	RG313
	Pi-27(t) Pi-28(t)	6 10	Est-2 RZ500
	Pi-28(t) Pi-29(t)	8	RZ617
	Pi-29(i) Pi-30(t)	0 11	RGA-IR14
	Pi-30(t)	12	O10-800
	Pi-32(t)	12	AF6
	Pi-33(t)	8	RM72-Y2643L
	Pi-b	2	RZ123
	Pi-h-1(t)	12	RG869
	Pi-Km (	11	R1506

Table 3 Continued

Traits	Gene	Chromosome	Linked marker
	Pi-ta	12	RZ397
	Pi-zh	8	BP127
	Pi-Co39(t)	11	G320
Brown pla	nthopper resista	nce	
	Bph-1(t)	12	XNpb248
	bph2	12	G402
	Bph9	12	G2140-G402
	Bph-10(t)	12	RG457
	Bph-?	12	RG463
	Bph12	4	RM261
	Bph13(t)	2	RM240-RM250
	Bph-?	11	RM287-RM209
Gall midge	e resistance		
	Gm2	4	RG329-RG476
	gm3	4	OPQ12
	Gm4(t)	8	R1813-S1633B
	Gm5	12	OPB14
	Gm6(t)	4	RG214
	Gm7(t)	4	F8-SA598
Submerge	nce tolerance		
	Sub1	9	RZ698-C1232
Fragrance	(aroma)		
	fgr	8	RG28, RM223
Amylopect	tin chain length		
	acl(t)	6	G200-C1478

strategy. The cloned gene encodes the  $\alpha$ -subunit of protein kinase CK2, but does not correspond to any of the genes identified by mutant analysis in *Arabidopsis* with a role in controlling flowering time. The QTL, Hd3a, is an ortholog of the *Arabidopsis* flowering-time gene FT which promotes transition to flowering downstream of Hd1 under short-day conditions.

### **Disease and Insect Resistance**

Resistance to disease There are two major types of disease resistance to plant pathogens, i.e., vertical (qualitative or complete) resistance and horizontal (quantitative or partial) resistance, both have long been recognized as a result of interactions between plant hosts and their pathogens. Vertical resistance in many plant host—pathogen relations is hypersensitive, race specific, and governed by interactions between avirulence genes in pathogens and resistance genes in plant hosts. Horizontal resistance is quantitative, presumably nonrace specific, and controlled by polygenes.

Because of the use of different genetic populations and different pathogen races in QTL mapping, the detected QTLs differ in number and effects in different studies. Even with the same population, different QTLs would result from different pathogen races used. No QTL can confer resistance to all the

 Table 4
 Summary of QTLs for some of agronomically important traits mapped on rice genome

Trait	Population	Population characteristics <sup>a</sup>	Related trait	No. of QTLs	Chromosome distribution <sup>b</sup>	$PVE^c$
Yield ar	nd yield related traits					
	9024/LH422	I/J, RIL	Grain yield	2	8,12	6.3, 9.2
		·	Panicles per plant	1	4	7.3
			Grains per panicle	3	3,4,5	14.3-22.3
			1000-grain weight	3	3,4,5	10.0-15.3
	V20/O. rufipogon	I/W, BC <sub>2</sub>	Grain yield	2	1,2	
	Zhaiyeqing 8/Jingxi 17	I/J, DH	Grains per panicle	2	4,6	12.9, 13.0
	, , ,		1000-grain weight	6	1,2,3,5,6,8	8.3-18.6
	Zhaiyeqing 8/Jingxi 17	I/J, RIL	Grains per panicle	1	5	12.9
	Tesanai 2/CB	$F_2$ , $F_3$	Panicles per plant	3	1,2,4	9.3-26.1
			Grains per panicle	2	1,2	17.2, 9.0
			1000-grain weight	7	1,1,4,5,5,10,11	7.6-14.8
			Grain weight per plant	5	1,2,4,5,8	6.4-11.4
	Waiyin 2/CB	$F_2$	Panicles per plant	2	5,6	9.9, 12.1
	•	_	1000-grain weight	2	2,10	14.8, 22.4
			Grain weight per plant	3	1,4,5	10.1-22.3
	Palawan/IR42	J/I, F <sub>2</sub>	Panicles per plant	1	4	5.4
		, <u>-</u>	1000-grain weight	1	1	9.5
			Tiller number	2	4,12	4.3, 6.8
	IR64/Azucena	I/J, DH	Tiller number	12	1,1,2,3,3,4,4,5,6,7,8,12	-,
	Zhenshan 97/Minghui 63	,			.,.,_,,,,,,,,,,,,	
	g	I/I, RIL	Yield per plant	5	1,1,2,3,6	4.9-10.0
		,	Grains per panicle	5	1,2,3,7,11	3.7-17.6
			1000-grain weight	9	1,1,1,3,3,5,6,9,11	2.5-20.8
			Tiller number	5	1,1,2,5,7	3.8-12.7
Plant he	eiaht				, , , ,	
	IR64/Azucena	I/J, DH		12	1,2,3,3,3,3,4,4,4,7,8,9	
	CO39/Moroberekan	I/J, DH		12	1,1,1,3,4,6,7,7,10,11,12,12	
	Lemont/Teqing	J/I, F <sub>2</sub>		4	2,3,8,9	7.9-25.1
	Tesanai 2/CB	I/I, F <sub>2</sub> , F <sub>3</sub>		8	1,2,3,4,5,8,11,12	10-21.3
	Palawan/IR42	J/I, F <sub>2</sub>		4	1,1,1,2	12.9-17.2
	9024/LH422	I/J, RIL		6	1,2,5,6,7,8	7.8–12.3
	Zhaiyeqing 8/Jingxi 17	I/J, DH		5	3,4,7,8,10	9.3-24.1
	Zhaiyeqing 8/Jingxi 17	I/J, RIL		2	1,4	11.9, 31.9
Looding		170, THE		-	.,.	11.0, 01.0
Headin	g date Lemont/Teging	J/I, F <sub>2</sub>	Days to heading	3	3,8,9	7.5-44.7
	9024/LH422	J/I, F <sub>2</sub> I/J, RIL	,			7.5-44.7 6.9-51.1
		I/J, HIL I/J, DH	Days to heading	3 4	3,8,11	6.9-51.1 9.3-35.4
	Zhaiyeqing 8/Jingxi 17		Days to heading		1,8,10,10	
	Zhaiyeqing 8/Jingxi 17	I/J, RIL	Days to heading	2	8,12	10.5, 35.2
	Nipponbae/Kasalath	J/I, — <sup>d</sup>	Days to heading	15	2,3,3,3,4,4,6,6,6,7,7,8,8,10,12	
Resista	nce to blight					
	Lemont/Teqing	J/I, RIL	Lesion length of leaves	11	2,3,3,4,4,8,9,10,11,11,12	

Resistance to blast					
IR64/Azucena	I/J, DH	Lesion score of leaves	9	1,1,5,6,8,10,11,12,12	
Lemont/Teqing	J/I, RIL	Percentage of diseased leaf area	9	1,2,3,4,6,7,9,12,12	5.0 - 32.0
Nipponbare/Owarihata mochi	J/J, F <sub>4</sub>	Field resistance	4	4,4,9,12	7.9 - 45.7
Chubu 32/Norin29	J/J, F3	Percentage of diseased leaf area	1	11	45.6
Co39/Moroberekan	I/J, RIL	Percentage of diseased leaf area	10	1,1,3,5,6,6,7,8,11,12	19.4-60.0
Resistance to brown planthopper					
IR64/Azucena	I/J, DH	Seedbox screening	5	1,2,4,6,8	5.6-10.1
		Field screening	3	2,4,6	5.6-16.6
		Feeding rate	1	3	13.0
		Antixenosis (settling)	3	3,6,8	5.6-8.1
		Antixenosis (oviposition)	3	1,6,8	6.0 - 7.4
		Tolerance	1	1	7.1
B5/Minghui 63	—, F <sub>2</sub>	Resistance score	2	3,4	14.3, 26.4
Lemont/Teging	J/I, RIL	Resistance index	7	1,3,5,5,8,11,11	3.3-16.9
, ,		Damage score	4	1,5,8,11	3.9-13.7
Tolerance to cool temperature					
Akihikari/Koshihikari	J/J, DH	Percent of floret sterility	3	1,7,11	5-21.7
M202/IR50	J/I, RIL	Percent of spikelet fertility	4	1,2,3,9	10.5-16.8
		Percent of undeveloped spikelet	4	5,6,7,12	11.6-12.8
Tolerance to drought					
CT9993/IR62266	J/I, DH	Osmotic adjustment	5	1,2,3,8,9	8.3-12.9
		Root penetration index	4	3,4,4,12	8.3-11.0
		Basal root thickness	6	2,3,4,8,9,12	9.2-37.6
		Penetrated root thickness	11	1,1,2,2,4,6,7,9,9,12,12	8.5-31.3
		Root pulling force	6	2,3,3,4,5,11	9.0-16.5
		Total root dry weight	5	1,2,4,6,10	8.6-20.2
		Penetrated dry weight	3	4,9,12	11.5-16.8
		Penetrated root length	1	11	17.0
		Cell membrance stability	9	1,3,7,8,8,9,9,11,12	13.4-42.1
Azucena/Bala	J/I, F <sub>2</sub>	Maximum root length day 21	5	2,5,6,10,11	7.8-13.5
	· =	Root volume	3	1,8,12	6.2-10.2
		Adventitious root thickness	3	2,3,5	7.0-21.3
Azucena/Bala	J/I, RIL	No. of tillers	1	1	12.4
	,	No. of roots	3	1,1,10	5.8-10.3
		No. of penetrated roots	7	2,2,3,3,5,10,11	5.2-16.7
		Ratio penetrated/total roots	7	2,2,3,3,5,10,11	6.8-18.0
Tolerance to submergence		·		· · · · · · · · · · · · ·	
IR74/FR13A	I/I, RIL	Tolerance score	4	6,7,11,12	19.4-26.5
IR74/Jalmagna	I/I, RIL	Plant height increment	3	1,2,4	11.2-29.6
,, 50	, · ··-	Internode increment	3	1,2,4	8.6-36.7
		Leaf length increment	3	4,6,7	6.8-18.0
		Ç			Continued
					00.1

Table 4 Continued

Trait	Population	Population characteristics <sup>a</sup>	Related trait	No. of QTLs	Chromosome distribution <sup>b</sup>	$PVE^c$
Tolerance	e to submergence					
	IR74/FR13A	I/I, RIL	Tolerance score	4	6,7,11,12	19.4-26.5
	IR74/Jalmagna	I/I, RIL	Plant height increment	3	1,2,4	11.2-29.6
	-		Internode increment	3	1,2,4	8.6-36.7
			Leaf length increment	3	4,6,7	9.4-14.2
Tolerance	to salinity					
	IR4630/IR15324	I/I, RIL	Na <sup>+</sup> uptake	1	1	8.9
			Na <sup>+</sup> concentration	2	4,6	6.4, 6.7
			$Na^+/K^+$ ratio	2	1,4	9.1, 9.6
			$K^+$ uptake	3	4,6,9	6.8-19.6
			$\dot{K}^+$ concentration	2	1,4	8.8, 10.6
Tolerance	to phosphorus deficiency					
	IR20/IR55178	I/I, RIL	Relative tillering ability	3	1,6,12	9.9-54
		• •	Relative shoot dry weight	4	1,6,9,12	10.8-60.8
			Relative root dry weight	3	1,6,12	8.9-44.2
	Nipponbare/Kasalath	J/I, BIL	Phosphorus uptake	4	2,6,10,12	5.8-27.9
			Phosphorus use efficiency	3	2,4,12	9.4-19.1
			Dry weight per plant	3	3,6,12	6.4-26.5
			Tiller number	3	4	9.5-20.6
Cooking a	and sensory quality					
	Zhaiyeqing 8/Jingxi 17	I/J, DH	Amylose content	2	5,6	11.8, 91.1
	, , , ,	•	Gel consistency	2	2,7	14.2, 20.2
			Gelatinization temperature	2	6,6	24.6, 82.4
			Peak viscosity	2	2,12	10.6, 14.0
			Hot paste viscosity	2	6,6	11.6, 33.6
			Cool paste viscosity	3	1,6,6	10.5-57.9
			Breakdown viscosity	5	1,5,6,7,12	10.4-29.0
			Consistency viscosity	4	1,6,6,7	10.0-63.7
			Setback viscosity	4	1,5,5,6	10.1-56.8
	Zhenshan 97/Minghui 63	I/I, RIL	Amylose content	1	6	
		,	Gel consistency	1	6	
			Gelatinization temperature	1	6	
	IR64/Azucena	I/J, DH	Amylose content	1	7	6.0
		,	Gel consistency	2	1,7	9.0, 13.0
			Gelatinization temperature	1	6	10.0
			Peak viscosity	2	6,7	11.0, 14.0
			Hot paste viscosity	2	2,7	15.0, 16.0
			Cool paste viscosity	<u>-</u> 1	3	8.0
			Breakdown viscosity	3	1,2,4	8.0-9.0
			Consistency viscosity	3	5,6,12	10.0-14.0
			Setback viscosity	2	4,6	9.0, 11.0

Grain appearance					
IR64/Azucena	I/J, DH	Grain length	4	1,3,3,10	13.4–23.3
		Grain width	2	1,2,3,10,11	10.1 - 13.5
		Length/width	က	2,3,3	14.9-17.2
Zhaiyeqing 8/Jingxi 17	I/J, DH	Percentage of white core	2	8,12	10.0, 21.9
		Area of white core	-	ന	8.8
Zhenshan 97/Minghui 63	I/I, F2	Grain length	က	2,3,7	6.5 - 63.8
		Grain width	က	1,5,6	10.4 - 55.2
		Length/width	2	3,5	36.4, 37.8
	I/I, RIL	Grain length	2	3,11	7.2, 57.6
		Grain width	2	5,6	4.6, 44.0
		Length/width	2	3,5	25.4, 33.3
Milling quality					
Zhenshan 97/Minghui 63	I/I, RIL	Brown rice percentage	-	5	10
		Milled rice percentage	2	3,5	4.8, 7.0
		Head rice percentage	-	ო	10.1

RIL = recombinant inbred line; W = wild rice <sup>a</sup> BC= backcross; BIL = backcross inbred line; DH = doubled haploid; I = *indica* subspecies; J = *japonica* subspecies; BIL = recombinant inbred line; W = wild ric <sup>b</sup> The value in this column indicates chromosome number, the two or three same values in the same line indicate two or three QTLs in the same chromosome. <sup>c</sup> Percentage of total variation explained by a single QTL.
<sup>d</sup> Different types of progeny. pathogen races. Some of the identified QTLs locate in the region where the major genes lie.

According to the protein sequences corresponding to the cloned plant resistance genes, a large number of resistance genes display similar characteristic domains such as a nucleotide binding site and a leucinerich repeat motif. Candidate gene fragments which involve in both recognition reaction (nucleotide binding site motif, called resistance gene analog marker) and general plant defense (putative defense response) are used as molecular markers to test for association with resistance to rice diseases. When co-localization is observed between the resistance gene analog and a putative resistance locus, the resistance gene analog markers can be assigned to the resistance gene locus and used to further identify the resistance gene. Clustering of these candidate genes in the rice genome has been observed at several chromosomal regions. Some of the clusters locate in regions where QTL associated with partial resistance to rice blast and bacterial blight are known to lie.

Among the diseases, resistance to bacterial blight, caused by Xanthomonas oryzae pv. oryzae (Xoo), has received detailed studies, because bacterial blight is one of the most serious rice diseases in Asia. Currently, more than 26 genes responsible for resistance to bacterial blight have been isolated, 13 of which (Xa1, Xa2, Xa3, Xa4, Xa5, Xa7, Xa10, Xa13, Xa21, Xa 22(t), Xa23(t), Xa25(t), and Xa26(t)have been mapped on chromosomes 4, 5, 6, 8, 11, and 12 (Table 3). Three bacterial blight resistance genes (Xa4, Xa5, and Xa13) have been physically mapped, which will facilitate the isolation of the target genes. So far, two bacterial blight resistance genes, Xa21 and Xa1, have been cloned via map-based strategy. The sequence of the predicted protein of Xa21 carries both a leucine-rich repeat motif and a serinethreonine kinase-like domain, suggesting a role in an intracellular defense response. The deduced amino acid sequence of the Xa1 gene product contains nucleotide binding sites and a new type of leucinerich repeat, indicating that Xa1 is a member of the NBS-LRR class of plant resistance genes but is quite different from Xa21.

Resistance to another disease, the blast fungus (*Pyricularia grisea*), has also received scrutiny. The genetic behavior of resistance to blast is very complex. It is genetically controlled by both major genes and QTLs. So far, more than 40 major resistance genes have been identified even though some of them are identical or allelic (**Table 3**). Some of the major genes have been tagged with molecular markers, and many of them tend to be clustered at particular chromosomal regions, such as those on chromosomes 6, 11, and 12 (**Table 3**). Two major resistance genes, *Pi-b* and *Pi-ta*,

have recently been cloned with the map-based cloning strategy. Both *Pi-b* and *Pi-ta* encode predicted nucleotide-binding-site-type proteins that are characteristic of products of major resistance genes. There are some QTLs conferring resistance to different races of blast. QTL mapping studies have permitted identifying some new major genes and QTLs as well. None of the major genes or QTLs mapped were found to confer resistance to all races. Inoculating different races of blast fungus, especially new races, can sometimes identify additional resistance genes to those previously reported.

Resistance to insects Brown planthoppers, whitebacked planthoppers, stem borers, gall midges, etc., are major insect pests of the rice crop in Asia. Investigations on resistance to insect pests are relatively more recent when compared to those on disease resistance. Rice resistance to insects is most often inherited as a quantitative trait. Some major genes for resistance to brown planthopper, Bph1, bph2, Bph10, etc., have been tagged with RFLP and RAPD markers and mapped on chromosome 12. Gall midge (Orseolia oryzae Wood-Mason) is a major dipteran pest of rice. Tolerance to gall midge has also received much genetic study. Seven major genes responsible for gall midge resistance (Gm1, Gm2, gm3, Gm4, Gm5, Gm6(t), andGm7(t)) have been tagged. Wild rice has valuable genes for resistance to various pests, and the populations derived from cultivated and wild rice crosses contribute to the identification of most of the major genes. For example, Bph12 has been identified from wild rice Oryza latifolia and mapped on chromosome 4 with microsatellite marker RM261, and Bph13(t) has been identified from Oryza eichingeri on chromosome 2 between microsatellite RM240 and RM250. The difficulty in trait measurement in the field hinders extensive investigation of QTLs underlying insect resistance. The threshold to declare a QTL for insect resistance is lower than that for other types of study, so that the QTLs identified are less accurate in position and explain a lower percentage of the total variation.

#### **Abiotic Stresses**

Tolerance to low temperature Low-temperature stress is common for rice grown in temperate regions and at high elevations in the tropics. Low temperatures dramatically reduce grain yield if the stress coincides with the sensitive period during the reproductive stage of rice by inducing spikelet sterility due to failure of microspore development or a decrease in the number of pollen grains per anther. Studies have

been conducted to locate QTLs on chromosome regions responsible for cold tolerance at the booting stage which would affect the floret sterility. The number of QTLs differ in the different populations (Table 4), indicating that useful QTLs are maintained in different rice materials.

**Tolerance to submergence** Rice is well known for its ability to grow under flooding, but most rice cultivars cannot survive if the plants are completely submerged for more than 7 days. In many genetic studies, this trait appears quantitative, but more careful testing reveals the effect of the major gene, designed Sub1 in the  $F_2$  population derived from a submergence tolerant indica line, IR40931-26, and a susceptible japonica line, PI543851. The Sub1 locus is tagged with two RFLP markers, RZ698 and C1232, on chromosome 9. QTL mapping for submergence confirms that the locus at Sub1 has a large effect on submergence tolerance, while four other QTLs have much smaller effects. High-resolution mapping of the region surrounding the *Sub1* has been undertaken, serving as the basis for map-based cloning of this important gene.

**Tolerance to drought** Drought is the major abiotic stress-limiting rice yields in rainfed environments. Tolerance to this abiotic stress is a complex process and is one of the crop genetic improvement's least understood genetic traits. Nevertheless, a few researchers have made significant progress in this aspect from the perspective of molecular marker mapping of components of drought tolerance-related root system parameters, such as root morphology, root penetration ability, osmotic adjustment, and cell membrane stability. Comparison of positions of QTLs across a few mapping populations reveals that there are several common regions associated with deep root morphology traits and root thickness traits, indicating a potential for marker-assisted selection for these traits.

Tolerance to adverse soils Adverse soil conditions hinder rice growth and development, such as high salinity, phosphorus deficiency, high aluminum content, etc. Genetic analysis of rice resistance or tolerance to adverse soil depends on the establishment of simple but quick and relatively accurate identification methods. Rice is sensitive to salinity, which affects one-fifth of irrigated land worldwide. Reducing sodium and chloride uptake into rice while maintaining potassium uptake are characteristics indispensable to aid growth under saline conditions. Genetic control of this trait appears to be quantitative. QTL mapping can dissect this complex physiological trait by

measuring its physiological components, such as Na<sup>+</sup> concentration, Na<sup>+</sup> uptake, K<sup>+</sup> concentration, K<sup>+</sup> uptake, and Na<sup>+</sup>/K<sup>+</sup> ratio. The QTLs for these traits have been mapped on chromosomes 1, 4, 6, and 9. Phosphorus deficiency occurs widely in rice soils with a high native phosphorus-fixing capacity, such as in acid soils, acid sulfate soils, and alkaline soils. Only two studies have been conducted on mapping QTLs for the traits responsible for phosphorus deficiency, enabling identification of a major QTL on chromosome 12 flanked by RFLP marker RG9 and RG241. Several other minor QTLs are mapped on chromosomes 1, 2, 3, 4, 6, 9, and 10 (Table 4).

## **Grain Quality**

Cooking and sensory quality The three most important parameters to evaluate the cooking and sensory quality of rice are amylose content (more accurately termed apparent amylose content), gelatinization temperature, and gel consistency. The change of starch viscometric properties during heating and cooling commonly tested with a Rapid Visco Analyzer simulates the cooking process, and serves as an indicator of the eating and cooking characteristics of milled rice and rice flour. Rice with aroma when cooked is often at a premium in many markets.

Amylose content has been found to be primarily controlled by an allelic series at one locus with major effects and by one or more modifier genes with minor effects. Through QTL analysis, amylose content is also reported to be controlled by a major QTL as well as other minor QTLs. This major QTL is at the *Waxy* (*Wx*) locus, which encodes granule-bound starch synthase (GBSS). The QTLs with large effects controlling gel consistency and amylograph characteristics are also at *Wx* locus, though there are many others with minor effects (Table 4).

Gelatinization temperature is a typical qualitative—quantitative trait. One to three genes with several modifiers reportedly control this trait. QTL analysis indicates that gelatinization temperature is controlled mainly by the *alk* gene loosely linked ( $\sim$ 28 cM) with Wx on chromosome 6. It is possible that the starch synthase IIa (SSIIa) gene is located at the *alk* locus, because *alk*, SSIIa, and amylopectin chain length (acl(t)) are mapped to the same locus (Table 3). In a case where the genetic population was derived from parents with similar gelatinization temperature ("IR64" and "Azucena"), it was found that the Sbe1 gene locus on chromosome 6 encoding starch branching enzyme I controlled gelatinization temperature.

The aroma of cooked rice contributes to consumer sensory acceptance. Several hundred compounds can

be observed in the volatiles of cooked rice, and more than 200 of these have been identified. The aromatic compound 2-acetyl-1-pyrroline is reportedly the primary component of the popcorn-like smell of aromatic rice. The major gene designated as *fgr* has been identified by several researchers on chromosome 8 flanked by RG28 and RM223 by 3.6 and 1.9 cM, respectively (Table 3).

Appearance The appearance of milled rice affects consumer acceptance of the product. Grain size and shape are the most stable properties of a rice cultivar. Some QTL studies have been conducted for grain length, grain width, and grain shape (length/width). Even though the number and effect of QTLs are different in different populations used, a QTL on chromosome 3 has been repeatedly identified for grain length, grain width, and grain shape. Two major QTLs are detected on chromosomes 8 and 12 for the percentage of grain with white core, and one minor QTL is found on chromosome 3 controlling area of white core. White belly seems to be primarily controlled by a major locus on chromosome 5, located in the same genomic region as a major QTL for grain width.

Milling quality The market value of rough rice, in part, reflects the percentage of head rice to total milled rice produced after milling. Milling yield is a complex trait affected by both genetic and environmental factors. A major QTL at the interval between markers RM42 and C734b on chromosome 5 controls brown rice yield, while a major QTL located at the interval of C1087-RZ403 on chromosome 3 controls head rice yield. These two loci are the major QTLs for grain width and grain length, respectively.

## **Future Prospect**

#### **Marker-Assisted Selection**

The potential usefulness of molecular markers linked to genes or QTLs is essential to the future success of molecular breeding programs. However, integrating DNA marker technology into conventional rice breeding has not been implemented widely. One reason is possibly due to the high cost of the techniques. The second is that many breeders are not so skillful in accessing the database information, and some are still stubborn to accept new methods and stick to phenotypic selection in the field. Strategy for molecular breeding requires introduction of target genes or QTLs of interest by using molecular biotechniques, including the alien genes of the donor traits from wild rice. Pyramiding more genes and QTLs into one

individual plant through marker-assisted selection is feasible for genes for disease and insect resistance, thus conferring high resistance to diseases and insects. The current development of new types of molecular markers, SNP, particularly those inside the genes, provides unlimited genetic markers for genetic linkage map construction. The quantitative traits can then be dissected into quantitative trait nucleotides (QTN) instead of QTLs by using this kind of genetic marker. Consequently, the SNP in relation to agronomically important traits can also be used in rice conventional or backcross breeding. In the next decade, more rice varieties will be bred successfully and efficiently with marker-assisted selection.

#### **Functional Genomics**

Positional cloning strategy has been demonstrated successfully in the cloning of eight genes and QTLs for traits of agronomic importance in rice. This strategy, however, is time, cost, and resource consuming, and fails to clarify the metabolic pathways underlying the phenotypic formation of traits of interest. The biological function of the cloned genes needs further investigation. The availability of whole genome sequence data of rice has emphasized the role of forward genetics, and the immediate may be characterized as an era of functional genomics.

Many artificial mutants have been generated with various approaches, such as the physical and chemical mutagens, AC/DS transposable elements of maize, retrotransposons, and T-DNA insertions. These mutants were previously utilized for forward genetics, and now are very popular for reverse genetics by providing the necessary link between structural or sequence data and gene functions. High throughput of gene expression analysis on microarrays facilitates quick acquisition of genes or expressed sequence tags in relation to phenotypic expression of different agronomic traits, and makes possible identification of metabolic pathways. This technology improves the methodologies in gene cloning with high efficiency. The early decades in twenty-first century will witness more genes cloned and gene functions clarified.

See also: Oil from Rice and Maize. Rice: Overview; Breeding; Chinese Food Uses; Wildrice, Zizania.

# Further Reading

Goff SA, Ricke D, Lan TH, et al. (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. Japonica). Science, 296: 92-100.

Goode JA and Chadwick D (eds.) (2001) Rice Biotechnology: Improving Yield, Stress Tolerance and Grain Quality. New York: Wiley.

Gowda M, Venu RC, Roopalakshmi K, Sreerekha MV, and Kulkarni RS (2003) Advances in rice breeding, genetics and genomics. Molecular Breeding 11: 337 - 352.

Khush GS, Brar DS, and Hardy B (eds.) (2001) Rice Genetics IV Proceedings of the Fourth International Rice Genetics Symposium, 22–27 October 2000. Los Banos, Philippines. Enfield, NH (USA): Science Publishers, and Los Baños (Philippines): International Rice Research Institute.

Matsuo T, Futsuhara Y, Kikuchi F, and Yamaguchi H (eds.) (1997) Science of the Rice Plant (Vol. 3): Genetics. Tokyo: Food and Agriculture Policy Research

Nanda JS (ed.) (2000) Rice Breeding and Genetics: Research Priorities and Challenges. USA: Science Publishers.

Paterson AH (ed.) (1998) Molecular Dissection of Complex Traits. New York: CRC Press.

Shimamoto K (ed.) (1999) Molecular Biology of Rice. Tokyo: Springer.

Steinmetz LM, Mindrinos M, and Oefner PJ (2000) Combining genome sequences and new technologies for dissecting the genetics of complex phenotypes. Trends in Plant Science 5: 397-401.

Yu J, Hu S, Wang J, et al. (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. Indica). Science 296: 79 - 92.

## **Relevant Websites**

http://www.gramene.org/ - This website, maintained at Cornell University, is a data resource for comparative genome analysis in the grasses. It collects molecular markers, genetic linkage maps, and genomic information for rice, particularly quantitative trait loci (QTL). The goal is to facilitate the study of cross-species homology relationships using information derived from public projects involved in genomic and EST sequencing, protein structure and function analysis, genetic and physical mapping, interpretation of biochemical pathways, gene and QTL localization, and descriptions of phenotypic characters and mutations.

http://www.irri.org/ - The International Rice Research Institute, based in Los Baños, Philippines, is a nonprofit agricultural research and training center established to improve the well-being of present and future generation of farmers and consumers, particularly those with low incomes. The Institute collects rice germ plasm worldwide, and carries out genetic and genomic studies for various traits in rice.

http://agronomy.ucdavis.edu/uccerice/ - The University of California Cooperative Extension Rice Project website introduces rice research in many aspects including agronomy, disease, quality, management, and utilization in California.

http://rgp.dna.affrc.go.jp/ - The International Rice Genome Sequencing Project was launched in 1997 with eight member nations and regions. This website delivers genomic sequences from a variety of japonica rice (Nipponbare), and offers many other services which are important for bioinformatic studies.

http://btn.genomics.org.cn/rice/ - Genome Database for Chinese Super Hybrid Rice delivers genomic sequences from a variety of *indica* rice (9311) and offers other services, such as facility to download contigs and do Blast searches, etc.

# **Breeding**

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It is difficult to trace man's earliest efforts to improve the rice plant. However, long before the advent of science, man undoubtedly had made good use of natural variation from spontaneous mutations and natural hybridizations. Susrutha (c., 1000 BC) in his Ayurvedic Materia Medica recognized the differences among rice cultivated in India and separated them into groups based upon their growth duration, water requirements, and nutritional values. Chinese classics show that Emperor Wen Ti of the Wei Dynasty (AD 186-226) discussed with his cabinet about quality rice having strong and fragrant aroma. Another Emperor, K'ang His (1662–1723) of the Ching Dynasty, selected an early maturing and aromatic mutant for a crop of rice grown in the Imperial Garden that later became the main staple of his household. The new strain was named Imperial Rice. The large-scale introduction, testing, and distribution of the early maturing Champa rice in central and east China during the eleventh century marked the first massive government-sponsored efforts to utilize efficient and productive genotypes.

Modern rice breeding is a major component of the Green Revolution which resulted in remarkable increases in rice production due to large-scale adoption of high-yielding semidwarf varieties. The development of hybrid rice further increased rice yield by 15-20% beyond the level of semidwarf rice varieties in China, and use of hybrid rice has recently been expanding in other countries. Modern varieties have had a dramatic impact on yield increases in rice production. World rice production doubled in a 25-year period, from 257 million ton (Mt) in 1965 to 520 Mt in 1990. However, to keep pace with an alarming rate of increase in the rice-consuming population, breeding of new rice varieties is an important and indispensable way to further enhance rice yield potential and stability, and adaptability to adverse soil and climatic conditions. New varieties will enable people to produce more rice from less land, with less water, less labor, and by using less pesticide. Recent breakthroughs in plant molecular biology and biotechnology have continued to revolutionize the methodology and technology of rice breeding, and will enable rice breeders to develop new varieties more efficiently.

# **Breeding for High Yield and** Wide Adaptability

Yield enhancement has long been one of the most important objectives in rice breeding. Efforts to combine a high yield with season-independent adaptability were initiated in subtropical Taiwan during the 1920s. Taichung 65 and other "Ponlai" series varieties represented a distinguished group (kêng) that is insensitive to changes in photoperiod and temperature, early maturing, nitrogen responsive, and high yielding. Ponlai varieties could be planted in both the first (spring) and second (summer) season of Taiwan and allow intensive multiple cropping between the rice crops. From a cross between a tall indica variety and the semidwarf Dee-geo-woo-gen (DGWG), the early maturity semidwarf variety Taichung Native 1 (TN1) was developed in 1956. It responded much better to heavy nitrogen fertilization and outyielded the popular Ponlai varieties. TN1 was widely used in the hybridization programs of India and by International Rice Research Institute (IRRI), the Philippines. Soon after IRRI began its research activities in 1962, the primary breeding objective was to develop high-yielding, short-and-stiffculmed, photoperiod-insensitive rice varieties that would resist lodging under heavy fertilization and would have wide adaptability in tropical areas. The famous semidwarf IR8, regarded as the most important rice variety leading to the Green Revolution, was bred from the cross of Peta/DGWG in 1966. From different country programs in tropical Asia and elsewhere, a large number of semidwarf varieties have been developed since 1968, either using TN1 or IR8 as semidwarfing parent. The more notable varieties are Jaya, Padma, Bala, Ratna, Sona, Cauvery, and Annapurna of India; RD1 and RD2 of Thailand; Meheran 69 of Pakistan; Chandina and Mala of Bangladesh; and CICA-4 and CICA-6 of Colombia.

Efforts to develop high-yielding semidwarfs on the China mainland began in the mid-1950s.

Ai-chio-nan-te and Chen-chu-ai were two of the initial releases in 1959. Ai-chio-nan-te (Ai-jiao-nan-te), Chen-chu-ai, Kwang-chang-ai (Guang-chang ai), Chiang-nan-ai (Chang-nan-ai), and Nung-k'en (Nong-ken) 58 were the principal varieties grown during the mid-1960s in central and south China, and they gave rise to improved semidwarfs or "kêng" types such as Ai-nan-tsao 1 (Ai-nan-zao 1) and Hu-hsuan 17 (Hu-xuan 17). The development of early maturing semidwarfs (100 days or less) such as Ai-nan-tsao 1 and its derivatives made triple cropping possible in Guangdong Province and double cropping in Shanghai-Nanjing area. The above semidwarfs have the same recessive gene for short stature as in DGWG.

# **Breeding for Stable Yield**

After the marked jump in yield potential was attained in the late 1960s, the next problem was to stabilize the high yield level in the face of intensified disease and insect pest pressure, which cause the loss of more than 200 Mt of rice each year. Rice fields in irrigated areas of the Asian tropics were ravaged by diseases and insects after increased nitrogen fertilization, dense planting, multiple cropping, and continuous planting of a few high-yielding varieties. Associated with the dominance of a few varieties of similar genetic backgrounds, a new or hitherto obscure biotype of a genetically plastic pest may appear in quantity, and the resistance in the major varieties breaks down. Successful progress has been made in transferring a major gene or a gene complex for resistance to specific diseases and pests into the high-yielding backgrounds. The transfer of the desired gene or genes was made by way of single or multiple crosses or a combination of them. The resistance genes for bacterial blight, blast, grassy stunt, tungro, green leafhopper, and brown plant hopper have all been incorporated into improved varieties. Among environmental factors affecting yield stability, resistance to drought and tolerance to submergence in flood waters are also essential to stabilize crop yields during seasons of erratic precipitation.

# **Breeding for Grain Quality and Improved Nutrition**

The consumer prefers rice with a clear (translucent) endosperm and pays a premium price for it. Grains with chalkiness in the endosperm, caused by loose packing of the starch and protein particles, break more easily than translucent grain during milling, thus greatly reducing the market value. The opaque areas are known as white belly, white center, or white back, depending on the location within the endosperm. For evaluation of breeding materials, it is most convenient to group them together as white belly rather than to individually rate each type. A few varieties have almost totally opaque endosperm. Other varieties are translucent or have only minute traces of white belly. The presence and degree of white belly are partially under genetic control although certain environmental factors (high temperature immediately after flowering) markedly affect their expression.

Preferences for grain length and shape vary among countries and marketing areas. Width and thickness, or shape, are less variable and less important than length, although the highest-quality markets usually demand a slender to medium width. Length and shape of grain are independently inherited and can be combined as desired with the possible exception of the extra long and bold characters. Furthermore, there are no barriers to recombination of any expression of grain length and shape with other quality traits, or with plant type, dormancy, or maturity period. Nevertheless, grain size and shape are relatively difficult traits to handle. The most important consideration is for the breeder to know which grain types are desired in the markets he serves and to stringently reject all segregates that do not meet those requirements. Improved semidwarf plant types with essentially all possible combinations of grain shape and length are available as parents in the germ plasm. Grain shape and length are quantitatively inherited. Grain size is highly heritable in most environments although low temperature after flowering can slightly reduce grain length. Despite the apparent complexity of their inheritance, grain length and shape appear to be fixed exceptionally early in the segregating generations. Preliminary selection for grain appearance can be based on visual evaluation in the field and laboratory based on examination of rough rice, and supplemented later with more accurate measurements on milled rice.

Careful evaluation of milling quality, particularly percentage of head rice, is critical in all rice breeding programs. Unfortunately, there is no simple, accurate technique to directly measure milling quality in segregating generations. It is imperative to run at least one evaluation of milling quality, preferably more, in a well-adjusted commercial mill before releasing a new variety.

Amylose is the linear fraction of starch in the nonglutinous varieties. Amylopectin, the branched fraction, makes up the remainder of the starch. Amylose content has a major influence on the characteristics of cooked milled rice, including cohesiveness,

tenderness, color, and gloss of cooked rice. Rice varieties are grouped on the basis of their amylose content into waxy amylose (1-2%), low amylose (8-20%), intermediate amylose (21-25%), and high amylose (more than 25%). Cooked glutinous or waxy rice is very moist, sticky, and glossy, and is the staple food in a few small areas in Asia. The nonwaxy varieties make up the bulk of the world's rice, ranging from 8% to 37% in amylose content. Low-amylose varieties are moist, sticky, and glossy when cooked, and readily split and disintegrate when overcooked. Rice with a high-amylose content such as IR8 cook dry and fluffy but become hard upon cooling. Intermediate types such as IR64 have the fluffiness of high-amylose types but retain a soft texture when cool. Japonica varieties tend to have low-amylose content and to be sticky when cooked. Indica varieties vary widely in amylose content according to regional quality preferences. High- and low-amylose types appear to differ by control of a single gene. The heterozygote has intermediate amylose but this cannot be stabilized. If intermediate amylose content is desired, one or both parents must be intermediate. Amylose content is partly modified by environment in largely unknown ways. High temperature during grain ripening lowers amylose content. Some varieties can vary as much as 6% in content from season to season.

Varietal differences in gel consistency exist among varieties of similar high-amylose content. Gel consistency of rice with less than 24% amylose is usually soft. The gel consistency test was developed to complement the amylose test in breeding programs for rice quality. Gelatinization temperature is partly associated with the amylose content of the starch, the major determinant of cooking behavior.

Milled rice has excellent digestibility and good protein quality, but the gross protein content (7-9%) is rather low for nutrition of children. Efforts have been made at IRRI and in China to increase the brown rice protein. High protein content appears to correlate with low yield in fixed genotypes. The use of protein per seed appears to be a more effective selection criterion than the brown rice protein content of a bulked seed sample of a plant or breeding line. Cultivation, especially use of nitrogen fertilizer, can affect protein content of milled rice to a great extent. However, high protein content is not always a preferred character, because it is very often negatively related to low eating quality.

Fragrance of cooked rice is preferred in many areas. Several chemical constituents are important to the aroma of cooked rice. However, 2-acetyl-1-pyrroline (2-AP) is the most important component. Aroma is a recessive trait that is controlled by a single recessive

gene. In aromatic rice, all parts except root contain 2-AP, and it could be detected by tasting the associated flavor in individual seeds or assessing the aroma of leaf tissues or grains after either heating in water or reacting with KOH. Therefore, selection of aromatic trait is not much difficult. Improved varieties having the aromatic quality — such as Sabarmati, Pusa 33, PR967-11, and Jasmine 85 — have been developed in South Asia and elsewhere. More recently, molecular markers, such as RM223 and RM42 in Australia, have been developed for this important trait, which may accelerate the breeding of aromatic rice.

Richness in micronutrients – such as Fe, Zn, and Ca - is a very important and desired trait in rice,because micronutrient malnutrition is a common phenomenon in countries with rice as staple food. Breeding of Fe dense rice has been initiated in several countries, but the progress has so far been very limited in conventional rice breeding. However, by using genetic engineering, transgenic rice plants reported with a ferritin gene form Phaseoulus vulgaris, and the Fe content was doubled in grains. Apart from increasing the mineral content in rice grains, reduction of antinutrient components is another way to increase the bioavailablity of micronutrients of rice. In this aspect, low-phytin rice might be the best choice, and some prototypic rice lines have been already developed, but their actual usefulness remains to be tested.

# **Breeding for Overcoming Stress Factors**

Productivity in upland, rainfed lowland, and even irrigated rice is limited by inadequate water at certain phases of growth. As much as 90% of the world's ricegrowing area is estimated to suffer from drought at critical growth stage. Under upland culture, thick and deep roots and ability to maintain leaf turgor are two of principal traits associated with drought resistance. Under rainfed lowland culture, the ability to maintain leaf water potential is clearly associated with drought resistance although the root system may play a somewhat smaller role. Incorporation of recovery ability from drought is also essential to the rainfed lowland culture.

Tolerance to submergence in flood water is different from tolerance to deep water. The two traits need to be evaluated separately, and specific qualities are needed for rice grown in deep water. To make breeding contribute more to productive and sustainable rainfed lowland rice system, genetic variation has been identified for both broad adaptation across target rainfed lowland environments and specific adaptations to some of the abiotic stress environments, e.g., late season drought, and submergence during vegetative development.

Tolerance to cool temperatures became necessary when population pressure forced farmers to cultivate rice at high elevations in tropical Asia. Good progress in breeding for cold-water tolerance in northern Japan and in California has been attained. Success appears to be attributable to long years of empirical testing and selection, use of diverse germplasm, and development of efficient cultural practices.

Millions of hectares in humid regions of Southeast Asia are left idle or are grown with very low yields because of salinity and abiotic stresses, though the climate is suited for rice production. Mineral deficiencies and toxicities frequently compound the problem of salinity. Rice varieties with partial tolerance to particular abiotic stress, e.g., salinity, alkalinity, iron toxicity or deficiency, zinc deficiency, phosphorus deficiency, manganese and aluminum toxicity, are available in rice germplasm. However, most of these varieties yield poorly and lack resistance to major disease and insects. Progress has been made recently in developing rapid and reliable screening techniques of saline tolerance, identifying and improving tolerant germplasm. Genetic analysis of the tolerance to most soil-related stresses has also been studied in rice, and elite lines have been developed with tolerance to multiple stresses. Many new varieties have been released with enhanced tolerance to such stresses and planted in salt-affected areas.

# **Breeding for the New Plant Type**

Quantum jumps in the yield potential of crop plants generally resulted from the modification of plant types. New plant architecture permitted the yield potential of rice to be doubled in the mid-1960s, which was characterized by short stature, high tillering, sturdy stems, and dark green, erect leaves that were largely contributed by DGWG or its derivatives and the tropical indicas. To make another quantum jump in the yield potential, modification of the present high-yielding plant type has been explored. The prototype is described as short stature (90–100 cm), low tillering (6–10 tillers under transplanting, no nonproductive tiller), dark green, and erect leaves. These new plant types are likely to have 20% higher yield potential than the highest currently cultivated. Heterosis between indica/japonica crosses is regarded as the most feasible way to achieve such a breakthrough.

# **Exploitation of Heterosis**

An  $F_1$  hybrid of two genetically dissimilar parents showing increased vigor at least over the mid-parent value (the average performance of two parents) is known as heterotic (shown heterosis). The use of  $F_1$  hybrids in commercial production hinges on the extent of heterosis, and the ease and costs of F<sub>1</sub> seed production. Since the early 1970s, a massive program of hybrid rice breeding has been operated in China, and recently in other rice-producing countries. Currently, more than half the rice area is planted to breed hybrid rice in China. The hybrid rice were described as having a vigorous root system, vegetative growth vigor, high tillering ability, large and dense panicles, heavy grains, and wide adaptiveness. Hybrid rice in China is mainly based on a cytoplasmic male sterility (CMS), which originated from a wild rice plant from Hainan Island, and fertility restoration system. Hundreds of CMS lines have been bred in China for hybrid rice production. These CMS lines could not be directly used as such to develop rice hybrids for the tropics because of their susceptibility to diseases and insects, and poor adaptability to tropical climates. New CMS lines were bred at IRRI and by various national programs using the wild abortive (WA) CMS system from China. New sources of CMS have also been identified at IRRI. There is no dearth of restorers among the elite indica rice germplasm in the tropics and subtropics, but the degree of restoration varies greatly and is sometime subject to environmental stresses, such as high temperature.

Use photoperiod-sensitive/thermo-sensitive genic male sterility (P/TGMS) has been extensively studied during the 1990s in China and elsewhere. P/TGMS line is used both for seed multiplication, which is done by a cross between a CMS line and its responding maintainer line in CMS system, and hybrid seed production, thus greatly simplifying seed production, because there is no need for maintainer lines. Hybrid rice facilitated by P/TGMS is named as two-line hybrid rice, and that facilitated by CMS as three-line hybrid rice. Several sources of TGMS and PGMS have been reported in China and other countries such as in Japan and the United States. In the two-line hybrid rice system, most conventional varieties are natural restorers for P/TGMS lines; thus, there is greater flexibility in selection of restorer line, and the fertility of hybrid plants is more stable than in the three-line system. Theoretically, two-line hybrids have more chance to gain greater heterosis, because there is less restriction on the choice of parents in comparison to the CMS system. However, because almost all indica two lines tend to be thermo-sensitive, and it is almost impractical to find a place where the temperature is absolutely stably higher than a certain degree because of raining and storms across different years, therefore, the seed production of the two-line hybrid rice has more restrictions than that of the three-line hybrid rice, which makes it inapplicable to many areas.

The genetic basis of heterosis is quite complicated, but it depends, in a large part, upon the genetic diversity between the two parents. The greater the genetic difference in the parents, the higher the potential heterosis. Since the 1970s, the genetic diversity among improved indica rice has narrowed due to massive international exchange of germplasm. Indica and japonica germplasm have, however, remained distinct, as there has been relatively very little gene flow between these two subspecies. As expected, the inter-subspecies hybrids showed greater heterosis for yield than did indica/indica hybrids. The new plant-type development program based on tropical japonica germplasm would also be utilized for producing hybrids with better heterosis. Successful instances are development of supervield rice varieties "Liang-you-pei-jiu (Pai'ai 64S/9311)" and "Xieging-zao A/T9308" with the highest yield potential of  $12 \text{ t ha}^{-1}$  in China.

# **Breeding Methods**

Development of new rice varieties always starts with the selection of parental varieties, no matter what kind of breeding method to be used. It consists of several important steps in rice breeding: the first is the creation of variation, the second is selection and obtains homozygous promising lines, the third is yield, quality, and other agronomic performance on plot basis, and finally participating official regional and national yield trials and registration of new varieties (Figure 1). Depending on the method of variation creation and homozygous line production, there are several methods in rice breeding (Figure 2).

#### **Cross-Breeding**

Genetic diversity among plants in a population is a prerequisite for successful plant breeding. Natural genetic variation has been used as a raw material in rice breeding. Hybridization has long been used to enhance genetic variability or to synthesize varieties combining desirable characters from two or more parents. The pedigree method of selection has been the predominant practice of rice breeders in handling segregating generations from the F<sub>3</sub> and beyond. The bulk method is particularly valuable where quantitative characters are concerned, such as yield of grain. It has the great potential advantage of reducing work in the early generations, as well as the demands on field space, so enabling the breeder to handle a larger number of crosses.

The backcrossing method may be expedient when a breeder wishes to make specific and limited improvement on a well-adapted and preferred variety. Backcrossing consists essentially of recurrent crossings to the hybrid progeny with one of the original parents (recurrent parent) with the objective of perpetuating a particular character possessed by the

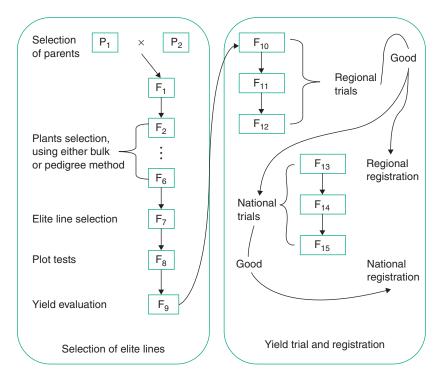


Figure 1 Flowchart of rice breeding and new variety registration.

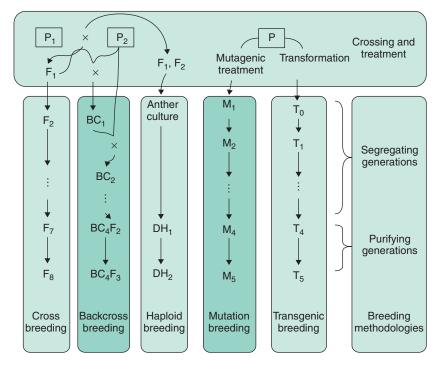


Figure 2 Flow chart of various methodologies for rice breeding.

donor parent. Backcrossing is particularly useful in increasing resistance to particular diseases. Recurrent selection following intercrossing could be helpful in improving traits that are under polygenic control and have low heritability.

#### **Induced Mutation**

Normally, the spontaneous mutation rate in rice is very low. The use of induced mutations has become a routine tool in rice breeding where convenient sources of radiation and chemical mutagens are available. Large number of mutants have been produced, most of which were by gamma irradiation of dried seeds. Chemical mutagens, mainly alkylating agents such as N-methyl-N-nitrosourea (MNH) and ethylmethanesulfonate (EMS), have been tested for their usefulness in mutation induction, but seldom used in practical breeding programs. A combination of chemical mutagens and radiation was applied in some cases. Semidwarf and early maturity are the characters most frequently achieved in released rice mutant varieties. Among other widely used mutated characters are those for grain quality, blast tolerance, salintolerance, and photoperiod insensitivity. Artificially induced mutations are very often accompanied by deleterious recessive genes, and even with chromosome damage leading to sterility. Therefore, mutants are also often used in cross-breeding as parents, which is known as indirect use of mutants.

Worldwide, numerous rice varieties have been developed by using induced mutations, and many of them have (had) been commercially planted. Zhefu 802, an indica variety developed by gamma irradiation, might be the most extensively grown mutant rice variety. Its accumulative planting area reached more than 12 Mha during 1986-94 in China. The semidwarf mutant variety Calrose 76, also induced by gamma rays irradiation from Calrose in California, USA, on the other hand, has been the semidwarf gene (sd1) donor of most modern American rice varieties, and of rice varieties grown in Australia recently. Induced mutations could be more readily used in crosses than exotic source when a desired trait could not be found in the local group of varieties. Meanwhile, mutagenic treatment could also serve as a powerful tool in breaking down the tight linkage and thus increasing recombination frequency in some wide crosses.

#### **Tissue Culture**

Tissue culture includes *in vitro* culture of embryos, protoplasts, somatic cells, anthers, and microspores. There are mainly three kinds of applications of tissue culture in rice breeding. The first is doubled haploid (DH) production, which consists of anther and microspore culture. Rice anthers, of which the microspores are at the middle to late single nucleus stage, often after subject to a 1 week cold pretreatment, are cultured on specific medium, and haploid plantlets are

induced from the microspores. The microspores could also be dispersed into culture medium and then cultured in liquid. A high percentage of regenerated plantlets is spontaneously doubled in chromosome number, and thus become DH plants. The main advantage of DH technology is its ability to produce homozygous plants from any heterozygous breeding materials; this greatly accelerates the breeding process and shortens the breeding cycle. Additional advantage of DH technology is that the dominant effects are removed in DH lines, which is of paramount importance for selection of traits contributed by recessive genes. Although anther culture has been practiced in many countries and a large number of rice varieties have been developed, its potential has not fully been realized. The majority of indica rice varieties, which cover most the rice cultivating areas worldwide, are recalcitrant to anther culture.

The second application comes with genetic transformation. In the 1980s, protoplast transformation had been unanimously believed to be the only way to produce transgenic rice plants. Therefore, protoplast culture has been widely studied in many countries, but only a few plants were regenerated. With the rapid progress of genetic transformation on rice intact cells and tissues in the 1990s, protoplast gradually phased out, and in vitro culture responsive explants, such as (immature) embryos and young panicles, are used in tissue culture for genetic transformation. A vast number of transgenic plants have been produced either for rice improvement or for genomic studies.

The observation on genetic variation among regenerated plants from in vitro culture has led to the third application. Such variation is now known as "somaclonal variations (SVs)." SVs are composed of genetic and epigenetic alterations in regenerated plants. Inheritable SVs become a unique source of genetic variation, and are used in rice improvement. In vitro culture combined with mutagenic treatment could further increase the frequency and range of variations in progenies of regenerated plants. This system is known as in vitro mutagensis. Instances of success with such a system is the development of a series of mutant hybrid rice varieties, e.g., II you 3027, Shanyou 371, and Xieyou 371 in China.

## **Genetic Engineering**

Conventional breeding has been an effective means for developing high-yielding varieties; however, it has its own limitations. The transgenic approach provides a novel way for rice breeders to use genes other than those from rice and its relatives. With this technology, the rice breeder is now able to transfer and

incorporate desirable genes into rice genome, no matter where the gene comes from. The evolutionary and taxonomic isolation is thus broken. Significant advances have been made in the genetic engineering of rice since the first transgenic rice plants reported in the late 1980s. Several transformation protocols have been deployed successfully for the introduction of foreign genes into rice.

More than 60 rice varieties, which belong to indica, japonica subspecies, and African elite rice, had been transformed with various of genes which confer important traits, such as herbicide resistance, disease and insect pest resistance, environmental stress tolerance, and nutritional quality. Among them, transgenic plants with the insecticidal gene from Bacillus thuringiensis (Bt) might be the best example on agronomical improvement.

Several insecticidal Bt genes have been transformed into various types of rice, and all showed great potential for controlling the most important insect pests in rice. The Bt gene encodes the delta-endotoxin highly toxic to lepidopteran insects. Transgenic rice plants with Bt gene have proven to be highly resistant to all lepidopterans, including stem borers such as yellow stem borer, striped stem borer and pink stem borer, and rice leaf folders in field trials. Therefore, it may lead to reduction of 1000 t of pesticide use in rice production every year. This is important not only for cost reduction in rice production, but also has huge health benefits.

The transgenic "Golden Rice" is another success of genetic engineering. The whole pathway of  $\beta$ -carotene biosynthesis was introduced into rice plants by simultaneously transforming of three foreign genes: one plant phytoene synthase (psy) gene originating from daffodil (Narcissus pseudonarcissus), one bacterial phytoene desaturase (crt1) gene originating from Erwinia uredovora, and the lycopene  $\beta$ -cyclase (lcy) gene originating from N. pseudonarcissus. The highest  $\beta$ -carotene content reached 1.6 µg per g in rice grains. It was estimated that 200 g of Golden Rice could give 100% of the recommended daily allowance of vitamin A; therefore, it has great potential for solving vitamin A deficiency related diseases, such as blindness, in developing countries.

#### **Marker-Assisted Selection**

Marker-assisted selection (MAS) is a novel technique of indirect selection of traits in rice breeding. Marker here refers to all kinds of DNA markers, usually visualized by the polymorphisms of a DNA sequence. Restricted fragment length polymorphism (RFLP) is the first extensively used DNA marker; many other types of DNA markers have been developed during

the past 10 years, mainly PCR-based markers, such as (randomly amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR), sequence tagged sites (STSs), and simple sequence repeats (SSRs) or microsatellite.

MAS has many advantages over phenotypic selection in rice breeding. DNA markers closely linked to important gene offer a tool for dissecting the genetics of complex traits and for transferring the identified loci into elite breeding lines. MAS is a more attractive approach when a few quantitative trait locus (QTLs) control a significant portion of the variability of one trait under selection. MAS is also favored when the traits being selected for have low heritability or are expensive and time consuming to measure. Compared to RFLP marker, microsatellite markers are probably the most promising markers for MAS in rice. These markers are highly polymorphic, and are now abundantly available for all rice chromosomes. Rapid DNA extraction methods developed for rice leaf tissues, or seeds are likely to work well with microsatellite markers for MAS. A prerequisite to using marker in such selection is that they should be closely linked to the precise phenotype or QTL of interest and polymorphism for the markers between the parents. MAS will certainly provide solutions to most problems associated with breeding improved for abiotic stresses. MAS techniques for bacterial blight and blast resistance, tolerance of flooding, and salinity are in progress.

See also: Rice: Genetics; Overview.

# **Further Reading**

Brunner H (1995) Radiation induced mutations for plant selection. *Applied Radiation and Isotopes* 46: 589–594. Dowling NG, Greenfield SM, and Fisher KS (1998) *Sustainability of Rice in the Global Food System.* Los Banõs, The Philippines: Pacific Basin Study Center,

International Rice Research Institutes.

Everson RE, Herdt RW, and Hossain M (1996) Prospects of approaches to increasing the genetic yield potential of rice. In: Everson RE and Herdt RW (eds.) *Rice Research in Asia: Progress and Priorities*, pp. 59–73. Los Banõs, The Philippines: CAB International and International Rice Research Institute.

Gowda M, Venu RC, Roopalakshmi K, Sreerekha MV, and Kulkarni RS (2003) Advances in rice breeding, genetics and genomics. *Molecular Breeding* 11: 227–352.

Grist DH (1986) *Rice*, pp. 126–146. Singapore: Longman. Jennings PR, Coffman WR, and Kauffman HE (1979) *Rice Improvement*. Los Banõs, The Philippines: International Rice Research Institute.

Kumar LS (1999) DNA markers in plant improvement: an overreview. *Biotechnology Advances* 17: 143–182.

Rao KK, Lakshminarasu M, and Jena KK (2002) DNA markers and marker-assisted breeding for durable resistance to bacterial blight disease in rice. *Biotechnology Advances* 20: 33–47.

## **Relevant Websites**

http://www.chinariceinfo.com — Information on rice-breeding initiatives, technologies, and new varieties developed in China.

http://www.gramene.org — A curated, open-source, web-accessible data resource for comparative genome analysis in the grasses, including rice.

http://www.hhrrc.com.

http://www.irri.org.

http://www.riceweb.org — An online encyclopedic resource of rice science and knowledge.

http://www.csrl.ars.usda.gov.

# **Chinese Food Uses**

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## Introduction

That rice culture has had a direct and significant impact on Taiwanese, and southern Chinese culture is a historical fact that could not be ignored. The Chinese proverb "Sow in spring, plow in summer, harvest in fall and store in winter" originated from and referred to rice culture which can be traced back to  $4000 \, \text{BC}$ . Of all rice,  $\sim 90\%$  is consumed as cooked whole kernels, and the rest is milled to produce flour, which is used to make cakes, desserts, and snacks for special feasts or celebrations.

In the USA, rice is usually classified by the length of grains, i.e., short, medium, and long. Both medium and long grain rice are produced in southern states and all grain types in California. In Taiwan, short grain *japonica* nonwaxy rice is produced throughout the island mainly in the southwestern plain area. Of this  $\sim 10\%$  is of long grain *indica* rice, waxy *indica*, and/or waxy *japonica* varieties. Most short grain varieties are round or bold, and the long grain varieties are slender.

The two topics covered in this article will be how to mill rice to flour and an introduction to rice-based foods. Most of the rice foods are made from either nonwaxy *indica* or waxy *japonica* rice only, and some others are made from waxy *indica* type of rice.

# Milling and Starch Isolation

Rice is milled or ground to flour or a coarse meal in some Asian countries as part of the process for making traditional rice dishes that resemble baked or steamed products. Rice flour used in processed food, which includes cereals, soup, snacks, candy and others, is estimated to have been 12.2 million cwt in 1990/91, over 21% of total domestic demand for milled rice. Official Taiwan markets statistics indicates that  $\sim$ 0.9 million cwt of rice flour is consumed annually, primarily in desserts and snacks. Of the total production,  $\sim$ 30% is used in making noodles, including rice noodles, "Bi-Tai-Ba," in main dishes.

One thousand years of milling history have produced three different milling processes: dry, semi-dry, and wet milling (Figure 1), which, depending on the amount of water used, yield different flour varieties. Properties and textural differences of rice flour are directly related to some of the inherent characteristics of starch, particularly the amylose content. Rice starch has more combinations of physico-chemical properties than other cereal starches. The amylose content of rice starch, which ranges from trace amount in waxy types to more than 30% in some nonwaxy indica varieties, plays an important role when rice flour is used as a thickener in food production. Some *indica* varieties, because of their higher amylose content (>27%), cause the product to be firmed or thickened, which produces a rigid gel during storage. Thin and wide rice noodle producers and rice cake manufacturers

prefer high amylose *indica* varieties, such as Tainung Sen 19 and Taichung Sen 17. The starch gel of the flours from medium or short grain japonica variety is more stable than that of high amylose indica rices and is preferred in the production of puffed rice cakes and rice cracker, such as popular snacks in Japan, "Sen Bei" and "Arare." The waxy japonica rice demonstrates a stickier cooked product than the waxy indica variety. Viscoamylography, a rapid viscosity analysis (RVA) or a differential scanning calorimeter (DSC), would test and determine the damage starch produced and gelling or pasting behavior. These measurements are important in choosing the type of rice for the manufacturing of the rice flour. In addition to inherent preprocessing starch properties and storage conditions, methods of milling or grinding and pretreated rice kernel profoundly affect the physicochemical and functional properties of rice flour. Complementary quality control tests – such as protein, ash, and microbial content tests – should be performed on the rice flour, as flour gleaned during the milling process is used to produce other food products, i.e., baby cereal and processed products.

## **Rice Starch**

Starch is a reserve carbohydrate found mainly in cereals, roots, tubers, and fruits and sometimes in the pith of plants. Usually it is associated with fats and inorganic salts, such as phosphorus. Rice starch granules are compound starches, varying in size from

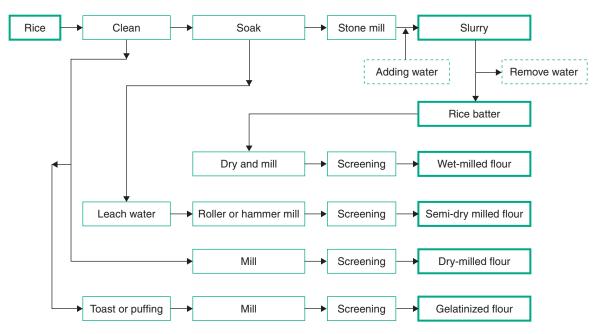


Figure 1 The flowchart of dry, semi-dry, and wet milling. (Reproduced with permission from Chen JJ (1995) Studies on the Physicochemical Properties of Waxy Rice Flours by Different Milling Methods. Master thesis, Chung-Hsing University, Taichung, Taiwan.)

3 to 10 µm. A starch granule is a homopolymer of  $\alpha$ -D-glucopyranoside units. The linear polysaccharide, amylose, has a degree of polymerization on the order of several hundred glucose residues connected by  $\alpha$ -D-(1,4) glucoside linkage. Amylopectin, a branched polymer, has a degree of polymerization on the order of several hundred thousand glucose residues, while the branched points are  $\alpha$ -D-(1,6)glucoside linkage.

Commercial preparation of rice starch is not a dominant product because of its higher cost, when compared to corn, wheat, and tapioca starches. In Belgium, Remy Industries produce 75% of the world supply of rice starch. Rice starch has more combinations of physico-chemical characteristics than other starches. The amylose content of rice starch, which ranges from trace amounts in waxy types, to more than 30% in some nonwaxy indica varieties, must be considered when using rice flour as a thickener in food production. Some *indica* varieties, with an amylose content of more than 27%, cause the product to harden or thicken and produce a rigid gel during storage.

The common methods of preparing rice starch are by alkali or DOBS extraction. An older process for manufacture of rice starch is dodecylbenzene sulfonate (DOBS) treatment. The newer process of preparing is by NaOH extraction of the protein. Milled or broken rice kernels are steeped in 5 times its weight of 0.1–0.2% NaOH for 24 h at 40°C to soften the kernels and aid protein removal at the first step. Caustic treated kernels are washed and mixed with five parts of 0.1% NaOH and then stirred for 24 h. Soaked rice kernels are ground into flour and cell walls are removed by screening. The starch slurry is allowed to settle and the supernatant solution, which contains most of the protein, is removed. Starch granules are washed with water; decanting is employed to remove soluble materials. Centrifuging dewaters washed starch; complete removal of residual alkali is an important step. The starch slurry is put in an oven to dry-down the moisture to  $\sim 10\%$ ; the rice starch cake is ground to the desired particle size and sieved.

The starch properties measured by differential scanning calorimetry (DSC) showed that gelatinization temperature and enthalpy values were significantly correlated for these flours. The lower enthalpy values for dry-milled flour give an indication of relative high starch damage that occurs during milling.

### **Rice-Based Food Products**

This article will not pay attention to the rice crackers, because they are the major and traditional baked snack food in Japan (Figure 2). Instead, the rice food which is consumed by the Chinese or overseas Chinese community will be emphasized. In terms of expanding into industrial scale, several discouraging factors come into play. The main obstacle is the lack of well-formulated machinery manufacturing processes. Other concerns include governmental controls over the distribution and price of rice, which limit the accessibility of raw materials to rice industry. The rice food products are classified into: (1) products using whole grains, such as puffed rice items, and (2) products using flours prepared before and after cooking.

#### **Puffed Rice**

Puffed rice products are common in Taiwan. The kernel puffing process involves frying or by high pressure.

Gun-puffing and Mi-Hua-Tung Gun-puffing cake and "Mi-Hua-Tung" are typical Chinese rice snacks made from puffed rice mixed and molded with syrup, sugar, peanut, and flavoring. *Japonica* type nonwaxy as well as waxy rice varieties are preferred for gun puffing. Waxy-type rice has higher water absorption index and water solubility, resulting in a soggy texture and eating quality, so the best choice for gun puffing is the low amylose content (below 20%) japonica rice. About 600 g of the rice with 14% moisture is fed to the gun, in which pressure is built up to 10-12 kg cm<sup>-2</sup>, and then suddenly released. The gun is preheated for a couple of minutes before the rice is put in. After a short cooking time, the gun is suddenly opened and puffed rice kernel is caught in a metal hopper. The puffed rice is mixed with sugar syrup or maltose syrup in a pan to make small square pieces, called gun-puffing cakes.

For Mi-Hua-Tung, the milled rice or brokens are washed, soaked in water, and passed through a steaming and drying oven. The cooked rice is dried to 10-15% moisture by a rotated dryer, and then fried at a temperature of 240-250°C for 10-12 s for puffing. The puffed rice kernel is mixed with maltose, starch syrup, and other ingredients and put through a molder, metal separator, and then packed.

**Guo-Ba** For "Guo-Ba," the rice grain is cooked first. There are two ways to cook the rice. Traditionally, the rice is soaked in water for 30 min, and then boiled or steamed by gas to obtain the whole grain cooked rice. All the water is absorbed by the rice during cooking period. For industry, the milled rice is soaked in an equal amount of water at room temperature for a couple of hours and then steamed in 18 psi pressure for 10 min. Selecting the proper *indica* waxy rice

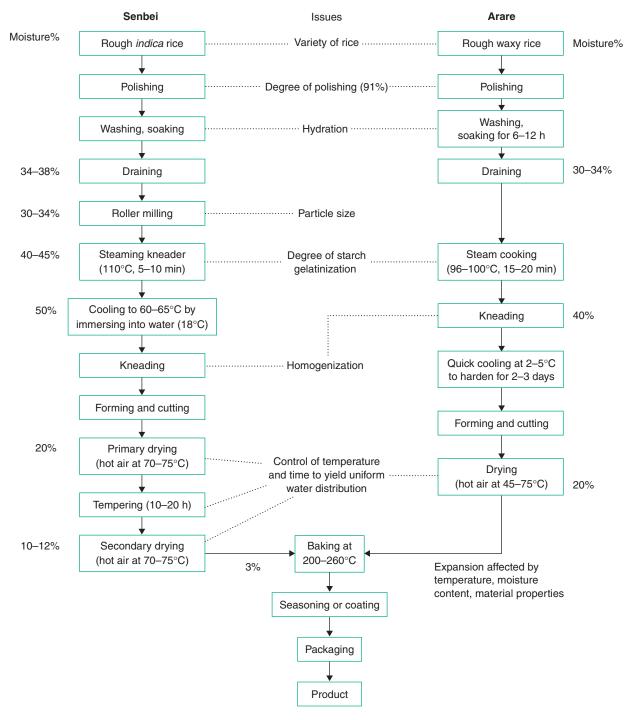


Figure 2 Manufacturing of "Senbei" and "Arare." (Courtesy of Yeh AY (2003). Preparation and application of rice flour. In: Champagne E (ed.) Rice: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.)

varieties and controlling the water-rice ratio are important in keeping the cooked rice from being too sticky or too soft. The heating conditions should be controlled to get the gelatinized rice or the cooked rice grain without scorches in the center location.

Cooked rice is weighed and cut to rectangular pieces (10 grams each), put into 5 cm squares, and dried to reduce the moisture content down to  $\sim$ 12–15%. Dried products are then fried at 220°C by a deep fry conveyor for 4–8 s and then packed.

### **Rice Desserts and Sweets**

Mogi In Taiwan, to the natives and to some extent immigrants, "mogi" is a very popular rice cake. It is prepared from *japonica* waxy milled rice (short grain) by washing, soaking, wet milling to flour, steaming at 100°C for ~45–60 min, kneading, cooling, dividing, and packing. Traditionally, the rice is pounded by wooden pestles in mortars. During this process, air is removed from the rice cake and a smooth texture is obtained. With mechanical kneading, the air bubbles easily get into the mogi which results in a rough surface of whiter appearance. Mogi is usually produced in a ball shape with mashed red beans or peanut grits inside and eaten as a snack.

New Year cake (Nien-Kuo) The short grain waxy rice is preferred to make "Nien-Kuo." The rice is soaked for a couple of hours and ground by stone mill to get slurry. The slurry is dehydrated either by centrifugation or by draining the slurry in a cotton cloth bag with heavy stones placed on the top of bag. The wet-milled rice flour containing  $\sim 45\%$  moisture is mixed with sugar and water to get batter, and the ratio is 100:80:70 for flour, sugar, and water, respectively (Table 1). The batter is cooked in a steamer for 4-5 h, then cooled and packed.

## Rice Noodle (Bi-Tai-Ba)

Indica rice varieties with high amylose content are preferred to make rice noodles because retrogradation is necessary. There are several rice noodle types, such as "Mi-Fen," "Bi-Tai-Ba," "Ho-Fen," etc., which are very popular in Taiwan, Japan, and among the overseas Chinese communities in Southeast Asian countries.

**Mi-Fen** Mi-Fen is a popular main dish in meals. Generally Mi-Fen is made in the dry state, and it can be steamed or cooked before serving. There are

two procedures to make Mi-Fen, process A or B. Either of them can be selected.

Process A Cleaned rice kernels soaked in water for couple of hours are ground into slurry, the rice slurry is filled into a cloth bag, and the filled bag is pressed with a mechanical press or centrifugation to remove water. The dewatered rice solids are put in a heated mixer for 50 min and the rice solids are partially cooked to a soft mass (addition of cornstarch and/ or wheat is optional). The soft rice mass is mixed a second time to further soften it. The soft rice mass is transferred to a presser to form thick sheets, followed by extruding the sheets into rice noodles. cooling, and loosening the extruded rice noodles immediately to avoid sticking together. The extruded rice noodles are steam-cooked for ~50 min, then cut into with a knife when still warm, shaped into bundles or blocks, and loaded onto trays in carts. Finally, shaped rice noodles are dried for 8h, and cooled thoroughly before packaging into specific containers for retail or storage.

*Process B* Instead of soaking, grinding, and dewatering the rice, rice flour is used directly in mixing and partial cooking. The rest of the steps are essentially the same as process A.

Bi-Tai-Ba Bi-Tai-Ba is produced as a wet form of noodles, coarser and shorter. It is eaten with syrup and ice water in the hot season, but it also can be served with meat, green onions, or other seasoning. The traditional procedure to make Bi-Tai-Ba is explained as follows: Soaked rice kernels are wet milled to slurry, centrifuged to dehydrate the batter, heated to pregelatinize one-third of batter, the remaining two-thirds of the batter is extruded to string (3.0 mm in diameter), and finally, steamed, cooled, and packed.

 Table 1
 The quality characteristics of Nien-Kuo with different formula

Quality characteristics		Ingredie	ents ratio (sucrose: wa	ter : flour)	
	11:4:10	10:5:10	9:6:10	8:7:10	7:8:10
Hardness	$3612 \pm 56^{a}$	2245 ± 35	1801 ± 65	1587 ± 67	1432 ± 24
Aw <sup>b</sup>	$0.79\pm0.01$	$0.81\pm0.01$	$0.86\pm0.01$	$0.87\pm0.01$	$0.90\pm0.02$
pH	$5.66\pm0.15$	$5.85\pm0.04$	$5.84\pm0.11$	$6.01\pm0.13$	$6.10\pm0.10$
Ĺ	$26.12\pm0.35$	$29.37\pm0.14$	$32.70\pm0.12$	$33.07\pm0.24$	$34.82\pm0.24$
Α	$1.01\pm0.12$	$0.56\pm0.11$	$0.45\pm0.09$	$0.31\pm0.05$	$0.05\pm0.04$
В	$12.34\pm0.20$	$9.99\pm0.21$	$9.12\pm0.16$	$9.25\pm0.12$	$10.69\pm0.11$

<sup>&</sup>lt;sup>a</sup>Mean  $\pm$  SD, n = 6.

<sup>&</sup>lt;sup>b</sup>Aw: water activity.

Source: Lin JS (1993) Studies on the Quality of New-Year Rice Cake Treated by Different Soaking Conditions and Dehydration Methods. Master thesis, Chung-Hsing University, Taichung, Taiwan.

Recently, the extruder method has been used to make Bi-Tai-Ba. Dry-milled flour with 38% moisture is fed to a single screw extruder, which has three barrel sections, with temperature set at 130°C, 100°C, and 50°C, respectively.

**Ho-Fen** Ho-Fen making is similar to Bi-Tai-Ba; it is produced in both wet and dry forms of noodle, more thinner (1-2 mm thick) and wider (1 cm) rice stripes.

The basic steps in the making of traditional Ho-Fen is described as follows: prepare rice slurry with rice flour and water, put a small amount of oil on stainless trays to coat the trays evenly, pour rice slurry on the trays to form a thin layer ( $\sim 1-2$  mm thick). Steam the trays of thin layers of rice slurry to gelatinize the starch for  $\sim 5$  min, then remove the trays as soon as possible from the steamer and cool down to the room temperature. Roll up the gelatinized rice sheet from each tray with a spatula ( $\sim 10$  cm in depth), and cut the layered rice sheets into 1 cm wide stripes. For long-time storage, dry the moisture to  $\sim 10\%$  water-content with a mechanical oven drier or any such aid.

# Fa-Kuo (Rice Muffin)

There is another kind of muffin-style rice food, "Fa-Kuo," in Taiwan and Southeast Asian countries. *Indica* rice is preferred to make this kind of food. Dried or wet-milled rice flour (100 g base) is mixed with 50-80% sugar and 3.5% leavening agent with or without red color additive, and water is added to the tune of 120% to make batter. The batter is put into a bowl, steamed for ~20 min, and then cooled. Fa-Kuo is prepared from wet-milling flour by two steps of high-speed mixing to get better eating quality than one-speed mixing procedure. The redcolored Fa-Kuo is usually prepared for celebration and festivals, since it is a symbol for luck and promotion in one's profession. During the time of Chinese New Year every family will have Nien-Kuo and Fa-Kuo.

#### **Bowl Rice Curd**

It is a very traditional and popularly consumed breakfast food item in the south of Taiwan. *Indica* rice flour is preferred to make bowl rice curd. The procedure is as follows: Milled rice kernels, homogenize, take 1/3 of the slurry to heat for partial gelatinization, mix remaining 2/3 portion, put in bowl and add other ingredients (chopped pork, salt, fried red onion, black pepper, seasoning etc.), steam for 15 min and, finally, pack the product.

### **Rice Cracker**

Japonica and/or waxy rice varieties are preferred to make rice cracker. Cleaned rice kernels are soaked in water for a couple of hours, and ground in a stamp mill or pulverized in a roller mill. The fine particle rice flour is fed into a steaming kneader or cooked in a steamer for 15-20 min to gelatinize the starch, then cooled down to  $60^{\circ}$ C, and the dough is sheeted to plate form and cut to pellets. The pellets are dried until only 20% moisture content remains, tempered at room temperature for  $\sim 1$  day, and then dried at a certain temperature until the moisture content drops to 10%. The dried pellets are then baked in an oven at a temperature ranging from  $\sim 200$  to  $260^{\circ}$ C, thereafter, the expanded final product has a moisture content of  $\sim 3-5\%$ .

#### **Miscellaneous Products**

There are several rice products, which are not major consumption for Asian people, and will not be attempted to introduce in this article. These products include parboiled rice, breakfast rice cereals, canned rice, easy-to-cook brown rice, rice bran oil and yeast-fermented rice cake.

# **Summary**

Rice-based food products require specific physicochemical characteristics to produce the required eating qualities. There are several major factors affecting the cooking or eating qualities as considered by the end user, which include the aging or storage conditions of rice grains, rice varieties, amylose content of starch, degree of milling, as well as milling methods.

See also: Noodles: Starch Noodles. Oil from Rice and Maize. Rice: Overview; Genetics; Breeding; Wildrice, Zizania. Snack Foods, Processing.

## **Further Reading**

Bean MM and Nishita KD (1985) Rice flours for baking. In: Juliano BO (ed.) *Rice Chemistry and Technology*, 2nd edn., pp. 539–556. St. Paul, MN: American Association of Cereal Chemists.

Chang SM and Chang TL (1995) The characteristics of explosion puffing rice produced with different amylose contents. *Journal of Food Science (Chinese)* 22: 465–478.

Chen JJ (1995) Studies on the Physicochemical Properties of Waxy Rice Flours by Different Milling Methods. Master thesis, Chung-Hsing University, Taichung, Taiwan.

- Huang RM, Chou MB, and Lii CY (1998) Effect of the characteristics of rice and the processing conditions on the expansion ratio of dry cooked rice. *Journal of Food Science (Chinese)* 25: 383–393.
- Jomduang S and Mohamed S (1994) Effect of amylose/ amylopectin, milling methods, particle size, sugar, salt, and oil on the puffed product characteristics of a traditional Thai rice-based snack food (Khao Kriap Waue). Journal of the Science of Food and Agriculture 65: 85.
- Juliano BO and Sakurai J (1985) Miscellaneous rice products. In: Juliano BO (ed.) Rice: Chemistry and Technology, 2nd edn., pp. 569–618. St. Paul, MN: American Association of Cereal Chemists.
- Lin JS (1993) Studies on the Quality of New-Year Rice Cake Treated by Different Soaking Conditions and Dehydration Methods. Master thesis, Chung-Hsing University, Taichung, Taiwan.
- Lu S and Lii CY (1989) The influences of various milling processes on the physico-chemical properties of rice flours and the rice flake preparation. *Journal of Food Science (Chinese)* 16: 22–35.
- Lu S and Yeh CP (1996) Laboratory preparation of bi-taiba by single screw extruder. *Journal of Food Science* (Chinese) 23: 650–661.
- Lu S, Lin MS, Lin TC, and Lii CY (1993, 1995) Studies on the quality of bi-tai-ba and its frozen stability addition with commercial starches. *Journal of Food Science (Chinese)* 20: 64–74.
- Lu S, Lin JS, and Lin TC (1995) The effects of physicochemical characteristics at different soaking and dehydration conditions on wet-milled rice flour. *Journal of Food Science (Chinese)* 22: 426–437.
- Sakurai J (1971) Rice as an Industrial Raw Material for Manufacture of Processed and Ready-to-Eat Rice Products. (Proceedings) International Seminar on the Industrial Processing of Rice Madras, India. United Nations Industrial Development Organization, Vienna. 65p.
- Taiwan Provincial Government (1998) *Taiwan Food Statistics Book*. Republic of China: Department of Food and Agriculture, Taiwan Provincial Government.
- Yeh AY (2004) Preparation and application of rice flour. In: Champagne E (ed.) *Rice: Chemistry and Technology.* St. Paul, MN: American Association of Cereal Chemists.
- Yeh AY, Hsiu WH, and Shen JS (1991) Some characteristics in extrusion cooking of rice noodle by twin screw extruder. *Journal of the Chinese Agricultural Chemistry Society* 29: 340–351.

## **Relevant Website**

http://www.irri.org — Website of the International Rice Research Institute, based in Philippines. Has information and links on rice utilization.

# Wildrice, Zizania

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### Introduction

Wildrice (Zizania sp.) is in its infancy in cultivation and domestication compared to other cereals such as rice (Oryza sativa L.). Rice has been under this process for a few thousands years BC. In the North American continent, the Native Americans did some expansion of natural stands by hand seeding new lakes and areas of lakes with sparse stands. But it was not until 1950 that a 0.5 ha area in Minnesota was specifically constructed to grow wildrice. Subsequently, more hectares were developed and some breeding efforts were initiated in the early 1960s with a full-scale breeding program initiated in 1972 by the University of Minnesota. Today wildrice is cultivated in Minnesota, Wisconsin, California, Oregon, and Idaho in the US, and in Hungary and Australia. The Canadians have expanded lake production by seeding new lakes in several provinces. The natural stands as well as newly seeded lakes in Canada are harvested by airboats with catchers attached to the front.

There are three species of wildrice that grow naturally in the North American continent: Zizania aquatica L., Zizania palustris L., and Zizania texana A.S. Hitchc. Z. aquatica grows along the eastern seacoast and has thin kernels. Z. palustris grows around the Great Lakes region and has large seeds. (It was gathered by Native Americans for hundreds of years as a food source.) Both of these species are annual aquatic grasses. Z. texana is a perennial with small seeds and grows only in a small area in Texas. Z. latifolia Turcz. grows in Asia, and the base which is infected with a fungus is harvested and used as a vegetable, "Makomo-taki."

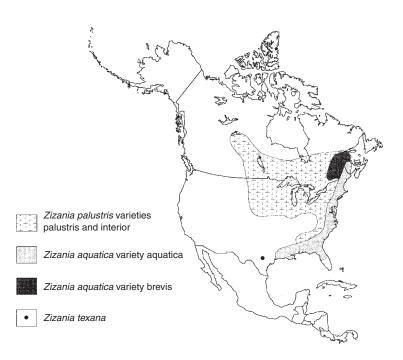
Since wildrice is a recently cultivated cereal grain and in the process of domestication, the purpose of this article is to acquaint the reader with this new crop. Wildrice is cultivated using similar practices as is used for lowland rice, i.e., it only grows in flooded soils. Harvesting the newly developed varieties is done with combines similar to rice in the US. Processing is different, however, giving wild rice its unique roasted flavor. The nutritional value of wildrice is considerably better than rice having nearly twice as much protein and an amino acid complex similar to oats. Wildrice is often mixed with rice and served as a blend. The pure product serves as a side dish similar to potatoes or rice.

Continued improvement in yield and increased production should make wildrice more available in stores and restaurants not only in the US but also in other countries.

# **Wildrice Species**

Wildrice was first classified by C Linneaus, in England in 1753. He used the description sent to him by JF Gronovius, Leyden, Holland. Gronovius described a plant sent to him by John Clayton which he collected in Virginia in 1739. Based on the plant's description, C Linneaus gave the plant the binomial Zizania aquatica L. The original plant specimen is preserved in the Gronovian Herbarium in the British Museum of Natural History in London, England. Zizania is a small genus of aquatic grasses in the tribe Zizanieae. Rice, Oryza sativa L., belongs to the tribe Oryzeae, which precedes the tribe Zizanieae. Both wildrice and rice belong to the grass family, Gramineae, and the subfamily, Poacoideae. Oats and barley are also in the Poacoideae subfamily. The above hierarchical classification is presented in Gray's Manual of Botany, eighth addition, by Fernald (1950). However, in more recent publications Grass Systematics by Gould and Shaw in 1983, second edition, and Flowering Plants of the World by Heywood (1993) both wildrice and rice are classed in the family, Poaceae, subfamily Bambusiodeae, and in the tribe, Oryzeae. Subsequently, wildrice growing naturally in lakes and rivers was classed into three different species by Linneaus and others. They are two annual species Z. aquatica L. and Z. palustris L. and one perennial species, Z. texana Hitchc. The distribution of these species in North America are shown in Figure 1.

There are two varieties of Z. aquatica, aquatica and brevis. They are annual plants that grow along the eastern Atlantic seacoast. The seed is long and thin and generally not harvested for food. There are two varieties of Z. palustris, palustris and interior. Both of these annual plants have relatively large grains and have been harvested as food for centuries by Native Americans. These two varieties grow naturally in the north central areas of North America. Today the best natural stands occur in the states of Minnesota and Wisconsin in the US and in the provinces of Manitoba and Ontario, Canada. A second perennial species has been identified in Asia. It is classed as Z. latifolia Turcz. There are accounts that the grain has been used by the elite as a special food. However, today in Asia the base of the plant, which is infected with a fungus, is used as



**Figure 1** The natural distribution in shallow lakes and rivers in North America of the three species of *Zizania*. (Reproduced with permission from Oelke EA, Porter RA, Grombacher AW, and Addis PB (1997) Wild rice – new interest in an old crop. *Cereal Foods World* 42: 234–247. Minneapolis: American Association of Cereal Chemists.)

Table 1 Description of Zizania species

Species name	Description
Zizania aquatica var. aquatica L. southern wildrice	Annual, tall, large panicles, many branched florets. Grows in muddy shores of streams in southern Ontario and Quebec, southward to Florida and Louisiana. Average seed size: 14.3 mm long and 0.9 mm diameter.
Zizania aquatica var. brevis, Fasset, estuarine wildrice	Annual, short, small panicle with few branched florets. Grows in tidal areas of St. Lawrence River estuary. Average seed size: 6.7 mm long and 1.0 mm diameter.
Zizania palustris var. palustris L. northern wildrice	Annual, height ranges from short to tall, slender few-flowered panicle. Grows in water up to 120 cm deep in southern Canada from New Brunswick to Manitoba and northern US states. Average seed size: 16.7 mm long and 1.4 mm diameter.
Zizania palustris var. interior (Fassett), Dore, interior wild rice	Annual, medium tall, medium to large panicle with numerous florets. Grows in water up to 30 cm deep along rivers in southeastern Manitoba and adjoining Ontario and in the North Central States. The germ plasm of this species and variety has been used to develop varieties for cultivation. Average seed size: 10.7 mm long and 1.8 mm diameter.
Zizania texana A.S. Hitchc., Texas wildrice	Perennial, decumbent with many long stems, panicles short, short seeds.  Grows only in a localized area in San Marcos River in Texas. Average seed size: 6 mm long and 1.2 mm diameter.
Zizania latifolia Turcz. Manchurian waterrice	Perennial, spreads by subterranean runners, tall, medium panicles, seeds short to medium in length. Native grass of Manchuria, Korea, Japan, Burma, and northeastern India. Base of plant often becomes infected with a fungus which is used as a "vegetable" delicacy. This species is commercially grown in Japan, Korea, and China for its vegetable called "Makomo-taki." Average seed size of collections from Japan: 7 mm long and 1.3 mm diameter.

a vegetable, "Makomo-taki." A brief description of each species is given in Table 1.

### **Historical Use of Species**

#### Zizania palustris L.

The large seed,  $8-16 \,\mathrm{mm}$  long and  $1.2-2.5 \,\mathrm{mm}$  in diameter, has been harvested from lakes and rivers in the Great Lakes Region by the Chippewa and Menomini Native American (First Americans) tribes for centuries. After harvest it was processed and stored and used as a carbohydrate source for the long, cold winters. Many battles were fought for control of the prized wildrice stands. In most cases the Chippewa ended up controlling the wildrice stands. The Ojibway name for wildrice is "Manoomin" meaning good berry or good seed, but the exact meaning is not agreed upon. The early explorers, both English and French, who often traded with the Ojibway, gave various names to this plant; some were Indian rice, wild rice, Canadian rice, squaw rice, water oats, march oats, and water rice. The French explorers called the plant folle avoine, fools oats or wild oats. The name which was most commonly used was wild rice and is used in the trade today. To avoid confusion with the weedy strain of rice, Oryza, writers often use the hyphenated, wild-rice, or the single word, wildrice. In this article the author is using the single word wildrice.

Today stands of wildrice on reservations in Minnesota and Wisconsin are controlled by Native Americans, but in lakes and rivers outside of the Reservations, the stands are controlled by the departments of natural resources. In Minnesota and Wisconsin, a license is required by everyone to harvest from lakes and rivers, not on Reservations. Today, natural stands in Minnesota are harvested, by law, in the traditional method using a flat bottom boat or canoe. The canoe is pushed through the stand by one individual using a long pole while the other individual uses two flails (knockers) to knock the grain into the canoe. Since the grain ripens at different times on the same panicle and easily falls (shatters) from the plant when mature, stands are harvested by this method every other day for a period of 2 weeks. Enough grain falls back into the water before and during harvest to reseed the lake or river for the next year. Figure 2 depicts the traditional harvest method. In Canada, natural stands are generally harvested by airboats fitted with a grain catcher onto the front.

The traditional method of processing the harvested wildrice was to lay it on the ground for drying since the grain has ~40% moisture when harvested. Later the Native Americans used an iron kettle over a fire to dry (parch) the grain



Figure 2 The traditional way (canoe and flail) of harvesting natural stands of wildrice. One individual (standing) propels the canoe through the stand while the other uses two flails (knockers) to remove the grain from the stalks. (Photograph by Oelke EA.)



**Figure 3** The traditional method of drying wildrice grain in a kettle above a fire. The grain is stirred while drying. (Photograph by Oelke EA, appeared in Oelke EA, Grava J, Noetzel D, Barron D, Percich J, Schertz C, Strait J, and Stucker R (1982) *Wild Rice Production in Minnesota*. Extension Bulletin 464, Agricultural Extension Service. St. Paul: University of Minnesota.)

(Figures 3 and 4). The hulls (lemma and palea) were removed from the warm grain by placing it into a round bottom container or leather lined hole and then walking in place (jigging) in the



**Figure 4** The traditional way (jigging) to remove the outer hull of the wildrice grains. The individual walks in place to rub the grain against each other and also the kettle. (Photograph by Oelke EA, appeared in Oelke EA, Grava J, Noetzel D, Barron D, Percich J, Schertz C, Strait J, and Stucker R (1982) *Wild Rice Production in Minnesota*. Extension Bulletin 464, Agricultural Extension Service. St. Paul: University of Minnesota.)

container. The chaff was removed by tossing the grain into the air (winnowing) and allowing the wind to blow away the lighter chaff. The heavier grain was caught in the container. Very little grain

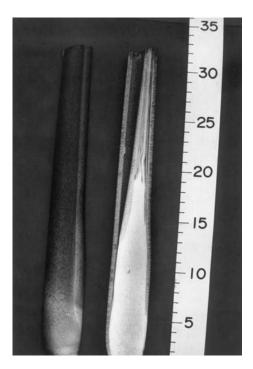
harvested from natural stands is processed in this manner today. Most of it is processed with mechanical equipment as described later.

# Zizania aquatica L. and Zizania texana Hitchc.

The seed from these species is either too thin or small to make it worthwhile to harvest as a food source. *Z. texana* is on the endangered species list.

#### Zizania latifolia Turcz.

This species is a perennial and is a native grass of Manchuria, Korea, Japan, Burma, and northeastern India. The plant generally is not used for grain production but grown commercially for its vegetable delicacy, especially in Japan, Korea, and China. The base of the plant becomes infected with a fungus. The infected base of the plant is harvested and eaten as a vegetable. In Japan they call the vegetable "Makomo-taki." The infected part is also dried and allowed to develop spores. The spores are then used by artists to develop lacquered,



**Figure 5** The base of the stem of *Zizania latifolia* which is infected with a fungus. This base is harvested especially in Japan and used as a vegetable "Makomo-taki" delicacy. Numbers are in centimeters. (Photograph by Oelke EA, appeared in Oelke EA, Bloom PR, Porter RA, and Liu Q (1999) Wild rice plant development and seed physiology. In: Williamson LS, Dlutkowski LA, and McCammon Soltis AP (eds.) *Proceedings of the Wild Rice Research and Management Conference*, pp. 54–67. Odanah, WI: Great Lakes Fish and Wildlife Commission.)

antique-looking woodwork, such as plates and small boxes (Figures 5 and 6).

# Cultivation of Zizania palustris L.

## **Domestication versus Cultivation**

The expansion of this species of wildrice represents one of the largest modern efforts to domesticate a cereal grain. Cultivation usually is defined as annually harvesting the grain from a plant without annual sowing, through the germination of seeds which fall onto the soil. Thus, even the Native Americans did some form of cultivation but under natural conditions. Most crops are now truly domesticated, which means they depend entirely on human care for their perpetuation. Wildrice is not far removed from the cultivation phase. Varieties are being developed that shatter less than the plants growing in the lakes and rivers. They also produce more grain per hectare but still only 10-15% of that produced by Oryza (rice).

## **History of Cultivation/Domestication**

Rice (Oryza) has been under the cultivation/domestication process for some thousands of years before



**Figure 6** The Japanese farmer on the right is growing *Z. latifolia* for its "Makomo-taki" production. (Photograph by Oelke EA, appeared in Oelke EA, Bloom PR, Porter RA, and Liu Q (1999) Wild rice plant development and seed physiology. In: Williamson LS, Dlutkowski LA, and McCammon Soltis AP (eds.) *Proceedings of the Wild Rice Research and Management Conference*, pp. 54–67. Odanah, WI: Great Lakes Fish and Wildlife Commission.)

Christ. Native Americans did some seeding of lakes, thus did some cultivation, but relied on natural lake structures. They seeded wildrice by mixing the seed into clay, rolling it into a ball and dropping the clay ball into the water resulting in some increase in natural stands.

Interest in cultivating this plant has been expressed for well over 100 years by businessmen and botanists. Early explorers collected seed for planting in Europe, but they failed. In 1828, Timothy Flint in *Geography and History* wondered why no attention was paid to this plant. In 1852 Joseph Bowron and in 1853 Oliver Kelly also thought about why this plant is not cultivated. In 1917 H B Williams and Z Durand started mechanically harvesting private land in Canada.

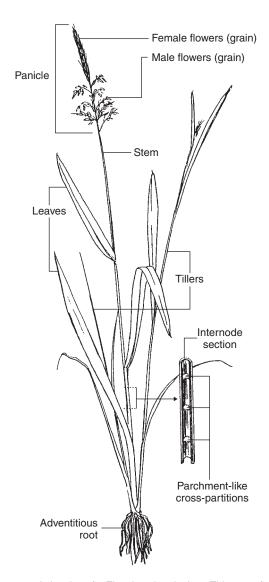
The first individuals to construct a field specifically to produce this aquatic grain were James and Gerald Godward. They grew a 0.5 ha field at Bass Lake near Merrifield, Minnesota. The first three years the Godwards had good crops, but disease destroyed the crop the fourth year. They continued their pioneering efforts and by 1958 had 50 ha diked for growing wildrice. Tom Godward, one of the sons, continues to grow wildrice today near Aitkin, Minnesota, with  $\sim$ 6-800 ha under cultivation. During the mid-1950s and early 1960s others started growing wildrice. In 1965, Uncle Ben, Inc. started contracting for production, which was a big impetus to produce wildrice. Today there are  $\sim$ 7000 ha in Minnesota, 5000 ha in California, with a few hectares in Wisconsin, Oregon, and Idaho in the US producing wildrice. Some production also is now taking place in Hungary and Australia (Figure 7).

### **Genetics and Breeding**

Domestication of a wild species requires that plants are selected from wild populations that maintain their grains on the plant even after maturity (nonshattering). The grain also needs to mature uniformly and have more grain production in relation to vegetative production. One of the important factors in the domestication process was the discovery, in 1963 in a grower's field, of a nonshattering plant by two University of Minnesota scientists (Figure 8). Subsequently improved varieties were developed from this germ plasm by individuals, companies, and the University of Minnesota. Up until that time fields were planted with seed collected from lakes. These fields were harvested with picker/harvesters on tracks. The fields had to be harvested several times during a 2 week period without cutting off the plants. Yields with this method were only 168-224 kg ha<sup>-1</sup> of unprocessed grain. Fields planted to the newer nonshattering varieties could be harvested

once with a regular grain combine and yielded over 1700 kg ha<sup>-1</sup>. The development of the nonshattering varieties resulted in a tremendous expansion of cultivatedhectares in Minnesota. Table 2 shows the cultivated wildrice production after processing for Minnesota and California since 1968.

Individual wildrice growers began their own selection programs to select better plant types in the 1960s and some are continuing today. The wildrice breeding program at the University of Minnesota began in 1972. It continues till today and is the only public wildrice breeding program in the US. To date, six varieties have been released all with some desirable characteristics needed to further the domestication of



**Figure 7** A drawing of a *Zizania palustris* plant. This type of plant is the one cultivated in commercial fields. (From Oelke EA, Elliott WA, Kernkamp MF, and Noetzel DM (1973) *Commercial Production of Wild Rice*. Extension Folder 284, Agricultural Extension Service. St. Paul: University of Minnesota.)



**Figure 8** The panicle on the left is a nonshattering type while the one on the right is a shattering type. The nonshattering type can be recognized by loss of male flowers soon after pollen shed on the branch (lower) portion of the panicle. (From Oelke EA, Grava J, Noetzel D, Barron D, Percich J, Schertz C, Strait J, and Stucker R (1982) *Wild Rice Production in Minnesota*. Extension Bulletin 464, Agricultural Extension Service. St. Paul: University of Minnesota.)

wildrice. Improved shattering resistance is the primary goal, but disease resistance particularly to fungal brown spot on the leaves is a close secondary goal. Another goal is shorter plants with higher vields. Yields have nearly doubled from the original "Johnson" nonshattering variety developed from the nonshattering plants found in 1963. A private breeding program exists today in California which has developed varieties for their warmer and longer growing season. The genetic material for that program basically came from Minnesota. Thus far, all of the breeding is done by conventional selection and not genetic engineering. A program at Lakehead University, Thunder Bay, Canada, is ongoing to select types of plants that do well in specific lakes.

Cultivated wildrice (*Zizania palustris* var. interior and *palustris* L.) of North America is an annual diploid with a chromosome number of 2n = 2x = 30; thus, it has 15 chromosome pairs. Compared to rice (*Oryza sativa*) wildrice has three more pairs of

**Table 2** Amount of cultivated wildrice produced (1000 kg) in diked fields for the two major producing states<sup>a</sup>

Year	MN	CA
1000	10	
1968	16	0
1969	72 165	0
1970		
1971 1972	276	0
1973	678 544	0
1973	470	0
1974	559	0
1976	820	0
1977	468	0
1978	799	45
1979	978	90
1980	1052	181
1981	1031	227
1982	1223	399
1983	1451	1134
1984	1633	1724
1985	1905	3584
1986	2313	4082
1987	1905	1905
1988	1814	1588
1989	1804	1814
1990	2177	1905
1991	2495	2495
1992	2767	3402
1993	2404	3402
1994	2404	2268
1995	2041	2921
1996	2722	3447
1997	2722	4082
1998	2649	3991
1999	2812	7065
2000	2449	5913
2001	1950	8165
2002	2517	5103

<sup>&</sup>lt;sup>a</sup> Data provided by Minnesota Cultivated Wild Rice Council and California Wild Rice Advisory Board.

chromosomes and twice as much DNA content as rice. Recent (2000) research indicated that when total wildrice DNA was used as a probe in Southern hybridization to different Poaceae genera such as rice, oat, barley, wheat, and maize, there was a strong hybridization signal with rice, but relatively little with oat, barley, wheat, or maize. The greater DNA hybridization of wildrice reflects wildrice classification in the Bambusiodeae subfamily to which rice also belongs. The close relationship of wildrice to rice will benefit the mapping of genes in wildrice. Similar probes used for mapping genes in rice have been used in wildrice and some of the same genes have been found to be located in the same area in wildrice as in rice. Locating important genes such as seed shattering will facilitate the wildrice breeding efforts for domestication.



**Figure 9** An aerial view of Minnesota wildrice fields after flooding in the spring. (Photograph by Oelke EA, appeared in Oelke EA, Grava J, Noetzel D, Barron D, Percich J, Schertz C, Strait J, and Stucker R (1982). *Wild Rice Production in Minnesota*. Extension Bulletin 464, Agricultural Extension Service. St. Paul: University of Minnesota.)

#### **Cultivation Practices**

Wildrice is well adapted to northern latitudes with its cooler climate. In southern latitudes of the USA, such as Arkansas, warm day and night temperatures speed up development resulting in a short, unproductive plant. The high humidity also results in severe leaf disease problems such as fungal brown spot (*Bipolaris* sp.). Varieties have been developed that grow well in the warm day but cooler night temperatures of northern California. Leaf diseases are not prevalent in northern California due to the low humidity.

The cultivation practices for wildrice are similar to rice in that the fields need to be kept flooded for the growing season except several weeks before harvest. Wildrice will grow well on organic or inorganic soils if nutrients are supplied. Wildrice requires less nitrogen than rice since it can get too tall and lodge if overfertilized. It will grow in deeper and cooler water than rice; thus, water depth can be used as a means of weed control. Handling wildrice seed is critical in establishing fields. Wildrice seed must be stored wet and in cold (3°C) water for 3 months to release seed dormancy. In Minnesota, new fields are seeded in the fall and the fields flooded either in the fall or spring to a depth of 30 cm. In Minnesota, fields can be kept in production for 2-4 years. The second and succeeding years the fields seed themselves from shattered seed even when seeded to the newer more shatter-resistant varieties. In the Sacramento Valley of California, the fields are seeded each year due to loss of viability of the shattered seed. Seed has to be stored in cool wet conditions over the winter and

then seeded the next spring. In the northern higher elevations in the valleys between Mount Lassen and Shasta, the production practices are similar to Minnesota (Figures 9 and 10).

Fungal brown spot (*Bipolaris* sp.) is a severe disease in Minnesota, but not a problem in California. The wildrice worm (*Apamea apamiformis* Guenee), which is the larval stage of the noctuid moth, is the most serious insect pest in the Upper Midwest but not a problem in California.

In Minnesota and California, fields are drained 3 weeks before harvest. Grain combines are adapted with large reels and tracks for harvesting wildrice. The grain has to be harvested at 30% moisture since shattering will occur if harvested at a lower moisture content. The grain is immediately transported to processing plants (Figure 11).

## **Processing**

Processing consists of drying (parching), hulling, scarification, cleaning, grading, and packaging. When the grain arrives at the processing plant, it is put into piles or containers and kept for 1 or 2 days. Then the grain is put into rotating, heated drums (parchers) that will hold  $225-370\,\mathrm{kg}$ . Drying the high moisture grain takes about an hour or more. The grain is dried to  $\sim 7\%$  moisture. This moisture level is reached when the grain temperature in the drum reaches  $135^\circ\mathrm{C}$ . Laser thermometers are used by some processors to monitor grain temperature. After parching, the hot grain is passed through a huller consisting of two



Figure 10 In Minnesota, fields in production for two or more years, the plant population is reduced by airboats. V-shaped knives mounted on the rear and spaced 15 cm apart are dropped into the water to the soil line which removes excess plants. Enough seeds, even from the nonshattering varieties, fall onto the soil before and during harvest to produce too many plants the following year. (Photograph by Oelke EA.)



Figure 11 Cultivated wildrice, nonshattering varieties, being harvested with a combine with full tracks and an enlarged reel. (Photograph by Oelke EA.)

rubber rollers going at different speeds. The light chaff is evacuated from the kernels and the hulled kernels are graded with graders and gravity tabels. Sometimes before grading, the kernels are scarified by passing through a tube with rubber paddles. This removes some of the darker, impermeable outer layer of the kernel to allow a faster cooking time. The parched, graded kernels can then be stored for future packaging (Figures 12 and 13).

# **Nutritional Properties**

The wildrice kernel consists of a pericarp, aleurone layer, endosperm, and embryo. The pericarp and embryo each represent  $\sim$ 5% of the kernel weight.

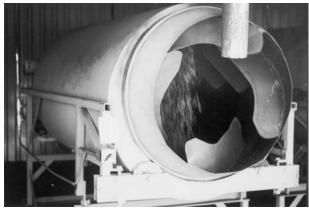


Figure 12 A rotating drum heated on the bottom to dry (parch) the wildrice grain. Modern processing is based on the principle of traditional methods. (Photograph by Oelke EA.)

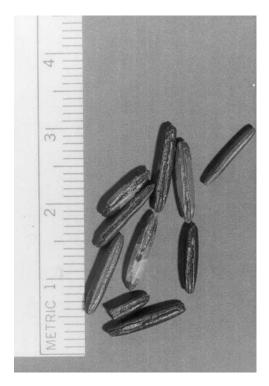


Figure 13 Wildrice grains after being parched and the hulls removed during processing. (Reproduced with permission from Oelke EA, Porter RA, Grombacher AW, and Addis PB (1997) Wild rice-new interest in an old crop. Cereal Foods World 42: 234-247. Minneapolis: American Association of Cereal Chemists.)

The rest, 90%, is made up of the endosperm and aleurone layer.

The nutritional quality is very good for a cereal grain. It is equal to or better than other cereals such as wheat, barley, or rice. The protein content is about that of wheat (13-14%) while brown rice has  $\sim$ 8%. The sum of lysine, threonine, and

Table 3 Composition of wildrice, cultivated brown rice, and wheat

Nutrient	Wildrice value	es	Cultivated	Wheat
	Early	1993	brown rice	
Protein (%)	13.8 (12.8–14.8) <sup>a</sup>	12.7	8.1	14.3
Ash (%)	1.7 (1.4–1.9)	1.5	1.4	2.0
Fat (%)	0.6 (0.5-0.8)	1.5	1.9	1.8
Fiber (%)	1.2 (1.0-1.7)	4.5	1.0	2.9
Carbohydrates	$NA^b$	76.6	NA	NA
Ether extract (%)	0.5 (0.3-1.0)	NA	2.1	1.9
Nitrogen (free % extract)	82.4	NA	87.4	78.9
Phosphorus (%)	0.28	0.37	0.22	0.41
Potassium (%)	0.30	NA	0.22	0.58
Magnesium (%)	0.11	NA	0.12	0.18
Calcium, (ppm)	20	76.6	32	46
Iron (ppm)	17	13.2	10-17	60
Manganese (ppm)	14	NA	30-39	55
Zinc (ppm)	5	34.8	24	NA
Copper (ppm)	13	NA	4–7	8
Sodium (ppm)	NA	30.1	NA	NA
Total kilocalories (per 100 g)	NA	372	NA	NA
Kilocalories from fat (per 100 g)	NA	14	NA	NA

<sup>&</sup>lt;sup>a</sup>Numbers in parentheses indicate ranges in values.

methionine content is similar to oat groats, which are considered one of the better cereals for humans. The mineral content of the wildrice kernel is similar to that of wheat, oats, and corn. Processed wildrice contains no vitamin A but is an excellent source of B-vitamins (Tables 3–5).

Wildrice has good antioxidant properties. There is considerable interest in antioxidants, which can slow diseases such as coronary heart disease and certain types of cancer. Hydrated wildrice mixed with ground beef had significant antioxidant activity. Recently, 2000, it has been found that consumption of cultivated wildrice by rats lowered their liver cholesterol. This needs to be confirmed in humans.

## **Markets and Uses**

The cultivation of wildrice provided a large, consistent supply of wildrice compared to the inconsistency of that from natural stands. Today  $\sim 95\%$  of the wildrice in the marketplace comes from cultivated fields. The wildrice market expanded rapidly during the 1960s through the 1980s primarily in the blend market. Sales increased an average of 15% per year when Uncle Ben, Inc., introduced the first package blend of wildrice, long grain rice, and herbs. Many other blends are now available including soups, vegetable-based side dishes, and convenience foods such as dehydrated mixes and frozen entrees. Today sales are increasing  $\sim 5\%$  per year. The wholesale

**Table 4** Amino acid composition (g per 100 g of protein) of wildrice, cultivated rice, and wheat<sup>a</sup>

Amino acid	Wildrice	Cultivated rice	Wheat
Lysine	4.1	4.0	2.8
Histidine	2.7	2.6	2.4
Ammonia	2.4	2.4	4.0
Arginine	7.3	8.8	4.7
Aspartic	10.3	9.7	5.4
Threonine	3.6	3.8	2.9
Serine	5.2	5.1	4.8
Glutamic	18.2	18.9	35.4
Proline	4.1	4.8	11.8
Glycine	4.8	5.1	4.3
Alanine	5.8	5.9	3.6
Cystine	$ND^b$	3.8	3.3
Valine	5.7	5.8	4.4
Methionine	3.0	2.1	1.4
Isoleucine	4.3	4.0	3.6
Leucine	7.3	8.3	7.2
Tyrosine	3.5	4.4	2.9
Phenylalanine	5.0	4.9	5.3
Tryptophan	1.6	1.8	1.6

<sup>&</sup>lt;sup>a</sup>Wild rice = Zizania palustris, cultivated rice = Oryza sativa.

price per US pound of processed grain ranged from US\$2.50 with a high of \$5.15 in 1978 and then decreased steadily to about \$1.40 in 2002 as increased supply came from cultivated fields.

<sup>&</sup>lt;sup>b</sup>Values not reported.

Reproduced with permission from Oelke EA, Porter RA, Grombacher AW, and Addis PB (1997) Wild rice – new interest in an old crop. *Cereal Foods World* 42: 234–247. (Minneapolis: American Association of Cereal Chemists.)

<sup>&</sup>lt;sup>b</sup>Not determined.

Reproduced with permission from Oelke E A, Porter RA, Grombacher AW, and Addis P B (1997) Wild rice—new interest in an old crop. *Cereal Foods World* 42: 234–247. (Minneapolis: American Association of Cereal Chemists.)

Table 5 Fatty acid and vitamin compositions of wildrice, rice, oats, and wheat

	Wildrice		Brown rice	Polished rice	Oat groats	Wheat
	Early	1993				
Fatty acids <sup>a</sup>						
Palmitic (16:0)	14.5	18.6	20.4	33.8	16.2	24.5
Stearic (18:0)	1.1	4.7	1.6	2.7	1.8	1.0
Oleic (18:1)	15.9	22.2	41.3	43.3	41.2	11.5
Linoleic (18:2)	37.7	29.1	34.5	18.0	38.8	56.3
Linolenic (18:3)	30.0	25.4	1.0	0.6	1.9	3.7
Vitamin content						
Thiamin (mg/100 g)	< 0.02	0.34	0.07	0.60	0.52	0.37
Riboflavin (mg/100 g)	0.26	0.05	0.03	0.14	0.12	0.12
Niacin (mg/100 g)	6.5	4.7	1.6	1.0	4.3	2.2

<sup>&</sup>lt;sup>a</sup> From hexane extracts. Percent of total. Number in parentheses indicates number of carbon atoms in the molecule, with the number of unsaturated carbons after the colon.

Increased use of wildrice as a pure product as well as in blends should continue. In Minnesota, by law, the pure product has to be labeled "cultivated" if it is produced in cultivated fields and sold in Minnesota. Wildrice is a nutritious food and even though it is considered a gourmet food, it is not very expensive on a per serving basis. Quick cooking wildrice is now being developed and should expand the market. Production of wildrice should increase as new, better yielding varieties are developed. Domestication of the plant is in its infancy compared to other cereals, but rapid progress can be made using modern breeding and genetic techniques.

See also: Cereals: Overview. Oats. Rice: Genetics; Breeding; Chinese Food Uses. Taxonomic Classification of Grain Species.

# **Further Reading**

- Aiken SG, Lee PF, Punter D, and Stewart JM (1988) Wild Rice in Canada. Winnipeg: Agriculture Canada.
- Archibold OW (1994) Wild Rice in Saskatchewan Agricultural Development in Harmony with Nature. Saskatoon: Saskatchewan Agriculture and Food.
- Cardwell VB, Oelke EA, and Elliott WA (1978) Seed dormancy mechanisms in wild rice (*Zizania aquatica*). *Agronomy Journal* 70: 481.
- Fassett NC (1924) A study of the genus Zizania. Rhodora 26: 153.
- Hass BL, Pires JC, Porter R, and Phillips RL (2003) Comparative genetics at the gene and chromosome levels between *Oryza sativa* and wildrice *Zizania palustris*. *Theoretical and Applied Genetics* 107: 773–782.

- Hayes PM, Stucker RE, and Wandrey G (1989) The domestication of American wildrice (*Zizania palustris*, Poaceae). *Economic Botany* 43: 203–214.
- Jenks AE (1900) The wild rice gatherers of the Upper Lakes: a study in american primitive economics. In: 19th Annual Report of the Bureau of American Ethnology, 1897–98, vol. 2, pp. 1013–1137. Washington DC: US Department of Interior.
- Kennard WC, Phillips RL, and Porter RL (2002) Genetic dissection of shattering, agronomic, and color traits in American wildrice (*Zizania palustris* var. interior L.) with a comparative map. *Theoretical and Applied Genetics* 105: 1075–1086.
- Kennard WC, Phillips RL, Porter RA, and Grombacher AW (2000) A comparative map of wild rice (*Zizania palustris* L. 2n=2X=30). Theoretical and Applied Genetics 101: 677–684.
- Nelson RN and Dahl RP (1985) Wild Rice Market Shows Vigorous Growth. Minnesota Agricultural Economics, No. 649. St. Paul, Minnesota: University of Minnesota.
- Oelke EA and Boedicker JJ (2000) Wild rice: processing and utilization. In: Kulp K and Ponte JG (eds.) *Handbook of Cereal Science and Technology*, pp. 275–295. New York: Marcel Dekker.
- Oelke EA, Bloom PR, Porter RA, and Liu Q (1999) Wild rice plant development and seed physiology. In: Williamson LS, Dlutkowski LA, and McCammon Soltis AP (eds.) *Proceedings of the Wild Rice Research and Management Conference*, pp. 54–67. Odanah, WI: Great Lakes Fish and Wildlife Commission.
- Oelke EA, Elliott WA, Kernkamp MF, and Noetzel DM (1973) Commercial Production of Wild Rice. Extension Folder 284, Agricultural Extension Service. St. Paul: University of Minnesota.
- Oelke EA, Porter RA, Grombacher AW, and Addis PB (1997) Wild rice new interest in an old crop. *Cereal Foods World* 42: 234–247 (Minneapolis: American Association of Cereal Chemists).

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Oelke EA, Grava J, Noetzel D, Barron D, Percich J, Schertz C, Strait J, and Stucker R (1982) Wild Rice Production in Minnesota. Extension Bulletin 464, Agricultural Extension Service. St. Paul: University of Minnesota.

Steeves TA (1952) Wild rice Indian food and a modern delicacy. *Economic Botany* 26: 107–142.

Vennum T (1988) Wild Rice and the Ojibway People. St. Paul: Minnesota Historical Society.

Weir CE and Dale HM (1960) A developmental study of wild rice, Zizania aquatica L. Canadian Journal of Botany 38: 719–739.

Williamson LS, Dlutkowski LA, and McCammon Soltis AP (eds.) *Proceedings of the Wild Rice Research and Management Conference*, July 7–8. Odanah, WI: Great Lakes Indian Fish and Wildlife Commission.

Wu K, Zhang W, Addis PB, Epley RJ, Salih A, and Lehrfeld J (1994) Antioxidant properties of wild rice. *Journal of Agricultural and Food Chemistry* 42: 34.

#### **Relevant Websites**

http://www.wildrice.org/iwra — This website is the one for the International Wild Rice Association (IWRA). IWRA consists mainly of producers, processors, marketers, and education/research professionals involved with wildrice. Members include individuals from the US and Canada. This provides a short history of wildrice and has links to other websites about wildrice, especially in the marketing area.

http://www.mnwildrice.org — This is the website of the Minnesota Cultivated Wild Rice Council whose members consist of Minnesota wildrice producers. Information on history, nutrition, and recipes can be found on this website.

# **RYE**

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## Introduction

Rye, (Secale cereale L.), is a grass plant cultivated worldwide and used as grain and straw. The grain is second after wheat in the production of bread. It is also important in the production of mixed feeds for livestock and as feedstock for the distillation of rye whisky. Straw is used in livestock feeds, as bedding in animal husbandry, and as a building material (e.g., roof thatch, particle board, etc.).

# **Center of Origin and History**

The primary center of origin of the rye plant is considered to be in the Anatolean Plateau of the Middle East, which is also the center of origin of wheat, barley, and oats. There is no evidence of rye cultivation in ancient Egypt. It is mentioned in early writings of northern Europe suggesting this as an important area of historic cultivation.

Cultivation of rye migrated from the center of origin to northern Europe in the first millennium BC. One possible route of migration is from Asia Minor

to western Russia and from there, westward to Poland and Germany where it is highly adaptable and the grain well liked by the inhabitants. Another possible route of migration is from Turkey by way of the Balkan Peninsula to north-central Europe.

Rye was brought to North and South America by European settlers during the sixteenth and seventeenth centuries. At about the same time, its cultivation spread eastward in Europe to Siberia. During the nineteenth and twentieth centuries, its cultivation began in Argentina, southern Brazil, Uruguay, Australia, and South Africa. Today, rye cultivation, like that of wheat, is worldwide but the largest production is in Germany, Russia, Poland, Belarus, and Ukraine.

## **Botanical Classification**

Rye belongs to the grass family Gramineae and the genus *Secale*. The most common cultivated species is *S. cereale*, which is presumed to have evolved from the wild perennial grass of the species *S. montanum*. Cultivated rye contains seven pairs of chromosomes belonging to a single genome designated by the letter R. Unlike the situation in wheat, the number of commercially grown rye varieties (cultivars) is relatively small. Rye cross-pollinates

extensively and is, therefore, difficult to maintain genetic purity.

Most of cultivated rye is fall-sown annual crop called "winter rye." Rye has excellent winter hardiness and therefore can be grown in areas where the climate is too severe for winter wheat. Spring rye is grown in some countries, e.g., Canada.

## **Worldwide Production and Trade**

Worldwide production of rye grain declined significantly over the past decade (Table 1) and stabilized at 20 million ton (Mt), grown on  $\sim$ 16 million hectares (Mha), over the past 3 years for which statistical data is available. The decline, mainly due to gradual decrease in demand for both human and livestock consumption, has been achieved by a gradual decrease in the area harvested. Russia is the leading rye producer followed by Germany, Poland, Belarus, and Ukraine. Together they produce almost 80% of world rye grain. United States, Canada, and Turkey are significant contributors to the world rye stocks.

Rye ranks last of the eight cereal grains grown for human food (Table 2), the position it has occupied over the past four decades.

Most of the rye grain is used domestically. The following data was derived from recent international statistics. In 2001-02, of the total exports of ~1.6 Mt, European Union (mostly Germany) contributed 61% of the total. Ukraine had an excellent crop and was able to supply 28% of the world market. Canada contributed  $\sim$ 5% and Russia 3.4%.

Major importer of rye in 2001–02 was Japan, accounting for 27.05%, where it is used mainly for animal feed. Other significant importers are South Korea (13.52%) and United States (8.45%).

# **Plant and Grain Morphology**

The mature rye plant has a slender, tough, fibrous stem (straw), and elongated leaves. Plant heights vary widely from  $\sim 30$  cm to over 2 m. The inflorescence (spike) is long and slender with stiff long awns (beards). Rye grain (caryopsis) is arranged in pairs alternately on a zig-zag shaped rachis (Figure 1). The grain is covered with a lemma, palea, and a glume (chaff) which is normally awned. Like wheat, rye grain is free-threshing; the mature grain separates easily from the glume during threshing.

Rye grain (kernels) is more slender and longer than wheat ranging in length from 4.5 to 10 mm and in width from 1.5 to 3.5 mm. The grains are normally of grayish-yellow color but the color can vary widely depending on rye cultivar, region of cultivation, and harvesting conditions. As in wheat, a crease extends the full length of the ventral side of the grain. The surface of the grain is usually shriveled and has a rough texture. A single grain weighs  $\sim 20$  mg.

Rye grains comprise three distinct morphological parts (Figure 2). They are starchy endosperm, 86.5%, bran (pericarp and testa), 10%, and germ (embryo and scutellum), 3.5%. In milling rye grain into flour, the bran and germ are separated from the endosperm, which is ground into flour.

# **Proximate Composition and Nutritional Properties of Rye Grain**

The proximate composition of rye grain (Table 3) is typical of cereal grains with carbohydrate (nitrogenfree extract) being the main constituent forming  $\sim$ 80% of the grain. Starch is the major carbohydrate component. As in wheat and barley, rye starch is stored in the endosperm in two types of granules, large lenticular granules measuring ~35 µm and small spherical granules  $\sim 10 \, \mu m$  in diameter. In gelatinization properties, rye starch is similar to wheat starch; they have similar functionality in bread-

Rye grain contains considerably more hemicellulose (pentosans) than wheat, 6-9% compared with

**Table 1** Rye production worldwide (in thousands of tons)<sup>a</sup>

	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
Russia	10 624	13 877	9161	6000	4500	5900	7478	3269	4781	5440
Germany	3323	2422	2984	3451	4521	4214	4645	4775	4329	4154
Poland	5899	3962	4992	5300	6288	5652	5300	5663	5181	4000
Belarus	1950	2500	2500	1900	2150	2000	2100	929	1024	1450
Ukraine	980	1156	1180	942	1216	1094	1148	1135	919	966
United States	248	304	263	279	254	250	250	260	320	350
Canada	339	278	319	394	299	309	320	398	387	260
Turkey	240	240	240	195	240	236	225	233	267	260
World total	28 669	28 804	26 191	22 681	23 447	23 140	25 410	20 318	20 334	20 488

<sup>&</sup>lt;sup>a</sup> Source: International Grains Council (2002) World Grain Statistics for 2000/01. London: International Grains Council.

Rye has some advantages over wheat in properties related to human nutrition. It is higher in lysine content to the extent that this amino acid is not the first limiting amino acid in rye as it is in other cereals. Tryptophan is considered the first limiting amino acid in rye. Rye flours are usually milled to a higher extraction rate, as they normally have higher dietary fiber content than wheat flours. The stronger flavor and gummier texture of rye-baked products are well liked by many consumers.

The mineral and vitamin content and composition of rye is similar to those of other cereals (Tables 4 and 5). Rye is considered a good source of thiamin, nicotinic acid, riboflavin, pyridoxine, panthotenic acid, and tocopherol. These constituents are mostly removed during milling of flour, as they are stored mainly in the germ and the aleurone layer of the grain.

Rye grain contains some constituents, which have antinutritional properties especially in animal nutrition where whole grain is used. These constituents include alkyl resorcinols, soluble hemicelluloses which interfere with feed digestion in monogastric animals, phytic acid which binds calcium and zinc, and trypsin inhibitor which interferes with the digestion of proteins.

Commercial rye grain is frequently contaminated with ergot bodies, sclerotia of the fungus *Claviceps purpurea* (Fr.:Fr.) Tul., which contain ergotamine (a toxic alkaloid), which, if consumed by livestock can cause abortions. The antinutritional constituents are of no significance in human consumption of rye products as they are either removed during milling or inactivated during baking.

**Table 2** World production of cereal grains (in thousands of tons)<sup>a</sup>

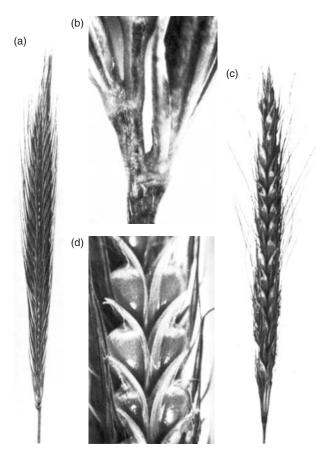
Cereal	Production
Corn	599 406
Rice, rough	580 790
Wheat	575 879
Barley	127 700
Sorghum	60 814
Millet <sup>b</sup>	43 000
Oats	25 088
Rye	19 927

<sup>&</sup>lt;sup>a</sup> Source: Canada Grains Council (1999) *Statistics Handbook*. Winnipeg, Canada: Canada Grains Council.

# **Genetics, Plant Breeding, and Agronomy**

Most of the commercial rye grown is the diploid *S. cereale* type. Attempts to develop tetraploid rye by doubling the number of chromosomes with colchicine have been unsuccessful. The resulting tetraploid was more susceptible to ergot and less cold-tolerant. Recently, breeders in Lethbridge, Canada, developed a hybrid between *S. cereale* and *S. montanum* as a potential perennial forage crop. Its productivity is relatively short-term (3–4 years) and this may limit its commercial production. Much of the research on hybrid rye is in Germany (Stuttgart) where most of the commercial crop is of the hybrid type.

Rye has been used extensively as a source of disease resistance in wheat-breeding programs. The disease-resistant genes are incorporated through a translocation of 1R chromosome into 1A or 1B



**Figure 1** The rye inflorescence: (a) inflorescence just prior to anthesis; (b) base of inflorescence just before flowering showing insertion of spikelets into rachis; (c) inflorescence at physiological maturity; and (d) physiologically mature inflorescence showing insertion of individual grains. (Reproduced with permission from Simmonds DH and Campbell WP (1976) Morphology and chemistry of the rye grain. In: Bushuk W (ed.) *Rye: Production, Chemistry, and Technology*, 1st edn., pp. 63–110. St. Paul, MN: American Association of Cereal Chemists.)

<sup>&</sup>lt;sup>b</sup>Millet datum from Food and Agriculture (FAO) Statistics, Rome, Italy.

**Figure 2** Diagrammatic view of rye grain in: (a) longitudinal section and (b) transverse midsection. (Reproduced with permission from Simmonds DH and Campbell WP (1976) Morphology and chemistry of the rye grain. In: Bushuk W (ed.) *Rye: Production, Chemistry, and Technology*, 1st edn., pp. 63–110. St. Paul, MN: American Association of Cereal Chemists.)

Table 3 Proximate composition of rye compared with other cereal grains

Cell wall

100 µm

	Rye (%)	Triticale	Wheat	Bar	ley	Corn	Oá	nts	Ri	ce
		(%)	(%)	Whole grain (%)	Kernel only (%)	(%)	Whole grain (%)	Kernel only (%)	Whole grain (%)	Kernel only (%)
Protein	13.4	14.8	14.3	13.1	14.5	10.4	13.0	17.0	8.2	9.4
Ether extract	1.8	1.5	1.9	2.1	2.1	4.5	5.5	7.7	2.2	1.8
Crude fiber	2.6	3.1	2.9	6.0	2.1	2.4	11.8	1.6	10.1	0.9
Ash	2.1	2.0	2.0	3.1	2.3	1.5	3.7	2.0	5.7	1.1
Nitrogen-free extract	80.1	78.6	78.9	75.7	79.0	81.2	66.0	71.6	73.8	86.8

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chromosome of wheat. Many commercial wheat varieties contain one of these two translocations.

Diploid rye is the starting species for the development of triticale, a hybrid of the alien genomes

of rye and wheat. Triticale is gradually becoming a significant grain for food and feed uses.

endosperm

Pericarp

Major rye-breeding programs are located in Germany and Poland. In most programs, grain

Table 4 Mineral composition of rye and other cereal grains (mg per 100 g dry-weight basis)

	Rye	Rye Wheat Barley Corn	Vheat Barley (			Oá	ats	Rice		
			Whole grain	Kernel only		Whole grain	Kernel only	Whole grain	Kernel only	
Phosphorus	380	410	470	400	310	340	400	285	290	
Potassium	520	580	630	600	330	460	380	340	120	
Calcium	70	60	90	80	30	95	66	68	67	
Magnesium	130	180	140	130	140	140	120	90	47	
Iron	9	6	6		2	7	4		6	
Copper	0.9	0.8	0.9		0.2	4	5	0.3	0.4	
Manganese	7.5	5.5	1.8		0.6	5	4	6	2	

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Table 5 Composition of B-vitamins and carotene in rye and other cereal grains (mg per 100 g dry-weight basis)

	Rye	Wheat	Barley (whole)	Corn	Oats (whole)	Rice (brown)
Thiamin	0.44	0.55	0.57	0.44	0.70	0.33
Riboflavin	0.18	0.13	0.22	0.13	0.18	0.09
Nicotinic acid	1.5	6.4	6.4	2.6	1.8	4.9
Pantothenic acid	0.77	1.36	0.73	0.70	1.4	1.2
Pyridoxine	0.33	0.53	0.33	0.57	0.13	0.79
Carotene	0	0	0.04	0.40	0	0

Reproduced with permission from Simmonds DH and Campbell WP (1976) Morphology and chemistry of the rye grain. In: Bushuk W (ed.) Rye: Production, Chemistry and Technology, 1st edn., pp. 63–110. St. Paul, MN: American Association of Cereal Chemists.

yield is the main breeding objective. The main quality characteristic that is selected for is high falling number (low alpha-amylase activity). As far as disease is concerned, the main efforts are on resistance to rust and *Fusarium* head blight. Snow mold is the third disease complex being tackled by breeding. A genetic engineering approach, which could potentially improve the baking quality of rye, is by replacing a secalin locus with the locus of wheat, which controls the 5+10 high molecular weight glutenin subunits in wheat. In wheat, these subunits play a key role in bread-making quality. Preliminary results by this so-called interstitial interchange approach to ryequality improvement are promising.

Agronomic practices and growing conditions for rye are similar to those for wheat, barley, and oats. Rye performs better than wheat on lighter soils and can tolerate higher levels of aluminum in acidic soils.

# Grading Rye Grain and Primary Processing

Agricultural products, including grains, vary in composition and processing quality caused by fluctuations

in weather conditions during the growing season. To facilitate marketing and processing the grain is graded, separated, or combined, into parcels of relatively uniform properties. In countries where rye grades exist, they are based on physical characteristics of the rye grain and on the presence of foreign contaminants. Test weight, a measure of bulk density related to milling quality, is the only grading factor that is measured objectively. All other factors are assessed subjectively by visual inspection. Normal or straight grades usually have a prescribed maximum moisture content; 14% in Canada and the United States and 16% in Germany. The number of grades of milling rye varies among countries. Germany (Table 6) has one grade, Canada has three, and the United States has five grades.

In grain commerce, rye is handled in bulk and transported by truck or locomotive. Rye for export must meet the importer standards for foreign contaminants and must be free of toxic substances. Export transfer is in bulk by cargo vessel or in containers if the shipments are small.

In transforming rye grain into food for human consumption, the primary processing step is milling the grain into a product called flour. Despite some

**Table 6** German standards for milling rye<sup>a</sup>

Moisture content	Max. 16%
Broken kernels	Max. 2%
Grains besatz (shrunken kernels,	Max. 1.5%
other grains, insect-damaged kernels)	
Sprouted kernels	Max. 1%
Black besatz (wheat seed, ergot,	Max. 0.5%
unsound grain, chaff, impurities)	
Hectoliter weight	Min. 71 kg

<sup>&</sup>lt;sup>a</sup>D. Weipert, personal communication.

Table 7 Rye flour grades in North America

Flour grade	Extraction	Ash (%)
United States White rye Medium rye Dark rye	Patent (80% of total rye flour) Straight grade Clears (20%)	0.60-0.70 1.00-1.50 2.50-3.00
Canada Light rye Medium rye Dark rye	75–80 83–85 92–95	0.70-0.90 1.00-1.25 1.35-1.80

Reproduced with permission from Bushuk W (ed.) (2001) Rye: Production, Chemistry and Technology, 2nd edn., 144p. St. Paul, MN: American Association of Cereal Chemists.

significant differences between the physical structure of rye and wheat, the milling processes for the two grains are similar. The first step is the removal of all undesirable contaminants. This is achieved by a series of special machines functioning consecutively. Differences in shape, size, density, and color are all used to improve the efficiency of the cleaning. The final step of the cleaning section is tempering which involves addition of water to toughen the bran and mellow the endosperm. Moisture levels for tempering rye are usually 1% lower than those used for wheat. Tempering periods are shorter because of the softer rve endosperm.

Because of the softer texture, rye endosperm releases more flour during the breaking operations and essentially no middlings require purification. Reduction is achieved by corrugated rolls instead of smooth rolls used for reducing wheat middlings into flour. Rye milling requires a larger sifting area than wheat because rye flour tends to clump because of its softer texture.

North American rye mills produce a small number of different grades of flour, usually three (Table 7). The main quality parameter is ash content. European rye milling is more precise. A modern German rye mill will produce as many as seven different flours designated by ash content and several types of meals processed by crushing and cutting over a range of particle size (Table 8).

Table 8 Classification of German milling products from rye used in bread making

Product	Mineral contents (% db)	Extraction (%)
Flour		
Туре		
815	0.90	78-82
997	0.91-1.10	82-86
1150	1.11-1.30	
1370	1.31-1.60	
1740	1.61-1.80	
Meal		
Type 1800	2.20	95-100
Whole-kernel		
Flour		100
Meals, various granulations		100
Special products		
Dietary brans		
With dehulled kernels (Steinmetz)		
Flakes, etc.		

Reproduced with permission from Bushuk W (ed.) (2001) Rye: Production, Chemistry and Technology, 2nd edn., 183p. St. Paul, MN: American Association of Cereal Chemists.

# **Uses of Rye**

Worldwide, over 50% of rye grain is used for the production of livestock feed. The key factors for this application are price, relative to other feed grains such as barley, energy per unit weight, and absence of antinutrients and toxicants. Ergot bodies must be removed by cleaning. Technology has been developed in the use of enzymes and other additives to mitigate the effects of antinutrients such as the soluble hemicelluloses. Rye grain imports by some countries, e.g., Japan and South Korea, are based almost entirely on the livestock-feed industry.

Bread and other baked products are the main food products in the major consuming countries – Austria, Belarus, Czech Republic, Germany, Poland, Russia, Scandinavian Countries, and Ukraine. Many types of baked products are made including bread from 100% rye flour, rye-wheat bread containing at least 50% rye, wheat-rye bread containing at least 50% wheat and at least 10% rye, whole meal rye bread, rye rolls, crisp bread, and pumpernickel. Crisp and pumpernickel breads have excellent shelf life. A new type of bread, made from partially sprouted rye, has been introduced in Moscow, Russia, for its unique flavor, texture, and nutritional quality. Many other products are available in regional markets made from various mixtures of whole meal, rye flour, and wheat flour.

Small quantity of rye is used worldwide in many other foods. These include rolls, sweet goods, breakfast cereals (mixtures, flakes, and bars) and as whole grain in specialty breads. The unique texture and flavor of rye is being used to advantage in many food products.

Worldwide, small quantity of rye is used in nonfeed and nonfood industries. Traditionally, rye straw is used on the farm as livestock bedding. In Canada, rye is fermented and distilled to give alcohol that is used as a beverage and in pharmaceuticals. Rye flour is used as an adhesive in the production of wallpaper, corrugated packaging materials, and plywood. The capacity of the rye plant to produce a large quantity of biomass on low-fertility soil has not been exploited by the biomass energy industry. The pentosan component of rye is used to make furfural, as starting material for many industrial chemicals. Rye plant has been reported as a significant component of a sustainable system of agriculture but its use in this way remains to be exploited. For additional information on rye, the reader is referred to the

recently published monograph "Rye: Production, Chemistry and Technology."

See also: Animal Feed. Breads. Cereals: Overview; Grain Diseases; Protein Chemistry. Contaminants of Grain. Grain Crops, Overview. Grain Production and Consumption: Europe. Nutrition: Effects of Food Processing. Taxonomic Classification of Grain Species. Triticale. Wheat: Breeding.

# **Further Reading**

Bushuk W (ed.) (2001) Rye: Production, Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Canada Grains Council (1999) Statistics Handbook. Winnipeg, Canada: Canada Grains Council.

Food and Agricultural Organization (FAO) Statistics, Rome, Italy.

International Grains Council (2002) World Grain Statistics for 2000/01. London: International Grains Council.

Simmonds DH and Campbell WP (1976) Morphology and chemistry of the rye grain. In: Bushuk W (ed.) Rye: Production, Chemistry, and Technology, 1st edn., pp. 63–110. St. Paul, MN: American Association of Cereal Chemists.

## **Relevant Websites**

http://www.usda.gov – United States standards for rye, grain inspection, packers, and stockyards administration, USDA (2002).

http://www.grainscanada.gc.ca. - Official grain grading guide, Canadian Grain Commission (2002).



# SCIENTIFIC SOCIETIES ASSOCIATED WITH GRAIN SCIENCE

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#### Introduction

The industries that grow, harvest, and process grain are big businesses worldwide, involving large numbers of people, including many scientists. It is important that these scientists should have the opportunities to interact with one another, to exchange ideas, to learn from one another, and importantly, to develop analytical methods that are accepted internationally (see Appendix: Test Methods for Grain and Grain-Based Products). Opportunities for these various activities are provided by specialist societies and associations ("learned societies"), with grain scientists as members, formed for the purposes of interacting with one another in their areas of expertise. In many instances, there is the added objective of providing a focus for interaction with the public in the area of specialization of each society.

Many such scientific societies have been formed, covering the wide range of aspects of grain science, from breeding, through agronomy and farming, to the chemistry of the harvested grain, to marketing and processing. Some of these societies are selected for description below, but the range covered is by no means comprehensive. In addition to scientific societies, there is an even greater number of trade associations, formed for the purposes of interaction at the technical and commercial levels, and for lobbying purposes. A list of many scientific and trade associations of the food industry has been assembled by the *New Products Magazine* (Stagnito Communications Inc., Deerfield, IL, USA, www.hoovers.com).

The activities of the scientific associations include the organization of conferences at which lectures are presented, where personal interactions can occur and where trade exhibits can provide information about the latest equipment and resources. Other activities include the dissemination of information via journals, books, and other publications, the establishment of professional standards of conduct and training, and the development of standard methods of analysis.

#### The Range of Societies

Prominent among these learned societies are those relevant to the quality and processing of grain. In particular, the American Association of Cereal Chemists (AACC) has a worldwide membership of scientists involved in grain production and processing. The International Association for Cereal Science and Technology (ICC), based in Vienna, Austria, is an international association committed to international cooperation through disseminating information and developing standard methods relevant to grain production and processing. There are also national bodies with similar objectives, such as the Chinese Cereals and Oils Association (CCOA), based in Beijing, China, and the Cereal Chemistry Division of the Royal Australian Chemical Institute, based in Melbourne, Australia. In addition, a range of societies caters for specific grain types or related processes, such as the National Oilseed Processors' Association.

Other societies have been formed for those involved in specific aspects of chemical analysis or individual classes of chemical compounds. For example, The American Oil Chemists' Society (AOCS), comprising some 5000 members worldwide, provides "a global forum for the science and technology of fats, oils, surfactants and related materials." Furthermore, AOAC International is an "internationally recognized organization with 120 years of experience in validating and approving analytical methods for foods and agriculture." In both these cases, involvement goes well beyond grain science, but grains are a significant part of their broader scope.

The American Society of Agronomy, the Crop Science Society of America and the Soil Science Society of America attract scientists involved in plant growth and production. While their interests include grain

production, the involvement of these societies covers the full breadth of plant-based agriculture. Complementary to this aspect of grain science is the International Seed Testing Association (ISTA), concerned with the earliest stage of plant production, namely, the provision of pure seed. Again, the scope of this society extends well beyond food grains to cover all types of plant seeds.

## The American Association of Cereal Chemists

The AACC is an international non-profit organization of ~3500 grain scientists and other professionals studying the chemistry of grains and their products or working in related fields. The word "cereal," used in the name of this and some other societies, is now less appropriate than the term "grain." Long ago, the scope may have been restricted to the cereal species, particularly wheat and barley, but the broad range of grains is now included in the activities of these societies.

The AACC was founded in 1915 for the purpose of standardizing methods of analysis among cereal laboratories. The association now provides a comprehensive and respected collection of methods in the field of grain science (*see* Appendix: Test Methods for Grain and Grain-Based Products). All methods must meet rigorous standards for approval by one of AACC's 23 technical committees, which are composed of scientists chosen for their specific area of expertise.

#### The Activities of AACC Members

Many AACC members are involved in food manufacture using grains, one of the fastest growing, most dynamic segments of the food industry. They are responsible for producing some of the most popular and profitable products, such as new types of cracker, cookies, pretzels, or other snack foods. Bringing such products into the market involves designing, developing, and marketing skills. Breads, tortillas, frozen doughs, and dry mixes all rely on cereal chemists for their success in the marketplace.

Other AACC members are involved "upstream" of the food industry. They may be associated with plantbreeding programs, with responsibility for ensuring that the grain from new varieties is suited for specific marketing and processing requirements. Research activities are prominent for other cereal chemists, with the objectives of elucidating the chemical and molecular bases of grain quality, thereby laying a foundation for the development of improved testing methods for grain quality. Other research activities are likely to lead to the development of new grain types with better processing and nutritional attributes.

#### **AACC Resources**

The AACC has provided resources in grain science for more than 85 years, supporting members' professional needs in private industry, academia, and government. The association provides an avenue of bringing together scientific information and technical research on cereal grains and related materials, their processing and utilization. This information is made available through outlets such as annual meetings, seminars, training sessions, and in publications. The range of published materials includes scientific journals, books, and data provided on the website.

AACC publishes Cereal Chemistry, a journal with peer-reviewed, original research on raw materials, processes, and products that relate to the utilization of cereal grains, oilseeds, and pulses, as well as analytical procedures and methods in the grains area. AACC also publishes Cereal Foods World, which includes feature articles and original research that focus on advances in grain-based food science and the application of these advances on current practices in baking, snack foods, breakfast foods, and other grain-based products. In addition to these two journals, AACC has published more than 65 titles on various food-science topics. Some titles are technically focused while others are designed for generalists. The books in the AACC handbook series offer a single source of practical information for the major ingredients used in food processing.

AACC's website offers members the opportunity to obtain information and resources in one common location. The website features more than 40 years of searchable *Cereal Chemistry* abstracts, an online catalogue of books, special reports, membership listing, a calendar of events, and on-line symposia. AACC Interactive is the place on AACC's website where members can update their member record, register for the association events and purchase books.

AACC's continuing education programs offer professional development services for food-industry professionals at any level in a variety of food-related industries. Core services include short courses taught by experts that offer comprehensive training and real-world applications. These courses provide basic training tools for new associates or as a resource for anyone working with a new ingredient or application. AACC's continuing education programs also focus on emerging issues and strategies for the future success in the food industry. Members receive special rates on technically focused, hands-on training. Courses are offered globally.

#### **AACC Meetings**

The AACC's annual meeting is attended by an international audience of  $\sim$ 2000 grain-based professionals, providing an education and networking event for the grains industry around the world. At this meeting, presenters have the opportunity to share their latest research on topics such as biotechnology, functional foods and ingredients, health and nutrition, structure and function of food components, quality enhancements, and more. The annual meeting also features a tradeshow, typically comprised of more than 250 exhibits.

AACC members work together on various initiatives through AACC technical and administrative committees. Committee participants help identify emerging issues, create definitions for critical industry ingredients, as well as investigating and developing analytical methodology. Members with appropriate expertise comment via AACC management on items of public interest, such as the definition of dietary fiber and the value of foods based on genetically modified crops.

Members have the opportunity to attend local section meetings, connecting them to other professionals within their own region. The AACC also conducts regional meetings, such as the series of Pacific Rim Meetings for delegates from countries bordering the Pacific Ocean. Several divisions within the AACC cover specialist interests, such as grain proteins and biotechnology, and these divisions arrange seminars focusing on these interests. Under the guidance of the International Executive Council, AACC also responds to the needs of its international membership working in more than 75 countries. AACC's European office, located in Belgium, is staffed with bilingual professionals, providing support and services to members throughout Europe and surrounding countries.

AACC recognizes excellence through its awards program. AACC awards include the Geddes Memorial Award, the Thomas Burr Osborne Medal, the C.W. Brabender Award, the Excellence in Teaching Award, AACC Fellowships, the Alsberg-French-Schoch Memorial Lectureship, and Honorary Memberships. In addition, the AACC Foundation provides scholarships and fellowships annually for students majoring in disciplines related to cereal science.

## The International Association for Cereal Science and Technology

The ICC was founded in 1955 on the occasion of the Third International Bread Congress in Hamburg, Germany, as the "International Association for Cereal Chemistry," with the initials ICC, which

have remained, despite the subsequent expansion of its title (above). The original objective was the development of internationally approved and accepted standard testing procedures for cereals and flour. The scope of the ICC has since expanded to include international cooperation and the dissemination of up-to-date knowledge. The Association has its head-quarters and its Secretariat-General near Vienna, Austria. More than fifty countries are represented in the ICC.

#### **ICC Objectives**

The ICC is a non-political, nonprofit-making organization. Membership is open to all interested countries that are prepared to offer their cooperation. The principal tasks of the Association are:

- to contribute to the advancement of cereal science and technology in all its aspects;
- to standardize test methods in cereal science and technology as well as in related fields; and
- to relate the results of scientific and technological research to the more efficient utilization of cereals.

ICC has the following special emphases:

- Cooperation with developing countries. ICC activities have involved the promotion and implementation of scientific and technical cooperation among and with developing countries through ICC regions in Asia, Africa, and South America.
- Cooperation with other international organizations. ICC activities so far have resulted in the conclusion of cooperation agreements with organizations working in similar or related fields.

#### **ICC Organization and Activities**

ICC is unique in that it does not provide membership to individual scientists. Instead it has country memberships (either Regular or Observer Country Membership). The ICC also has Corporate Membership, involving companies involved in the grain industry. ICC affairs are financed via fees paid by Country and Corporate Members. The ICC is governed by its Executive Committee, Subcommittee, and Technical Committee, supported by the Secretariat General and Chief Executive Officer, based in Vienna.

Many of the ICC's activities are carried out by some 40 Working and Study Groups, each of which is chaired by an expert qualified in the specific field of activity. Participation in the work of these groups is open to all qualified persons and guided by a Technical Director. Congresses and symposia, which ICC holds at regular intervals, offer opportunities for a direct exchange of views about developments and progress

in the various disciplines, and the reports about these meetings help disseminate the most recent findings.

ICC information is also disseminated via its website and via many publications, including Standard Methods, a newsletter, a multilingual dictionary, a book about people in Cereal Science titled Who is Who in the World of Cereal Science, a calendar of events and literature about ICC meetings.

#### **National Cereal Chemistry Societies**

#### **Chinese Cereals and Oils Association**

The CCOA, based in Beijing, China, provides opportunities of interaction for a large number of Chinese scientists working with grains, edible oils, and related commodities. Whilst its activities are mainly centered within China, the Association also hosts international conferences, thereby involving the wider range of grain scientists. The CCOA has over 6000 personal members and has over 570 organization members. The CCOA is a member of the ICC. The CCOA is divided into four sub-associations, namely, grain storage, grain food, edible-oil processing, and animal-feed production. The CCOA publishes a journal, the Journal of the Chinese Cereals and Oils Association. The Chinese version is published every two months, while the English version appears annually.

#### **Australian Cereal Chemists**

The Cereal Chemistry Division of the Royal Australian Chemical Institute (RACI), based in Melbourne, Australia, is a focus for grain scientists in Australia to meet in annual meetings, to exchange information and to interact with colleagues internationally. The Cereal Chemistry Division was one of the first specialist groups to form within the broader range of chemists of the RACI. From these beginnings early in the 1950s, the Cereal Chemistry Division has been one of the strongest of the various specialist divisions of the RACI, with a current mailing list of over 300 scientists. Attendances at Annual Meetings average  $\sim$ 150. At regular intervals, these meetings are combined with other international societies, especially the AACC and the ICC. Other activities include conducting workshops and training seminars on specific aspects of grain science, the provision of standard methods of analysis, and facilities for checking samples with known analytical results by which laboratories are able to compare the results of their analytical methods with others.

Throughout the life of the Cereal Chemistry Division, presentations at annual meetings have been recorded in published form, in recent years under the titles Cereals 2003, Cereals 2002, Cereals 2001, etc. Details of these publications and other activities are provided on the website.

#### Societies Involved with Grain Growth

#### The International Seed Testing Association

The ISTA is a worldwide, nonprofit association, whose main activity is to provide methods and services for the testing of seed traded internationally. The primary purpose of the Association is thus to develop, adopt, and publicize standard methods for sampling and testing seeds of all types, not only the edible grains. The ISTA's secondary purpose is to promote all areas of seed science and technology. These purposes are largely served by the publication of relevant handbooks and bulletins, scientific journals (Seed Science and Technology and Seed Symposium Abstracts), Proceedings of Symposia and Workshops, and training booklets.

The primary ISTA "instrument" for promoting uniformity of seed testing procedures is *The International* Rules for Seed Testing, a publication that is updated annually with amendments and additions resulting from ISTA Meetings. This publication is available in several languages, including English, German, and French. The ISTA Secretariat is based in Bassersdorf, Switzerland. Details of publications and membership are available on the ISTA website.

#### The American Societies of Agronomy, Crop, and Soil Science

Founded in 1907, the American Society of Agronomy is "dedicated to the development of agriculture enabled by science, in harmony with environmental and human values." The Society supports scientific, educational, and professional activities to enhance communication and technology transfer among agronomists and those in related disciplines on topics of local, regional, national, and international significance. The Society's secretariat is in Madison, Wisconsin, USA.

The Crop Science Society of America is an educational and scientific organization comprised of more than 4700 members dedicated to the advancement of crop science. Founded in 1955, the Society is international in scope with members in more than 100 countries who are advancing the discipline of crop science by acquiring and disseminating information about crops in relation to genetics and plant breeding, crop physiology and production, germplasm resources, and environmental quality. An important function of the society is the production of its main journal Crop Science.

The Soil Science Society of America is the professional home for over 5700 professionals throughout the world involved in soil science. The primary purpose of the Society is to advance the discipline and practice of soil science by study and education concerning soils in relation to crop production, environmental quality, ecosystem sustainability, bioremediation, waste management and recycling, and wise land use.

## **Societies Involved with Grain Analysis**

#### **AOAC International**

AOAC International is an internationally recognized organization with 120 years of experience in validating and approving analytical methods for foods and agriculture. It is "committed to be a proactive, worldwide provider and facilitator in the development, use, and harmonization of validated analytical methods and laboratory quality assurance programs and services."

The organization was founded in 1884 as the Association of Official Agricultural Chemists, under the auspices of the US Department of Agriculture. Its initial role was to adopt uniform methods of analysis for fertilizers. In the following year, a Convention established the AOAC as an independent organization, but membership was restricted to analytical chemists in state and federal positions of the US government, and this membership requirement remained for  $\sim 100$  years. In 1965, to recognize the expansion of AOAC's scope of interest beyond agricultural topics, the Association's name was changed to the Association of Official Analytical Chemists.

During the 1970s, membership was extended to scientists outside the United States, and to nongovernmental scientists. Now over 60% of members are working in industrial laboratories. During the 1980s and 1990s, the scope of analyses expanded and there was increasing demand for quality control of laboratories and international laboratory accreditation. Consequently, the name of the Association was changed to AOAC International, thereby retaining the initials by which the Association had been known for over 100 years, while eliminating reference to a specific scientific discipline or profession.

The scope of the AOAC includes not only methods for the analysis of grains, but also for a much wider range of materials. AOAC provides a number of key publications, hosts technical meetings and conferences, and offers training courses in the areas of laboratory management, quality assurance, accreditation, statistics, and measurement uncertainty. Information is readily accessed at the website.

#### The American Oil Chemists' Society

The AOCS is another American scientific association that has become international, growing and expanding in scope since its origins in 1915. This scope now includes oil- and fat-related commodities, oilseeds, oilseed meals and edible fats. Grains are thus prominent in this range of materials for analysis, but the scope goes beyond grains, including other sources of edible fats, such as fish and animals. The AOAC technical services are based in Champaign, IL, USA.

#### **Celiac Societies**

Societies have been formed in many countries and regions by people with celiac disease and related forms of dietary intolerance to gluten (see Celiac Disease). These are not associations of scientists, but rather of people with celiac disease, together with their relatives and friends. They may, in turn, rely on scientists and medical workers to provide advice in the forms of publications, consultation, and conference presentations for the benefit of the members. A few web addresses are provided in the list below, but there are many more such societies worldwide.

See also: Celiac Disease. Cereals: Overview; Grain-Quality Attributes. Consumer Trends in Consumption. **Cultural Differences in Processing and Consumption.** Genetically Modified Grains and the Consumer. Research Organizations of the World: Europe and North America; Asia/Pacific, Central/South America, and Africa/ Middle East; CGIAR; Global Trends and the Commercial Sector. Starch: Analysis of Quality. Wheat: Dough Rheology. Appendix: Foods for Celiac Diets; Test Methods for Grain and Grain-Based Products.

#### **Further Reading**

American Association of Cereal Chemists (2002) AACC Methods, 10th edn. St. Paul, MN: American Association of Cereal Chemist.

American Oil Chemists' Society (2002) Standard Methods of the American Oil Chemists' Society. Champaign, IL: American Oil Chemists' Society.

AOAC International (2002) AOAC Official Methods. Gaithersburg, Maryland: AOAC International.

Black CK and Panozzo JF (eds.) (2003) Cereals 2003. Proc. 53rd Australian Cereal Chemistry Conference. Melbourne, Australia: Royal Australian Chemical Institute.

Association for Cereal Science International Technology (2002) Standard Methods. Vienna, Austria: International Association for Cereal Science and Technology (ICC).

International Seed Testing Association (1999) *International Rules for Seed Testing*, 1999. Zurich, Switzerland: International Seed Testing Association.

Westcott T and Ross D (2002) Official Testing Methods of the Cereal Chemistry Division of the Royal Australian Chemical Institute. Melbourne, Australia: Royal Australian Chemical Institute.

Wootton M, Batey IL, and Wrigley CW (eds.) (2001) Cereals 2000. Proceedings of 11th International ICC Cereals and Bread Congress and of the 50th Australian Cereal Chemistry Conference. Melbourne, Australia: Royal Australian Chemical Institute.

#### **Relevant Websites**

http://www.aoac.org - AOAC International. http://www.aaccnet.org - The American Association of Cereal Chemists.

http://www.aocs.org — The American Oil Chemists' Society.

http://www.agronomy.org - The American Society of Agronomy.

http://www.asbcnet.org — The American Society of Brewing Chemists.

http://www.canola-council.org — The Canola Council of Canada.

http://www.raci.org.au — The Cereal Chemistry Division of the Royal Australian Chemical Institute. http://www.csaceliacs.org — The Celiac Sprue Association, USA.

http://www.coeliac.org.au - The Coeliac Society of Australia Inc.

http://www.crops.org - The Crop Science Society of America.

http://www.icc.or.at — The International Association for Cereal Science and Technology (ICC).

http://www.nopa.org – The National Oilseed Processors' Association.

http://www.soils.org - The Soil Science Society of America.

http://www.spcouncil.org - The Soy Protein Council.

### **SNACK FOODS, PROCESSING**

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This article deals with different aspects of snack food production. It covers the areas of snacking patterns, types of snacks, raw material for snack foods, and different snack food manufacturing details. This is intended to be useful for the snack food industry as well as for marketing professionals to understand different types of snack and trends, and for consumers who wish to gain more knowledge about snacks.

#### **Background**

Snack foods have always been a significant part of modern life; they represent a distinct and constantly widening and changing group of food items. Sales in 2000 were over \$19.37 billion and per capita snack consumption was 10 kg. According to a rough working estimate, annual worldwide sales including the US was \$30–35 billion. American Heritage dictionary defines snack as "hurried or light meal" or "food eaten between meals." Traditional snack foods appeal to consumers on a number of levels. Snacks can be

considered a treat or reward. Designing snack foods today can be a complex process to meet changing consumer's taste and expectation, e.g., "good for your health," "rich source of soy protein," "offering a unique flavor," and the elusive search for something unique that also appeals to a wide variety of people. Most snack manufacturers use some form of existing technology as the basis for creating snack products, but incorporate variations that increase the resulting snack's health image appeal by lowering fat and calories or adding nutrients.

#### **Snack Consumption Patterns**

The snack food market is constantly changing relative to product types, and although most snacks are not primarily consumed for their nutrients, many snacks are made with nutrition in mind. The snack food industry is experiencing extraordinary changes from the consumers' point of view. Consumers want snacks to not only taste good, but also smell good, feel good, and look good. Snacks should give the consumer a homemade/fresh feel. Some of the snacks are developed with a special theme in mind like world soccer. These snack pellets are soccer ball shaped, which on



Figure 1 Different shaped snacks.

frying or treating in a microwave oven become soccer balls (Figure 1).

Snacking is increasing from factors such as increases in one-person households, higher proportions of working spouses and more school age children obtaining their own meals and refreshments, a highly mobile population, and availability of snack foods in vending machines and convenience markets. Various products, which were once consumed mainly on impulse, are becoming accepted as side-dish items, for example, corn chips or potato chips served in place of mashed potatoes. The established position of snack foods in the diet is demonstrated by the continuous growth in sales.

In the last ten years, changes in lifestyle and eating patterns have led to a gradual increase in demand for snack foods. The pattern of snacking in different countries can be affected by several factors such as the lifestyle in each area, the economic climate, rival foods, and public receptiveness of current views on nutritional matters. Snacks can provide an increased dietary intake of essential amino acids and other nutrients for developing countries.

Some of the most recent factors driving the newer snack food trends are -(1) availability of healthy snacks like energy bars or soy fortified bars, (2) better taste and flavors, (3) better and attractive shapes for children and packaging, and (4) better varieties of snacks like soy nuts or yogurt bars, etc.

#### **Types of Snack Foods**

Although it is not possible to discuss all types of snack foods available in the market in this article, only major types of snack foods like potato chips, corn and tortilla chips, and extruded snacks (expanded and pellets snacks) will be discussed in detail. A broad variety of snacks made by different processes

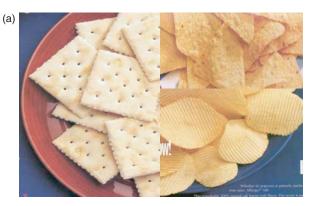




Figure 2 (a) First generation snacks. (b) Multi-grain chips.

are available in the market. These include potato chips, tortilla chips, corn chips, ready-to-eat popcorn, extruded snacks, pretzels, snack nuts, meat snacks, pork rinds, party mix, multigrain chips, granola products, variety packs, etc. Most recently, a variety of health snacks made with soy protein have been seen. These soy-based snacks contain 6.25 g of soy protein per serving to qualify for the health claim of the Food and Drug Administration. Along the same line, snacking on soy nuts is becoming very popular among the health-conscious consumers. Nowadays, soy nuts can be found with different flavors in most grocery stores. Snacks with different spices and flavors are becoming very popular with US consumers. There is a substantial growth in ethnic snacks from Mexico and India in the markets. Snacks from India are mostly fried lentils and chickpeas, and similar types of pulses with different flavors (mostly with chilli powder) are entering the US market.

Each snack processor may use a specific unit operation and somewhat different technologies to produce unique snacks. There are many ways to classify the snacks. Snack manufacturers use three main terms to identify the snacks (see Figures 2-4):

1. First generation snacks. In this category all the natural products used for snacking, nuts, potato chips, and popped popcorn are included;

- 2. Second generation snacks. Majority of the snacks fall in this category. All the single ingredient snacks, simple-shaped products like corn tortilla chips and puffed corn curls and all
- directly expanded snacks are included in this category;
- 3. Third generation snacks (also called half-products or pellets). In this category, snacks and pellets



Figure 3 Second generation snacks.



 $\textbf{Figure 4} \quad \text{(a-d) Third generation snacks. (e) Example of co-extruded snacks. (Courtesy of Wenger Manufacturing, Sabetha, KS.)}$ 

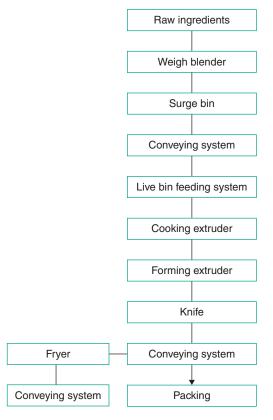
formed using multi-ingredients, made by extrusion cooking are included.

#### **Production of Snacks**

It is not possible to discuss every snack-manufacturing procedure in detail (see Figure 5). Manufacturing of only major snacks will be discussed here.

#### **Potato Chips**

The potato chips form the largest snack food sector in all markets. Traditional potato chips start with whole, raw potatoes sliced from between 0.035 and 0.070 inches thick. These cuts can be straight, grooved, crinkled, or flat surface. The most important point is that slice surface should be uniform for all these cuts. These slices are washed with water to remove starch and eliminate sticking, or can be blanched to reduce the level of reducing sugars to avoid browning before frying. The critical parameters for the chipping potatoes are moisture, starch, and sugar level. For a good potato chip, the dry matter should be  $\sim 20-23\%$ , giving a specific gravity of 1.080–1.095. Potatoes with a lower specific gravity require a longer frying time and absorb more oil.



**Figure 5** Third generation snack-manufacturing procedure.

In general, potatoes contain 80–85% water, depending on variety. The rest is (15-20%) dry matter, which is  $\sim 90\%$  carbohydrates, mainly starch. The major concern with the carbohydrates portion is reducing sugars. The sugar contents may be as high as 10% of the dry matter. However, for potato chip processors, a sugar level above 2% is a concern as it induces the Maillard reaction during frying and makes unacceptable dark color potato chips.

Potato chips can be fried using batch or kettle frying or continuous frying. In batch operation, chips have a higher fat level, more blistering, and a harder surface due to the presence of starch on the surface. Whereas with a continuous fryer, the potato slices are continuously fed into the fryer, and cooked as they are conveyed and removed at the end. After frying, before the product cools and the surface oil solidifies, the chips receive an application of salts and or seasoning. Typical salt levels are from 1.5% to 2.5% and the seasoning levels range from 4% to 8%. The typical potato chip has a fat content of 35-40% making it difficult for most consumers to categorize it as a health snack.

#### **Corn and Tortilla Chips**

The increased popularity of certain ethnic snacks, such as corn and tortilla chips, has significantly increased this segment of the snack food industry. These snacks are very popular in South America. In recent years, their visibility has increased in North America also. After potato chips, corn and tortilla chips represent the second largest category of salted snacks. The main difference between tortilla chips and corn chips is the extra baking step required in the manufacture of tortilla chips.

The key to the process is initial nixtamalization or alkaline cooking and steeping step involving whole corn. In the past, not much attention was given to corn type in the manufacturing of fresh "masa" that was to be immediately converted to chip products. However, corn quality is quite critical in the manufacture of dry masa. In the actual process, good quality whole corn is cooked for up to 3 h at 80-100°C with frequent stirring in 120-300% excess water containing 0.1-2.0% hydrated lime. The cooked corn is then permitted to steep, usually overnight. The cooking and steeping steps permit the endosperm to hydrate and soften, which encourages partial starch geletinization, and disrupts and partially dissolves the pericarp. Subsequent washing removes the pericarp and residual lime. The resulting cooking/steeping liquor normally contains 2-6% dissolved and suspended solids, which are usually discarded. The washed material is then traditionally stone ground, resulting in fresh masa, which is then

sheeted, cut, and either baked and fried to produce tortilla chips or just fried after sheeting and cutting to make corn chips. In the case of corn chips, the masa can be directly extruded and cut into the frying oil instead of being sheeted and cut.

If reconstituted masa flour is used, a large particle size should be used for corn chips. This will provide for interruption in the dough structure so that air and water can escape during frying. If small particle flour is used, excessive puffing occurs during frying, resulting in a chip that will absorb more oil during frying and can be easily broken. On an average basis, corn chips contain 35% fat, whereas good quality tortilla chips have 25% fat. This is because of the preliminary baking step with tortilla chips, which set the structure, therefore minimizing the oil absorption during frying. Usually, corn chips are made with a blend of white and yellow corn masa, which produces chips that are light in color. Corn chips made from 100% yellow corn masa usually have an objectionable burnt flavor, which can be attributed to the degradation of carotenoids during the frying step. Flavors and colors can either be added to the dough, which can result in significant flavor loss due to volatilization or degradation during frying, or they can be added either as a powder or in the form of an oil spray after frying.

The traditional corn cooking techniques to prepare masa or corn/tortilla chips are being changed by more efficient, large scale operations where corn is cooked and ground immediately with little or no steeping. New and simple methods of producing corn and tortilla chips, and other masa-based snacks have been developed. Many snack food manufacturers like to use dry masa flours that come in a variety of color and particle sizes. By adding water in precooked masa flour, different shapes of snacks can be made by using a forming extruder. These snacks can be fried, flavored, and packaged for sale. Frying has increased the masa-based snacks market, because after frying. the final product has an excellent taste and texture and a long shelf life. Typical flow diagrams of tortilla and corn chips production are shown in Figures 6 and 7.

#### **Extruded Snacks**

This category has the greatest potential for growth among the snack foods. The snacks can be produced using innovative methods (see, e.g., Figures 8–10) which capture the consumer imagination. Some of the examples are three-dimensional snacks, a variety of animals, cartoon, and alphabets shapes etc. Producing a successful snack is a fine balance between the consumer's needs, like tastes and interests versus a manufacturer's production abilities, economics, and quality control. Raw material cost plays an

important role in the finished product's selling price. Therefore, it is an advantage to use the lowest cost raw material to produce a successful snack.

Common ingredients used for extruded snacks Presently, snack products are being made from a variety of ingredients (see Table 1 where typical properties of common raw materials are listed). However, the selection of the ingredients was limited by the equipment availability. The introduction of the

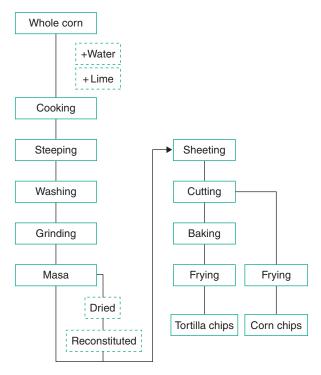


Figure 6 Flow diagram of tortilla and corn chips.

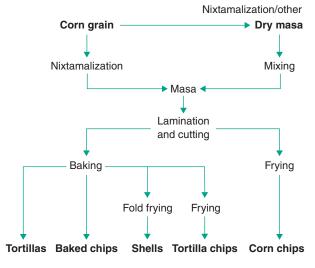


Figure 7 Corn and tortilla chips production.

extrusion process, other processing equipments, and better knowledge of extrusion technology have led to more diverse and complex formulations for snack foods. The most common source of ingredients are corn, wheat, rice, potato, tapioca, and oats. This is

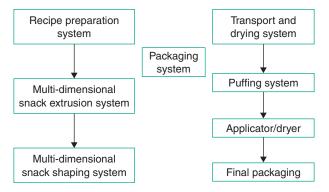


Figure 8 Multi-dimensional snack production.

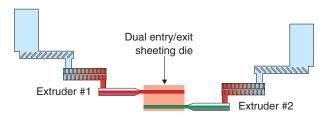


Figure 9 Multi-dimensional snack extrusion system.

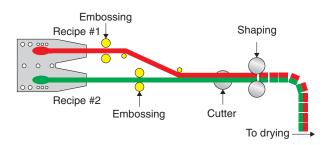


Figure 10 Multi-dimensional snack shaping system.

**Table 1** Properties of different raw material in relation to snack food production

Raw material	Granule size (µm)	Flavor	Expansion	Texture
Corn	5-20	Definite	Very good	Crispy
Wheat	20-40	Mild	Good	Crispy
Rice	2-8	Bland	Good	Crispy
Oats	2-12	Very strong	Poor	Soft to hard
Barley	Medium to large	Mild	Poor-good	Soft to hard
Potato Tapioca	60-100 5-35	Definite Bland	Very good Good	Crispy Crispy

not an inclusive list and one should not limit his/her snack food formulation based on these ingredients. There are several other sources of ingredients for snack food all over the world. A major ingredient in snack food formulation is starch. In its natural form, the starch is insoluble, tasteless, and unsuited for human use. To make it digestible and acceptable, it must be cooked.

Cereal sources Almost any cereal can be extruded, but if expansion is a major objective, the numbers of functional cereals are limited to de-germed corn/grits and rice. Cereals that have high amounts of lipids are more difficult to expand due to dough slippage within the extruder barrel. This type of cereal usually requires high moisture and high temperature before significant puffing can occur. In general, starches with 5-20% amylose content will significantly improve expansion as well as texture of the snack foods. The most common cereals used in snack food formulations are described below.

Corn Extruded snacks are a growing segment of the corn-based market (see Figure 11). Corn (also called maize) is a primary ingredient for corn collets and many pellet products. For most corn-based extruded snacks, dry-milled corn meal is used. Large quantities of corn meal are used in puffed extruded snack production and some are used in corn chips. Corn meal, corn grits, corn flour, and corn cones are all a different form of dry-milled dent corn, and in general vary only in particle size distribution. Selection of the granulation depends upon the type of snack and type of extruder. For example, for fine texture and cell structure, or softer bite, a fine granulation of corn meal should be

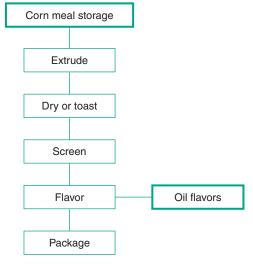


Figure 11 Flow diagram of baked collets.

used. Whereas for crunchy texture with a slightly larger cell structured snack, a more coarse granulation of corn meal is desired. Similarly, a twin screw extruder can handle fine as well as coarse granulation corn flour, while collect extruders require coarse granulation. Mostly, de-germed corn is used in extruded snacks because it expand better than whole corn. Yellow and white corn are most commonly used in snack foods. Cornstarch granules are medium in size (5-20 μm) and have very good expansion characteristics. Protein content of corn ranges from 6-10%. Snack food formulations with corn have a definite flavor. Cornstarch is usually cooked at a medium to higher temperature during extrusion. The function of the starch in snack foods is to achieve various textural attributes and characteristics. Changing the amylose/ amylopectin ratio in the starch can change these attributes. Today, we can find cornstarches with high amylose or high amylopectin in the market. High amylose cornstarches are used when crunchiness and strength is required in the snack. To increase the expansion of the snack, high amylopectin cornstarches (waxy starch) can be used. Waxy corn contains very little amylose, whereas the normal corn contains  $\sim 25-35\%$  amylose. Under high shear and high temperature cooking, a cross-linked waxy cornstarch is recommended in snack foods, since it exhibits an improved property of resistance to amylopectin breakdown.

Wheat In general, wheat can be classified into two types: hard and soft. Hard wheat is higher in protein, produces stronger flour, and is better for bread making. On the other hand, soft wheat is lower in protein, yields weaker flour, which is better for cake making. In the snack food industry, wheat flour is used in formulation for making baked and fried snacks, flavored crackers, snack cakes, pretzels, bread, and the like. Semolina, (coarse particle) usually produced from hard wheat milling, is also used in snack food formulation. The semolina product has an expansion ratio and bulk density about the same as corn meal. Snack foods with all-semolina will produce a very crispy texture. Wheat starch granules are fairly large (20-40 μm) as compared to other cereal grain starches. In wheat, amylose and amylopectin are found within a narrow range of 20-25% amylose. It gives good expansion during extrusion cooking. Wheat is relatively higher (8-15%) in protein than other cereals. Sometimes it is difficult to expand due to the presence of gluten. In extruded snacks, wheat gluten provides nutritional value, crispness, and desired texture. In general, 1-2% wheat gluten is used in snack foods. Hard wheat is commonly used in bread rolls, pretzels, and fabricated or pellet type snacks. Wheat varieties with a lower gluten level, give a more tender expanded product than semolina, or hard varieties. Snack products made with wheat usually have mild flavor and white to off-white color. It needs medium to low cooking temperature during extrusion cooking. Milling byproducts (bran) can be used with sov protein and some other ingredients to produce expanded snack foods of high nutritional and fiber value. The use of wheat in snack food formulation is limited because of cost.

*Rice* Rice is one of the largest crops grown in the world. Four types of rice are produced in the United States: long, medium, short, and waxy grain. In the US, rice ingredients are not commonly used in snack food formulation. In Japan, most of the snacks are made with rice or rice flour. One major reason is the cost of rice as compared to that of other snack food ingredients. Broken rice can be used as ingredient in expanded or puffed snack products, since rice has good expansion qualities. Rice starch granules are the smallest  $(2-8 \,\mu\text{m})$  of all grain starches and can digest very easily. Its functional properties are very different from corn or wheat starches. The primary difference is in amylose/amylopectin ratio in the starch. Flours from different rice varieties have major differences in physical and chemical properties, which can affect the snack cell structure and expansion. For example, long grain rice flour can increase the crispiness in snack foods, whereas waxy rice flour can reduce chip hardness and at the same time can provide a melt-in-the-mouth texture usually achieved with extra fat. Rice is commonly used as a carrier product for other flavors, since it is bland in flavor. In comparison with other products, rice requires the highest temperature during extrusion to cook a snack. Selection of the rice starch in the snack foods formulation will depend upon the amylose content of the common rice varieties. Long grains have 22-23%, medium grain 15-19%, and waxy grain < 1% amylose. This difference in amylose/amylopectin ratio greatly affects the gelatinization temperature of rice flour. The protein content of rice ranges from 6% to 8%. Rice flour could be used for texture improvement in multi-grain snack foods. Rice flour can be mixed with masa flour, potato flakes, or bean flakes. Chips made with 100% rice flour absorb 20-30% less oil during frying. In a formulation where rice and potato blend is used, the potato flavor and texture remains distinctive even though it is mixed with the less costly rice blend. A mixture of bean flake and rice flour produces a distinct visual appearance of the beans while creating a well-blended bean flavor with no bitter aftertaste.

Oats In general, oats are marketed as rolled oats or as an ingredient for breakfast cereal. Oats have not been used in grain-based snacks as wheat and corn. Recent discoveries, that oat bran can reduce serum cholesterol level in humans, have boosted the market for oats in the snack food industry. The major problem with oats is high oil content (7-9%) and lipase enzyme. Before using oats in the snack food formulation, it is desirable to inactivate the lipase. Otherwise, lipase will catalyze the hydrolysis of oil, which would lead to the production of bitter tasting free fatty acid. Oat starch granules are comparatively small (2-12 μm) in size as compared to other starches. Amylose content of oats varies from 16% to 27%. Oat starch has a very strong flavor and it gives light brown color to the product. It requires a relatively low gelatinization temperature, but a higher amount of energy input for cooking because of higher amounts of oil content. Oats contain high levels of fiber. Snacks extruded with oat starch expand poorly. For this reason, it has only found its way into products at low levels. By using longer barrel extruders with preconditioner, a higher level of oats can be used in snack foods. Among the snacks that have traditionally included oats in their formulation are cookies and granola. With new technologies and more interest in oats due to health claims, oatbased snack products may be popular in the future.

Barley Barley is used in small quantities in some snack food formulations. It has a mild flavor, and nutritionally, it is almost the same as wheat, except it contains considerably more fiber. Barley starch granules are medium to large in size as compared to other cereals. A reasonable amount of expansion can be obtained during extrusion of snack foods using barley starch. It gives a light brown to gold color to the product. Snack food formulation containing barley starch needs a low cooking temperature during extrusion. Barley fiber can be used in healthy snack foods as a fiber supplement. Sometimes, manufacturers use barley in multigrain snack foods in order to add one extra cereal on the label.

Other cereal sources Cereals such as rye, sorghum, millet, amaranth, and triticale have been used in snack foods. Presently, these cereals are not major ingredients in the snack food formulation.

**Tuber sources** Roots and tubers belong to the class of foods that basically provide energy in the human diet in the form of carbohydrates. According to a recent estimate by Food and Agriculture Organization (FAO), virtually every country in the world grows some species of root crop. Potato and tapioca (also

called cassava) are two main tuber crops used for extruded snack foods.

Potato Different forms of potatoes (granules, flakes, flours, and starches) are used in snack food formulations. Potato starch is often used in snacks to provide extra expansion. Potato starch has a wide range of sizes with some granules (60–100 μm) larger than the other cereals. This starch contains 20–25% amylose and has very low oil contents. Potato starch develops high viscosity during extrusion cooking. It has an excellent swelling and binding power. In snack food, it has a definite flavor and it gives gold to light brown color to the product. It requires low cooking temperature since its granules breakdown easily. Potato flour is the major ingredient for two common snack products, i.e., direct expanded snack (product looks like French fries) and fabricated chips.

Tapioca Tapioca (cassava) is a basic source of low calories, or a supplement to cereal. In general, tapioca starch is used in third generation snack foods formulation. Tapioca starch grains vary in shape, and size from 5 to 35  $\mu$ m. The amylose content is  $\sim 17\%$ . Good quality starch should have a pH of 4.7–5.3, a moisture content of 10–13.5%, and should be uniformly white in color. Tapioca starch develops a very high viscosity and it is an excellent binder. It has a bland flavor and requires moderate cooking temperature during extrusion cooking.

#### **Expanded Snacks**

The majority of extruded snacks are in this category. This group is also referred to as "collet" or "second generation snacks." In general, expanded snacks are made on high-shear extruders. These are high-fiber, high-protein, and low-calorie snacks. Some examples are corn curls, onion rings, three-dimensional snacks, and potato sticks. These types of snacks can be seasoned with a variety of different flavors, oils, salt, sugars, etc. The quality of an expansion-cooked product depends upon the conditions of operation of the extruder and the main raw material used in the formulation. Several other factors can influence the degree of puffing of snacks during extrusion, i.e., amount of moisture in the feed material, dough residence time in the extruder barrel, and cereal particle size.

Fried collets These are the most familiar extruded snacks in the market. A special die arrangement gives the product a twisted and puffed shape. These collets are made on collet extruders. The product is then fried in vegetable oil, and coated with cheese and some

other flavor. During frying, the moisture level reduces from 8% to 1-2% in this product. The most common material used for fried collet is corn meal. Typical corn meal specifications are given in Table 2. Some other cereal grains can also be used for this type of product.

Baked collets Baked collets are another example of the expanded extruded snacks. These include products such as baked corn curls, onion rings, and potato sticks. Baked collets can be made with different cereal grains and tuber flours. Protein, fibers, cellulose, and bran can be blended with cereal grain up to 20% to make healthy snacks. Potato sticks are usually made by mixing potato flour with corn or rice flour. A typical formulation for baked snacks is given in Table 3.

Third generation snacks Third generation snacks, also referred to as "half products" or pellets provide an alternative to fully prepared puffed snack foods. Third generation snacks, or half products are extrusion cooked, and formed at low pressure to prevent expansion, and then dried to a final moisture content of  $\sim 10\%$  to form a glassy pellet. In developing third generation snacks, "half" of the process is completed to prepare "pellets" which are shelf-stable for periods of up to a year without refrigeration, provided they are properly packed to retain their moisture. These products are economical to run and have built-inmarketability due to their high-bulk density. Third generation snacks can be prepared in homes or restaurants. Unlike typical snack foods, half-products do not yet contain oil that can oxidize to give offflavor to the products. These pellets can be shipped from a central manufacturing distribution point, held until needed for the market, and then puffed, flavored and packed fresh and locally. New variations of the third generation snacks expand using infrared heating, hot air, or microwaving. The use of hot air systems reduces the oil uptake that occurs in frying and allows a controlled addition of oil to be made as required for flavoring. With consumer concerns about fats and oils, a half product snack that expands using hot air, offers snack food manufacturers an oil free snack with perceived health benefits. Elimination of frying oils reduces calories and allows a marketing opening for snacks with a "lite" image. A typical flow diagram for the production of third generation snacks is shown in Figure 12.

With the multidimensional snack system, a wide range of raw ingredients can be used to blend together to make an excellent formulation for many types of third generation snacks. The extruder feed must contain a high level of starch to maximize expansion

Table 2 Typical corn meal specifications for fried collets

Granulation (mesh)	Percent retained on screer		
16	0		
20	0-2		
25	0-10		
30	25-50		
40	45-65		
50	0-8		
60	0-2		
Moisture	11-13%		
Fat	<1%		

**Table 3** Typical formula for baked snacks (second generation snacks)

Ingredients	Amount (%)
High-protein snack	
Rice flour	35
Wheat flour	35
Soy concentrate	20
Sugar	6
Corn starch	2
Vegetable oil	2
Potato-stick snack	
Potato granules	64
Degermed corn meal	35
Vegetable oil	1
Corn curls	
De-germed corn meal or grits	100

of the collet during exposure to hot oil or air. Levels of 60% or less total starch in the formula give only slight expansion in the puffing step and yield a final product with a crunchy, hard texture. Wheat, corn, and tubers are widely grown crops in developing and industrialized countries, and they are cheaper and more easily available in the market than other cereal crops. Several formulations for third generation snacks are given in Table 4.

#### **Co-Extruded Snacks**

This is a relatively new technology introduced in 1984 for the snack food industry. In this process, two different materials are extruded from one die. The two materials can come from two extruders or from one extruder and one pump. This process can produce a snack with two different flavors, or two textures or two colors. The most common snack produced by co-extrusion is a cereal based outer tube with a cheese filling inside. There are three basic types of co-extruded snacks in the market; cereal-based tubes with fat-based fillings, and cereal-based tubes

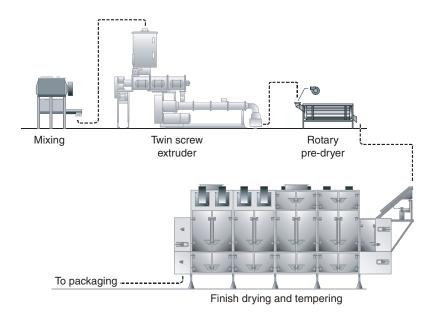


Figure 12 Flow diagram for third generation snacks.

Table 4 Typical third generation snack formulations

Corn based           Hard, crunchy texture           Ground corn         94.5           Corn starch         5.0           Monoglyceride         0.5           Soft, frothy texture           Corn starch         55.2           Wheat starch         27.5           Tapioca starch         14.0           Liquid shortening         2.5           Monoglyceride         0.8           Potato based         49.0           Hard and crunchy         Potato flakes           Potato flakes         49.0           Durum flour         30.0           Wheat starch         20.0           Monoglyceride         1.0           Crispy         Potato flakes         47.0           Drum flour         30.0           Wheat starch         20.0           Vegetable oil         3.0           Soft         20.0           Potato flakes         49.0           Corn flour         30.0           Wheat starch         20.0           Monoglyceride         1.0           Speciality snacks         Fresh shrimp recipe           Tapioca starch         64.0           Fresh shrimp	Ingredients	Amount (%)
Hard, crunchy texture         Ground corn       94.5         Corn starch       5.0         Monoglyceride       0.5         Soft, frothy texture       27.5         Corn starch       27.5         Wheat starch       14.0         Liquid shortening       2.5         Monoglyceride       0.8         Potato based       49.0         Hard and crunchy       49.0         Potato flakes       49.0         Durum flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Crispy       20.0         Potato flakes       47.0         Drum flour       30.0         Wheat starch       20.0         Vegetable oil       3.0         Soft       20.0         Potato flakes       49.0         Corn flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Speciality snacks       Fresh shrimp recipe         Tapioca starch       64.0         Fresh shrimp       20.0         Rice flour       10.0         Vegetable oil       3.0		
Ground corn       94.5         Corn starch       5.0         Monoglyceride       0.5         Soft, frothy texture       27.5         Corn starch       27.5         Tapioca starch       14.0         Liquid shortening       2.5         Monoglyceride       0.8         Potato based       49.0         Hard and crunchy       49.0         Potato flakes       49.0         Durum flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Crispy       20.0         Potato flakes       47.0         Drum flour       30.0         Wheat starch       20.0         Vegetable oil       3.0         Soft       49.0         Corn flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Speciality snacks       7         Fresh shrimp recipe       7         Tapioca starch       64.0         Fresh shrimp       20.0         Rice flour       10.0         Vegetable oil       3.0         Salt       3.0		
Monoglyceride       0.5         Soft, frothy texture       55.2         Corn starch       27.5         Tapioca starch       14.0         Liquid shortening       2.5         Monoglyceride       0.8         Potato based         Hard and crunchy         Potato flakes       49.0         Durum flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Crispy         Potato flakes       47.0         Drum flour       30.0         Wheat starch       20.0         Vegetable oil       3.0         Soft       20.0         Monoglyceride       1.0         Speciality snacks       20.0         Fresh shrimp recipe       1.0         Tapioca starch       64.0         Fresh shrimp       20.0         Rice flour       10.0         Vegetable oil       3.0         Salt       3.0		94.5
Soft, frothy texture         Corn starch       55.2         Wheat starch       14.0         Liquid shortening       2.5         Monoglyceride       0.8         Potato based         Hard and crunchy         Potato flakes       49.0         Durum flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Crispy       20.0         Potato flakes       47.0         Drum flour       30.0         Wheat starch       20.0         Vegetable oil       3.0         Soft       20.0         Monoglyceride       1.0         Speciality snacks       1.0         Fresh shrimp recipe       1.0         Tapioca starch       64.0         Fresh shrimp       20.0         Rice flour       10.0         Vegetable oil       3.0         Salt       3.0	Corn starch	5.0
Corn starch       55.2         Wheat starch       27.5         Tapioca starch       14.0         Liquid shortening       2.5         Monoglyceride       0.8         Potato based         Hard and crunchy         Potato flakes       49.0         Durum flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Crispy       Potato flakes       47.0         Drum flour       30.0         Wheat starch       20.0         Vegetable oil       3.0         Soft       Potato flakes       49.0         Corn flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Speciality snacks       Fresh shrimp recipe         Tapioca starch       64.0         Fresh shrimp       20.0         Rice flour       10.0         Vegetable oil       3.0         Salt       3.0	Monoglyceride	0.5
Wheat starch       27.5         Tapioca starch       14.0         Liquid shortening       2.5         Monoglyceride       0.8         Potato based         Hard and crunchy       49.0         Potato flakes       49.0         Durum flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Crispy       70         Potato flakes       47.0         Drum flour       30.0         Wheat starch       20.0         Vegetable oil       3.0         Soft       49.0         Corn flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Speciality snacks       Fresh shrimp recipe         Tapioca starch       64.0         Fresh shrimp       20.0         Rice flour       10.0         Vegetable oil       3.0         Salt       3.0	Soft, frothy texture	
Tapioca starch       14.0         Liquid shortening       2.5         Monoglyceride       0.8         Potato based         Hard and crunchy       49.0         Potato flakes       49.0         Durum flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Crispy       70         Potato flakes       47.0         Drum flour       30.0         Wheat starch       20.0         Vegetable oil       3.0         Soft       49.0         Corn flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Speciality snacks       Fresh shrimp recipe         Tapioca starch       64.0         Fresh shrimp       20.0         Rice flour       10.0         Vegetable oil       3.0         Salt       3.0	Corn starch	55.2
Liquid shortening       2.5         Monoglyceride       0.8         Potato based         Hard and crunchy       49.0         Potato flakes       49.0         Durum flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Crispy       70         Potato flakes       47.0         Drum flour       30.0         Wheat starch       20.0         Vegetable oil       3.0         Soft       49.0         Corn flour       30.0         Wheat starch       20.0         Monoglyceride       1.0         Speciality snacks       Fresh shrimp recipe         Tapioca starch       64.0         Fresh shrimp       20.0         Rice flour       10.0         Vegetable oil       3.0         Salt       3.0	Wheat starch	27.5
Monoglyceride         0.8           Potato based           Hard and crunchy         49.0           Durum flour         30.0           Wheat starch         20.0           Monoglyceride         1.0           Crispy         Potato flakes         47.0           Drum flour         30.0           Wheat starch         20.0           Vegetable oil         3.0           Soft         Potato flakes         49.0           Corn flour         30.0           Wheat starch         20.0           Monoglyceride         1.0           Speciality snacks         Fresh shrimp recipe           Tapioca starch         64.0           Fresh shrimp         20.0           Rice flour         10.0           Vegetable oil         3.0           Salt         3.0	•	
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Table 5 Typical formulation for co-extruded snacks

Ingredient (sweet snack)	Amount (%)	Ingredient (savory snack)	Amount (%)
(Sweet Shack)	(%)	(Savory Snack)	(%)
Tube		Tube	
Wheat flour	70	Corn meal	80
Sugar	20	Wheat bran	10
Milk powder	9	Milk powder	8
Salt	1	Salt	2
Filling		Filling	
Powdered sugar	50	Cheese powder	24
Vegetable oil	21	Vegetable oil	30
Corn starch	11	Shortening	14
Shortening	11	Corn starch	10
Cocoa powder	7	Milk powder	10
		Dairy powder	10
		Salt	2

with water-based filling. The shelf lives of these snacks are limited, because of migration of moisture and/or oil from the filling to the outer shell. A typical formulation of co-extruded snacks is given in Table 5.

In conclusion, snacks can be processed by a variety of different methods and techniques. Several new raw materials containing nutraceutical and functional properties are being introduced in the market every day for snack food products. Snacks can be made with a combination of different raw materials containing different properties. The role of snacks in a healthy life style is only now being developed. The recognition of snacks as healthy foods will increase as industry changes products from those having merely good tastes to nutritious ones.

See also: Extrusion Technologies. Maize: Dry Milling; Foods from Maize. **Tortillas**.

#### **Further Reading**

Gomez MH, Rooney LW, Waniska RD, and Plfugfelder RL (1987) Dry corn masa flours for tortilla and snack food production. Cereal Food World 32(5): 372-377.

Huber GR (2002) Recent developments and trends in the snack food industry. In: Riaz MN and Rooney L (eds.) Snack Food Processing Short Course Manual. College Station, Texas: Food Protein Research and Development Center, Texas A&M University.

Huber G and Rokey GJ (1990) Extruded snacks. In: Booth RG (ed.) Snack Food, pp. 107-138. NY: Van Nostrand Reinhold.

Kuntz L (1996) Creating healthful salty snacks. Food Product Design 6(9): 31-56.

Lusas EW and Rooney LW (2001) Snack Food Processing. Lancaster, PA: Technomic Publishing.

Moore G (1994) Snack food extrusion. In: Frame ND (ed.) The Technology of Extrusion Cooking, pp. 110. Glasgow, G64 2NZ: Blackie Academic and Professional.

Riaz MN (1997) Technology of producing snack food by extrusion. In: Ranhotra G (ed.) Technical Bulletin, vol. XIX(2), pp. 1–8. Manhattan, KS: American Institute of Baking.

Riaz MN, Rooney EL, and Barron M (2002) Snack Foods Processing Manual. College Station, Texas: Food Protein Research and Development Center, Texas A&M University.

Sheng DY (1995) Rice-based ingredients in cereals and snacks. Cereal Food World 40(8): 538.

#### **Relevant Websites**

http://www.aibonline.org http://www.sfa.org http://www.aaccnet.org

### **SORGHUM**

Contents

**Breeding and Agronomy** Harvest, Storage, and Transport Utilization

### **Breeding and Agronomy**

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#### Introduction

Sorghum breeding and agronomy as components of sorghum improvement have been the critical issues being addressed by scientists, educationists, researchers, producers, consumers, and other end users including industry and policy makers. The emphasis has been on increased productivity, adoption and sustained production, environmental and biodiversity management, and livelihoods of people in the semiarid and drought-prone subhumid areas of the world. The present plateau in the production levels of sorghum across the globe and disenchantment of commercialization forces and agents, have all combined to generate continued interest in the improvement of sorghum through breeding and agronomy.

This article critically reviews the current state of knowledge of the topic and puts in perspective old and new breeding methods and tools. The article also pioneers the new paradigm of breeding for impact, highlighting the need for strategic collaboration and partnerships among stakeholders in generating new methods, tools, and improved products from breeding of sorghum. This is put in a scenario of traditional and classical breeding and agronomy

approaches as compared with nonconventional and applied breeding and agronomy.

## General Description and Botany of Sorghum

#### Characteristics, Morphology, and Phenology

Sorghum is a single- to multi-culmed C4 plant with perfect flowers; grass species cultivated in diverse and adverse environments from subhumid, hot and dry agro-ecologies, to drought-prone low-to-medium altitudes of the tropics and subtropical regions of the world. This very versatile crop is truly multipurpose, and is used as: (1) grain for food, livestock feed, and industrial products like malt, alcoholic and nonalcoholic beverages, lager beer, stout, and malt drinks; (2) crop residue and silage for livestock feed; (3) chewing cane of the sweet stalk sorghums, (4) household appliances (in fencing and roofing with the dried stalks and as a broom for sweeping with the broomcorn types); and (5) sources of industrial alcohol and household brown sugar with the sweetstalk sorghums.

The sorghum plant is composed of two major sections: (1) the vegetative part consisting of the fibrous root system, the culm (stem), and leaves with leaf sheaths wrapping around the node and internode of the culm; and (2) the reproductive portion called inflorescence (panicle) carried on a peduncle (neck, which can be straight or curved (goose neck))

which can be well exerted (short or long neck) or poorly exerted with panicle partially covered by the boot (flag leaf and sheath). The peduncle extends into a central axis of the panicle called rachis, from the nodes of which several branches originate, which bear racemes. The racemes carry one or more spikelets (flarets), which bear the seeds subtended by glumes. Figure 1 shows the sorghum plant and its components.

The sorghum seed is a caryopsis composed of pericarp, endosperm, and embryo. Each of these consists of complex sections and constituents. The sorghum seed can be white, gray, red or brown in color, based on combination of pericarp color (which can be white or red only) and the presence or absence of testa (seed-coat which is always dark in color). The endosperm is always white and can be corneous (hard and translucent) or floury (soft and opaque). Thus, a seed having:

- white pericarp with no testa is described as having white seed color;
- white pericarp with testa is described as having gray seed color;
- red pericarp with no testa is described as having red seed color; and
- red pericarp with testa is described as having brown seed color.

There are several shades of red and brown seed color in sorghum due to expressivity and intensifier gene actions.

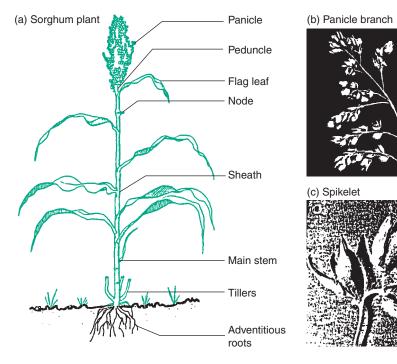


Figure 1 Diagram of the sorghum plant (a) and its components (b and c).

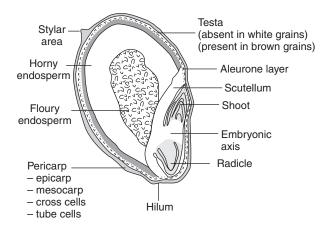


Figure 2 Longitudinal section through sorghum seed.

Figure 2 shows the longitudinal section through a mature sorghum seed. The pericarp is three-layered with epicarp, mesocarp, and endocarp of cross cells, tube cells, and aleurone cells. The aleurone layer cells could have been absorbed and absent, or in other circumstances, this layer persists and is present in mature seed as the testa. Below the pericarp is the corneous endosperm, which may surround (fully or partially) the inner floury endosperm. At the base of the seed, in an angle, is the scutellum (towards the endosperm) covering over the embryo. The embryo consists of the embryonic axis at the top of which is the shoot (develops into stem, leaves, and inflorescence) and at the bottom is the radicle (develops into roots). The physiologically mature seed has a dark hilum at the base, where it was connected to the ovary. The hilum consists of dried up transfer cells, placento-chalazal pod, and phloem parenchyma.

#### **Biodiversity and Genetics**

Sorghum biodiversity and genetic resources acquisition, maintenance, characterization, and utilization (curation) are the basis of breeding in the crop. Two organizations, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and International Sorghum and Millet Collaborative Research Support Program of USA (INTSORMIL), play global roles in the curation of sorghum. The diversity in sorghum is very unique and large in size. For example, ICRISAT has a collection of 35 643 accessions and the National Plant Germplasm System (NPGS) collections in the USA include 40 477 accessions of sorghum. These collections include both active (working collections) and base collections, which are preserved in medium-term conventional storage (about −18°C) and long-term cryo-storage (vapor phase above liquid nitrogen at about  $-160^{\circ}$ C).

In the preserved germplasm accessions, the unique diversity of sorghum has been classified according to species. There are the cultivated sorghum (Sorghum bicolor L. Moench) and the wild weedy species. Within cultivated sorghums, there are five basic races (race guinea, race candatum, race durra, race bicolor, and race kafir) and ten stable hybrid races. The hybrid races are guinea-caudatum, guinea-durra, guinea-bicolor, guinea-kafir, candatum-bicolor, durra-candatum, dura-bicolor, kafir-candatum, kafirbicolor, and kafir-dura. These 15 races of cultivated sorghum can be identified by a combination of seed (size, shape, plumpiness, and color) and glume characteristics (grain covering and glume color) with some help from panicle (inflorescence) traits. Among the wild and weedy sorghum, there are:

- Sorghum halepense (L.) Pers. (2n = 40), a rhizomatous (perennial) autotetraploid species;
- Sorghum almum also a forage grass is autotetraploid (2n = 40);
- *Sorghum propinguum* (Kunth) Hitchc. (2n = 20);
- Sorghum arundinaceum (Desv.) Stapf. (Shattercane);
- Sorghum aethiopicum (Hack.) Roger. Ex Stopf;
- Sorghum drummondii Stapf; and
- Sorghum verticilliflorum (Steud.) stapf.

The taxonomy and evolution of sorghum is well known and documented. The cultivated sorghum, S. bicolor (L. Moench) is made up of two crosscompatible subspecies, bicolor and arundinaceum. Bicolor is derived from the domestication of arundinaceum. Arundinaceum has five races; two of these are proposed to be the progenitors of cultivated sorghum in different parts of Africa. Race arundina*ceum* is a forest grass in West Africa and proposed to be the progenitor of one specific race of bicolor. Race verticilliflorum is a savannah grass, which is proposed to be the main progenitor of cultivated sorghum. The ease of cross-compatibility of wild with cultivated sorghum leads to continuous gene flow in the domestication continuum, which also results in weedy intermediates. Such a genetic scenario has implications for breeding sorghum, especially in the use of wide crosses to incorporate specific traits (e.g., disease or pest resistance) into improved varieties. Molecular breeding and some new biotechnological tools would be required for such difficult crosses.

#### **Conventional Approaches/Classical Breeding**

A generalized sorghum breeding scheme is presented in Figure 3. This is a flow diagram for a systematic

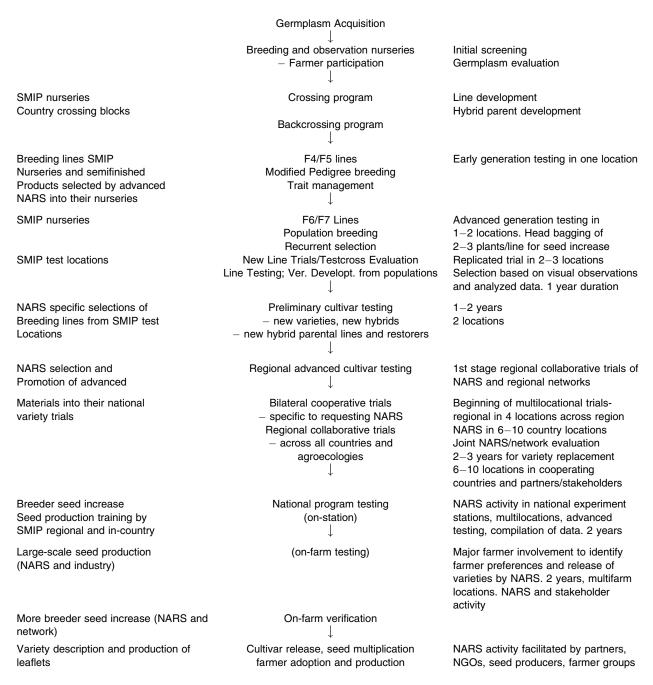


Figure 3 A generalized example of sorghum breeding scheme for achieving impact.

and logical breeding and selection approach for both short-term and long-term (Table 1). The procedures include:

- germplasm acquisition and assemblage, characterization, evaluation, and trait identifications (pre-breeding);
- identification of parents followed by crossing program, including hybrid development;
- the selection and testing process;
- population improvement and recurrent selection;

- line testing, variety identification/development, verification, and release; and
- seed systems and issues for a breeder.

#### **Germplasm Assemblage and Utilization**

Traditional breeding has been mostly limited to agronomically significant and specific genetic resources. Such sources have been, for example, the zera zera (which are mostly caudatum race) from Gambella

Table 1 Breeding activities and strategic partnerships in an impact-oriented breeding program

Event	Activities	Collaboration partners
Technology generation	Germplasm movement exotic introductions indigenous collections distribution Germplasm utilization and trait management plant genomics germplasm evaluation with field and molecular approaches crossing block trait and marker identification initial field and genomic selections marker assisted selection variety development and line breeding development of populations	Breeder Genetic resources scientist Farmers/farmer groups Soil fertility/water mgmt., scientist Entomologist, nematologist Pathologist, virologist Molecular biologist Biotechnologist Advance Research Institutes (ARIs) Networks
Technology testing and development of methodology	On-station trials multi-locational effects year effects; marker testing and selection On-farm evaluation and verification Farmer preference tests and selection Industry preference, market traits identification Laboratory screening for grain and food quality Processing and utilization research, testing of equipment	Breeder/molecular biologist Technology exchange specialists/agronomist/physiologist Extension specialist/NGOs Process technologist Farmers Processors-millers, maltsters, bakers Food/feed technologist/biochemists Resource economists Networks/ARIs
Technology exchange and commercialization	Variety releases line/parent, marker, and population releases breeder and foundation seed production Linkages and collaboration Industrial perspectives and needs analyses Product development and testing Development of quality standards Pilot testing Test marketing of products Seed and raw materials issues availability, quantity supply consistency, access quality	Breeder Seed Producers/Seed specialist Technology exchange specialist Biotechnologist/molecular biologist Extension specialist Economist (policy and markets) Farmers Traders/middlemen Industrial users Processors/machinist/innovator Policy makers/institutions Networks
Value addition for new technology/product niches	Institutional research for commodities Research on novel traits and novel products Regional breeding and spillovers Regional seed and market issues	National/Regional institutions, SROs, ARI scientists, Private/public sector R&D staff, Donors, Networks

Hills of Ethiopia, the kaffir sorghums from southern Africa giving rise to the converted combine kaffirs in the USA, and the guinea race sorghums from West Africa. The guinea race sorghums are also used extensively in the conversion programs of United States Department of Agriculture — Agricultural Research Service (USDA-ARS) and INTSORMIL. There is a greater need now, more than ever, to use more of the photo-sensitive West African guineas; the yellow endosperm sorghums (Kaura types) from Nigeria, Mali and parts of southern Sudan; the very hard grain sorghums Kenike (called rice sorghums) of Mali and parts of West Africa; the durra race (mostly from West Africa); and the Maicellos Creolos (of Central America) to benefit from their unique

attributes. Using these genetic resources, *per se* or in crosses, will also result in more diversification of presently available genetic bases of improved sorghum.

Classic examples of germplasm utilization from acquired resources in sorghum include:

- 1. the Texas A&M US Department of Agriculture (USDA) sorghum-conversion program (623 converted lines released globally),
- the ICRISAT/ Southern Africa Development Community (SADC) sorghum and millet improvement program working collections at Matopos (total 12 350 accessions from across the globe, leading to 27 improved varieties released, 748 breeding lines, and hybrid parental lines made available

- to national program scientists and universities in Africa), and
- 3. the ICRISAT/ Latin American Sorghum Improvement Program (LASIP)- Latin American Commission of Sorghum Researchers (CLAIS) regional sorghum program for Central America.

#### Choice of Parents, Crossing Program, and **Hybrid Development**

Parents, be they varieties, breeding lines, or populations, are identified and chosen from information derived from characterization and evaluation of acquired (locally collected or introduced) genetic resources, and also from previous information and experimental results. The choice of parents to be used in a crossing program depends on the objectives of the program, traits of concern, and product desired.

Normally a self-pollinating plant with perfect flowers (both male and female organs are present in the same floret), crossing (hybridization) in sorghum is achieved mostly by removal of the three anthers from the floret to be crossed, before dehiscence. Alternately, the anthers could be prevented from dehiscing and releasing pollen. With the two alternatives, only foreign pollen from other selected plants or panicles (male parent) will be allowed to pollinate the plant selected as female parent. The two alternatives are referred to as emasculation and can be done by hand (physical removal of anthers) or by double plastic bag methods. The latter method allows hot moist air build-up in the plastic bag and prevents the bursting of the already swollen and mature anthers at the time of pollination with outside pollen. Crossing (setting up of panicle to be crossed and actual pollination) using these methods requires a skilled breeder or trained breeding technician. Success from emasculation or plastic bag methods is variable, depending on the sorghum type, the environment, and skill.

Crossing can be done either by hand-pollination, following emasculation in normal male-fertile plants (to develop lines from F1), or by use of male-sterility systems. Male sterility is used to make crosses for developing commercial hybrids (cytoplasmic genetic male sterility (CMS) is used) and populations (genetic male sterility, which allows for segregation in resulting crossed plants, is used). In the development of hybrids using male sterility, several CMS systems found earlier can be used, but with differential successes. The distinct CMS systems which are available are A1, A2, A3, A4, and 9E – all with diverse trait combinations, and characteristics (advantages and disadvantages) but having a common trait of no-pollen shed. A5 and A6 cytoplasms are not distinct. Fertility restoration is a problem in A2

cytoplasm; testcrosses (experimental hybrids) developed with it are mostly partially sterile. A3 cytoplasm male-sterile plants have plump yellow anthers that are difficult to distinguish from fertile anthers, though no pollen is shed. A4 and 9E are not commonly used in sorghum though it is easier to develop seed parents (male-steriles) with A2, A3, and A4 cytoplasms, than with A1.

There are different types of sorghum hybrids that can be developed using the CMS systems, depending on the expected output and the use to which the products would be put. These include: two-way F1  $(A \times B)$ , three-way F1  $[(A \times B) \times C]$ , and four-way or double-cross F1  $[(A \times B) \times (C \times D)]$  hybrids. Another form of hybrid is a top-cross hybrid, derived from pure line parent crossed unto a random-mating population for synthetic variety development. Commercial hybrids are mostly developed and produced using the single-cross F1 (A  $\times$  B). In a few instances, three-way cross F1  $[(A \times B) \times C]$  hybrids are still produced but are less productive than single cross hybrids; they can, however, be more stable and easier to produce in the field. Restorers (male parents) can be either improved varieties, pure breeding lines, or naturally existing germplasm accessions. Among the basic sorghum germplasms, studies have shown a general trend of  $\sim 20\%$  to be natural maintainers (B-lines), 5-8% natural restorers (R-lines), and the rest,  $\sim 70\%$ , segregated for male-fertility restoration (useful in developing new A-/B- parental lines for hybrid development).

#### **The Selection and Testing Process**

Several selection methods have been described based on expected progress and output products from selection, the skill, capacity, knowledge, and funding of the breeder. Selection methods already described in several books and published proceedings, and used for sorghum, a self-pollinated cereal crop, include:

- pure-line breeding and selection,
- individual plant selection (mass selection),
- single-seed descent selection,
- pedigree selection,
- modified pedigree selection, and
- bulk breeding and selection.

These methods usually follow a selected and advanced process in segregating generations derived from the F1 cross or backcross. Visual observations of selectable materials are used in the nursery, which should be arranged such that the two parents used in the initial cross or backcross are planted intermittently (in 1 or 2 rows) among several (6-10) rows of the segregating generations, for comparison and

ease of selection of desired recombinants. The pedigree selection method is most laborious with regard to detailed documentation of selected recombinants or derivatives from generation to generation and is mostly required in developing new male-sterile and maintainer hybrid parents. Individual plant selection is usually used in late filial generation stages (e.g., F4–F6) to purify selected new lines for purity and uniformity of desired traits. The best method to use depends on the program funding level, capabilities, experience, and knowledge about the traits being combined or managed, of the breeder.

With the identification and selection of desired recombinants, through a systematic generation advance process starting from among the F1 crosses, testing activities follow. Testing can be in the early generations when segregation is still operating, or in late generations when the lines and selections are almost pure. The best stage to carry out testing is still a debatable point, and depends on the breeder and the breeding program objectives. It is, however, necessary to understand the theory of selection among segregating populations of self-pollinated crops like sorghums and also the roles of genotype—environmental interaction.

Line, variety, or hybrid testing recognize and characterize the reaction of the test genotypes to environmental variation, which plays a significant role in determining the usefulness of a new improved genotype derived through cross-breeding. According to the generalized sorghum breeding scheme (Figure 3), a testing program begins with a preliminary replicated trial with hundreds of test entries, in one location. The testing progresses through intermediate trials (with less number of selected entries) in two to four locations, and finally to series of advanced trials and multilocational testing (now composed of few selected and elite lines, varieties or hybrids) in four to ten or twenty locations. The adaptation areas or production zones, which the new improved genotypes are meant for, would have been identified during the series of trials, from observations and recorded data.

Following a crossing program in the breeding nursery, a systematic and progressive selection system and testing program follows, for the identification and development of the most productive and adapted new lines, varieties, hybrid parents, and hybrids. Several selection methods have been described based on expected progress and output products from selection.

#### **Population Improvement and Recurrent Selection**

Random mating populations (RMPs) or composites of sorghum are developed and improved for variety of

reasons. Population improvement involves generation of broad-based gene pools (population development), improvement of the RMPs through recurrent selection, and utilization of the improved RMPs for line, variety, hybrid parent, and top-cross hybrid development. The cyclic selection and recombination process simultaneously improves several traits and is important in improving polygenic traits, resulting in better performance of the superior families derived hitherto. This breeding method is supplementary to classical or conventional breeding. The improvement of populations is appropriate where there is a long-term breeding strategy, and where the myriads of recombinants and possible line developments can be capitalized upon. It, however, requires working knowledge of population genetics, genetic statistics, and quantitative genetics for understanding the theory and nature of populations. This knowledge is also essential for the ability to choose and implement the improvement cycles and recurrent selection methods.

The development or synthesis of RMPs or composites in sorghum uses genetic male sterility that allows for segregation at each stage following intercrossing. The male-sterile genes used are mainly *Ms3* or *Ms7*.

The synthesis of new populations using Ms3 or Ms7 takes three or four random matings with or without selection. At each cycle of synthesis, sterile plants are identified and tagged before anthesis, so that they are harvested and bulked (in equal quantities of seed) for the next cycle of synthesis. ICRISAT, INTSOR-MIL universities, and national programs have described several methods for population synthesis and numerous sorghum populations have been developed employing these methods, with different traits/ trait combinations for different agro-ecological environments.

At the end of the third or fourth cycle of synthesis, the newly developed population is improved by recurrent selection. Recurrent selection is cyclic in nature and four main recurrent selection and two reciprocal recurrent selection methods have been described. These are S1 testing, S2 selection and testing, recurrent half-sib-selection, reciprocal recurrent selection (RRS), reciprocal full-sib, and reciprocal half-sib recurrent selection.

Both recurrent mass selection and S1 selection and testing have been most commonly used to improve sorghum populations or composites. The S2 selection is used in specific instances for multiple traits selection or to break difficult and tightly linked traits of concern. A three-stage S2 progeny testcross procedure is relatively new. This method combines full-sib selection with S1 testing and testcrossing to a desired tester. Reciprocal recurrent selection procedures use and enhance the heterosis between two populations.

It maximizes the genetic divergence between the two populations used in the improvement program focused on parental lines and hybrid development as end products.

Responses to and progress from selection in population improvement program depend on the base genetic diversity in the original (CO) population, the recurrent selection method used, the selection intensity/pressure exerted on the population cycles, the period of recurrent selection (C1, C3, or C5), type of gene action controlling the selected traits, the population size (number of plants carried forward) at each cycle of selection, and the experience of the breeder. Responses of 10-40% are common for grain and stover yields, while keeping other traits constant. These traits, kept constant normally, are associated with increases observed due to selection and testing.

#### **Seed Issues Related to Breeding**

The new lines, varieties, hybrid parents, hybrids, and populations selected are described after a series of testing and release, purified and multiplied for seeds which are provided to users. The issues of seed increase and multiplication (breeder seed) is the main job of the breeder at the end of breeding. The integrity, purity, and trueness-to-type of the product as described by the breeder must be delivered. In sorghum, of concern to the breeder are the critical maintenance of seed color, seed size and shape, glume color, panicle shape and size, plant color and height, and maturity. The production of next-stage foundation seed is a joint responsibility of the breeder, who described the material, and the seed company (small, farmer-level, medium, or large-scale), who will produce, in larger quantities, the seed provided by the breeder. The breeder need not be involved in producing certified seed for market and larger-scale accessing. The distinction between seed and grain must be understood. A seed is a reproductive organ having a living embryo and must be able to germinate. A grain is not a reproductive organ and does not have to germinate, but must be clean and containing described qualities. They are raw materials in food and feed processing but not in malting.

#### **Nonconventional Approaches**

#### **Biotechnology and Molecular Tools**

In recent times, more nonconventional breeding approaches have been used to improve sorghum. These include molecular breeding, biotechnological approaches, and farmer participatory plant breeding

(PPB). These three nonclassical breeding methodologies, which are more recent scientific and socioeconomic (especially PPB) tools, complement the classical breeding methods. Each of these has their specific uses, which can overlap in some instances in their complementarity with, and enhancing research in conventional breeding.

In sorghum, biotechnology tools are now being used in drought-resistance breeding by tagging quantitative trait loci (QTLs) associated with the different types of drought resistance (seedling, pre-flowering, and postflowering stages), for Striga resistance, and genetic mapping for linkage and genomic maps. They are also used for stem borer and midge resistance, grain quality improvement for increased protein, better digestibility, better processing, and incorporation of vitamin A (enhancing yellow endosperm sorghums) and micronutrients (especially Fe, Zn, and Ca); fodder and crop residue quality and digestibility by incorporation of bmr gene for brown midrib and stay-green trait gene. Newer biotechnological techniques have recently been used in developing alternative foods and industrial applications (as in using sorghum nondigestible protein character in developing biofilms for fruits and vegetable preservation for exports). These several methodologies have been described and recorded, especially for striga resistance and control, herbicide resistance, molecular and linkage mapping, population dynamics of striga, and biodiversity studies in wide crosses.

#### **Farmer Participatory Breeding**

The role of farmers and indigenous knowledge in plant breeding are combined with PPB becoming "farmer participation in plant breeding and selection (farmer participatory breeding - FPB)." This is a practical process of bringing together farming community knowledge and research capabilities with that of scientific research organizations in an interactive way. It involves breeding activities with shared responsibilities and benefits farmers and scientists/ breeders, in the characterization and description of indigenous germplasm, identification of useful parents with specific traits for a crossing program, selections, testing (on-station and on-farm), and in the use of new improved breeding materials. This approach contrasts with conventional breeding methods and tools in that the farmers involved are not treated as passive subjects, but rather as active partners. Some successes have been achieved using this FPB or PPB approach in eastern and southern African countries by Centro Internacional de Agricultura Tropical (CIAT) and ICRISAT for beans, sorghum, and millets.

#### **Production**

The world production of sorghum has ranged between 54.32 million tonnes (Mt) (in 1994) and 61.39 Mt (in 1998) and decreasing to 55.00 Mt in 2002. Table 2 shows the sorghum area and production across the globe in 1994. Relative to wheat, rice, maize, and barley, sorghum ranks fifth in importance, quantitatively, accounting for 5% of world cereal production. Africa, where sorghum is mostly used for food and beverages, was the largest producer in 1998 (20.1 Mt), followed by Asia (13.5 Mt) and North America (13.2 Mt), where it is mostly used for livestock feed. Cultivated sorghums are annuals adapted to the semiarid areas and drought prone subhumid agroecosystems, with ability of the roots to continue living after harvest to support ration crops (second and third rations possible from the same old roots), a common practice by farmers in some parts of eastern and southern African semiarid areas. Among the cultivated sorghums, there are three main types: (1) grain sorghum for food and feed, (2) forage sorghum for silage and crop residue (fodder) mainly for livestock feed, and (3) sweet sorghum with use in brown sugar, syrups, and alcohol production. Some of the weedy and wild sorghums together with their intermediates (mostly shatter cane) are used as grazing silage and hay.

#### **Agronomy and Protection Breeding**

There is a range of diseases and pests that constrain sorghum production. Sorghum diseases can be caused by bacteria, viruses, nematodes, and parasitic plants (witchweeds = Striga species). Bacterial diseases in sorghum are few (leaf stripe, leaf streak, and leaf spot) and cause minor yield losses. Fungal diseases are most common causing minor to very significant yield losses. They include:

- 1. foliar diseases leaf blight, leaf anthracnose, gray leaf spot, sooty stripe, zonate leaf spot, rust, downy mildew;
- 2. panicle and inflorescence diseases head anthracnose, smuts (head smut, loose kernel smut, covered kernel smut, and long smut), ergot, grain mold, head blight; and
- 3. root and stalk diseases Fusarium root and stalk rots, charcoal rot, Acremonium wilt, twisted top (pokkah boeng), and Pythium root rots.

Control of these diseases, which can be simply or complexly inherited with one or multiple genes, includes use of resistant varieties and integrated disease management with crop rotation and field hygiene. Depending on the inheritance gene action for the

Table 2 Sorghum area and production across the globe in 1994

Region/country	Area	(Mha)	Produc	tion (Mt)
Africa (30 countries)	21.80		17.10	
East and Central Africa (7 countries) <sup>a</sup>		9.15		7.11
Southern Africa (11 countries) <sup>b</sup>		1.60		1.27 <sup>b</sup>
West Africa (12 countries)		11.05		8.72
Asia	13.91		16.84	
India		12.55		11.23
China		1.36		5.61
America	4.90		20.38	
United States of America		4.05		17.50
Argentina		0.70		2.60
Brazil		0.15		0.28
World	40.61		54.32	

<sup>&</sup>lt;sup>a</sup>Includes Sudan.

disease, mass selection or backcrossing are most commonly used resistance breeding methods.

The common virus diseases include maize dwarf mosaic and sugarcane mosaic viruses. Root-knot nematodes and pratylenchus species which cause stunting are the common nematode diseases. The major parasitic weed in sorghum are Striga species (of economic significance are S. hermonthica, S. asiatica, and S. ferbesii. Integrated management including host plant resistance (HPR) seems to be succeeding for control of these set of diseases. Striga HPR is not yet completely successful. After ~60 years of developing resistant varieties combined with fertilizer and herbicide use, no sustained and high-level control with stable resistance has been achieved. Presently, maker-assisted selection with farmer involvement, and biotechnological tools (like restriction fragment length polymorphism (RFLPs) and QTLs) are being used to develop sustained resistance in sorghum and effective management of striga. It has and still is a scourge in African sorghum production (Figure 4).

There are large number of insects that attack sorghum. There are the soil insects, stem and head feeders and stored sorghum grain insects. Breeding for host plant resistance as control measures for insect pests has not been very successful. Avoidance and appropriate cultural practices (like timely planting, field hygiene, and use of non-toxic and cheap insecticides) have been the management for control measures used so far in sorghum. Presently however, biotechnological tools (like Bt gene) and biological control (using parasites of the insect pests) are being developed for use in sorghum insect pest control. In all, 21 soil

<sup>&</sup>lt;sup>b</sup>Includes Tanzania and South Africa that grow hybrid sorghums.

Period	Breeding strategy
1977–84	Bilateral conventional breeding with NARS in Sudan and Tanzania.
Phase 1	Focus: (i) Crossing and testing program using landraces as parents in sorghum improvement; (ii) Variety and hybrid development for Striga resistance and grain yield.
1984-93	Collaborative conventional breeding through networks (EARSAM, SMIP); regional breeding followed by national and regional testing.
Phase II	Main priorities: (i) Technology generation, i.e., germ plasm assembly and exchange, crosses, segregating generations, initial testing and evaluation for variety, population and line development; (ii) technology testing and utilization, i.e., on-station and on-farm trials, farmer participation in breeding and selection, focus on earliness, yield, disease and pest tolerance/resistance and dual-purpose varieties; (iii) capacity building of NARS breeding.
	Secondary priorities: (i) Grain-quality screening and evaluation; (ii) beginning technology transfer and exchange with producers/farmer groups and extension, for national variety releases and seed production.
1993-2002	Rationalization of breeding objectives from conventional approaches in first 20 years, to impact-oriented breeding.
Phase III	Main priorities:
	<ul> <li>(i) Technology exchange:</li> <li>on-farm trials and on-farm verification;</li> <li>farmer-participatory breeding;</li> <li>release of farmer-acceptable varieties;</li> <li>breeder and foundation seed production involving private seed companies;</li> <li>training of farmer groups in seed production.</li> </ul>
	<ul> <li>(ii) Target technology generation:</li> <li>breeding for increasing productivity through development of higher yielding varieties, hybrids, and hybrid parents;</li> <li>incorporating end-user perspectives in breeding programs;</li> <li>regional breeding and testing;</li> <li>continued variety releases;</li> <li>spillovers across countries.</li> </ul>
	<ul> <li>(iii) Commercialization and impact: <ul> <li>linking producers with processors through provision of cultivars with known and use qualities;</li> <li>documenting, disseminating and modifying, processing and utilization technologies with improved cultivars for food, livestock feed, malting;</li> <li>expanding linkages and partnerships with private sector (millers, maltsters) and farmers' groups, for pilot testing of end use qualities;</li> <li>linking/identifying improved or released varieties with market products;</li> <li>closer collaboration between breeders, economists and processors/private sector seed companies;</li> <li>seed systems and improving farmers' access to improved technologies.</li> </ul> </li> </ul>
	Secondary priorities:
	<ul><li>(i) promotion of improved varieties and hybrids for food products, use in industry and livestock, through breeders' involvement in pilot product development and testing with small- and large-scale commercial farmers and farmer groups;</li><li>(ii) Expanded linkages with food technologists, processing industries, economists, NGOs, and ARIs for processing and utilization and market systems.</li></ul>

Figure 4 Sorghum research in eastern and southern Africa: an example of evolution of breeding strategies as shown in ICRISAT.

insects, 22 stem and head (panicle feeders), 8 stored sorghum grain insects, and 5 predators of sorghum insect pests, have been identified and described.

#### **Breeding Sorghum for Specific End Uses**

There is ample natural genetic variation in sorghum for several adaptive, productivity, survival value, and

end-use traits. These - including storage; mechanical and chemical processing (de-hulling, milling, malting, popping, fermenting, brewing, baking, extruding); carbohydrate, fiber, protein, oil, polyphenols contents; food and livestock feed use - can be improved and selected for, through breeding. Methodologies, including conventional and nonconventional, can be used to screen, evaluate, and enhance the quantity and quality of these normal end use and also novel traits.

**Table 3** Range of grain qualities in different sorghum types based on grain colour; usable in defining limits for end uses in industry (cottage or large scale)

Grain trait	Range of values		
	White sorghum	Red sorghum	Brown sorghum
Testa	Absent	Absent	Present
Hardness score <sup>a</sup>	2.6-4.8	1.7-4.7	1.4-3.8
Flour yield (%)	72.60-90.82	69.23-88.20	64.20-86.20
Water absorption (%)	3.8-11.8	4.2-13.1	5.1-14.8
Flour color: (Agtrons) <sup>b</sup>			
Dry Agtron reading	68.2-82.5	59.5-76.8	50.7-72.1
Wet Agtron reading	48.8-63.6	32.2-55.4	24.4-48.8
Malting quality (SDU values)	14.68-73.34	15.90-72.62	28.28-74.17
Tannin content (% ce)	0	0-0.5	0.5-5.0
Crude protein (%)	10.9	10.9	10.9
Popping quality	Very good	Very good	Poor
Visual hardness	2.4-3.0	3.0-3.4	1.0-2.5 <sup>c</sup>
Grain size <sup>d</sup>	Medium-large	Medium-large	

<sup>&</sup>lt;sup>a</sup> Hardness score on a 1–5 scale where 1.0–2.5 = soft, 2.6–3.4 = intermediate, 3.5–4.5 = hard, and 4.6–5.0 = very hard.

Table 4 Range of whole plant qualities in different improved sorghum types for silage (Animal feed) in drought prone and adverse environments

Traits	Silage use		
	Dairy cattle	Beef cattle	
Plant type	Tall, long season, bulky, and tillering; purple or tan color	Semidwarf, short to medium season, bulky or tillering, tan plant color	
Green biomass	High (70–120 t ha <sup>-1</sup> )	Medium to low $(60-40 \text{ t ha}^{-1})$	
Dry matter (DM)%	Low to medium (28-35)	Medium to high (35-55)	
Metabolizable energy (ME)	9-11 MJ	11–13 MJ	
Convertible protein (CP)	5.0-8.0%	5.0-7.0%	
Crude fiber (CF)	25-30%	22-35%	
Examples of improved cultivars with	PATO	PHOFU/MACIA	
the desirable traits combination	SDS 2690-2	Town	

The evaluation of grain quality of improved sorghum cultivars for several end uses, should be a complementary activity of breeding programs. Thus, while breeding improved genotypes for adaptation and productivity, their grain qualities together with those germplasm accessions or genetic stock used in their improvement, should also be assessed for processing and utilization technologies. Simplified methodologies for grain and product quality evaluation of sorghum has been described. These include qualitative and quantitative methods, chemical analyses, product preparation, and testing. One such database was generated on 2500 genotypes (including improved and farmer varieties) for 14 grain quality traits, analyzed in a period of 2-6 years. Such databases have been used to characterize and classify sorghum genotypes for what end uses, develop grading systems and quality standard for sorghum as raw

materials in industry. This complementary breeding activity has been found to enhance and increase adoption rates for sorghum varieties and hybrids and generated impact in farmers' fields and industry.

Tables 3 and 4 show the ranges for grain qualities in different sorghum types based on grain color, and for whole plant qualities in different sorghum types for silage in adverse environments, respectively. These are examples from mostly African sorghums that define limits for some end use qualities for product development and sorghum commercialization.

See also: Animal Feed. Cereals: Overview. Cultural Differences in Processing and Consumption. Nutrition: Effects of Food Processing. Sorghum: Harvest, Storage, and Transport; Utilization. Taxonomic Classification of Grain Species.

<sup>&</sup>lt;sup>b</sup>The higher the reading, the lighter is the product color.

<sup>&</sup>lt;sup>c</sup>Brown sorghums do not pop well as the grains are too soft.

<sup>&</sup>lt;sup>d</sup>Grain size: large = grains > 4.00 mm, medium = grains 4.00-2.60 mm, small = grains < 2.60 mm.

#### **Further Reading**

Allard RW (1966) Principles of Plant Breeding. Sydney: Wilev.

Cooper M and Hammer GL (1996) Plant Adaptation and Crop Improvement. UK: CAN International with IRIR and ICRISAT.

Doggett H (1988) Sorghum, 2nd edn. Tropical Agriculture Series, UK ed USA: Longman Scientific and Technical.

Gomez MI, Obilana AB, Martin DF, Madzvanuse M, and Monyo ES (1997) Manual of Laboratory Procedures for Quality Evaluation of Sorghum and Pearl Millet. Technical Manual no. 2. Patancheru, India: ICRISAT.

House R (1985) A Guide to Sorghum Breeding, 2nd edn. AP, India: International Crops Research Institute for the Semi-Arid Tropics.

IBPGR and ICRISAT (1993) Descriptors for Sorghum (Sorghum bicolor (L.) Moench, 38pp. Rome, Italy and Patancheru, India: International Board for Plant genetic Resources, and International Crops Research Institute for the Semi-Arid Tropics.

INTSORMIL and ICRISAT (1997) Proceedings of the International Conference on Genetic Improvement of Sorghum and Pearl Millet. Texas, USA and Patancheru, India: INTSORMIL and International Crops Research Institute for the Semi-Arid Tropics.

Musselmann LJ (1987) Parasitic Weeds in Agriculture, Vol. 1, Striga. Boca Raton, Florida: CRC Press.

Obilana AB (2003) Sorghum breeding research in Africa. In: Sorghum Genetic Enhancement: Research Process, Dissemination and Impacts. Patancheru, India: ICRISAT.

Van Der Plant JE (1968) Disease Resistance in Plants. London: Academic Press.

Wricke G and Weber WE (1986) Quantitative Genetics and Selection in Plant Breeding. New York: Walter de Gruyter and Co.

Witcombe JR and Duncan RR (1994) Use of Molecular Markers in Sorghum and Pearl Millet Breeding for Developing Countries. Bangor, UK: Overseas Development Administration.

### Harvest, Storage, and **Transport**

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#### Introduction

Sorghum, Sorghum bicolor (L.) Moench, is an important food and feed crop in developing and developed countries respectively. Sorghum, the fifth most important cereal after rice, wheat, maize, and barley, is generally grown in marginal areas where other cereal crops would normally fail. It is, therefore, critical for food security in the semiarid tropics of Africa, Asia, and Latin America. Strides have been made in developing improved technologies (management practices and varieties) for farmers but average grain yields have remained low in developing countries. Harvesting is carried out manually and mechanically by smallholder and commercial farmers respectively. While a number of technologies exist to increase production of sorghum grain, the crop suffers damage and weight losses during storage to ravages of insect pests. It is difficult to quantify the losses due to handling and storage, especially in the developing countries, but most literature on the subject cite up to 20%. Therefore, the issue of inadequate storage is paramount. There is need to preserve the quality by preventing insect pests from getting into the grain. Farmers use various ways to store the grain. Both traditional and improved storage structures have their advantages and disadvantages. Improved grain storage techniques are an improvement on the inadequacies found in the traditional methods so that the stored grain does not deteriorate in quality. Therefore, the primary aim of grain storage is to prevent crop and monetary losses resulting from various agents such as rain, insect, fungi, and rodents by maintaining the quality and quantity of the grains from the beginning of storage up until it is consumed or sold. Transport of sorghum is restricted in developing countries as the crop rarely reaches the market. The crop is usually grown by smallholder farmers, largely for domestic consumption. Commercial producers use trucks, rail, and barge for grain movements to domestic and foreign markets.

#### **Harvesting of Sorghum**

The total sorghum area harvested worldwide in 2002 was 45 566 239 ha, 55% of which was harvested in Africa (Figure 1). India had the largest area harvested (22% of total) among all countries. Thus, developing countries have the greatest sorghum area harvested and harvest practices vary. Timely harvest is important to preserve grain quality and reduce mold damage, bird damage, insect pest infestation, and loss due to bad weather conditions. Traditionally, sorghum grains are harvested during the beginning of the dry season when the grain moisture content is low  $(\sim 15-20\%)$ . It is critical for farmers to know when to harvest the grain to minimize losses. Grain should be harvested when it has become physiologically

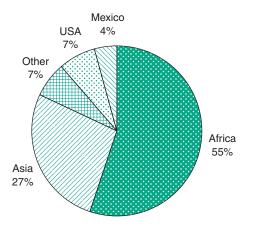


Figure 1 Sorghum area harvested (ha) in Africa, Asia, USA, Mexico, and other in 2002. (Source: FAOSTAT 2003.)



**Figure 2** Manual harvesting of sorghum in Zimbabwe. The panicles are cut from the stalk when the grain moisture content is  $\sim 15-20\%$ .

mature. Three indicators of maturity include the following:

- 1. a black layer forms at the tip of mature grains,
- 2. mature grain cannot be indented by the thumb nail, and
- mature grain breaks cleanly when bitten with the front teeth.

Premature harvesting results in reduced grain yields while grains with lower starch, protein, and sugar content are obtained when harvesting is delayed.

In developing countries, most of the harvesting is done manually and in a few cases mechanically by combine harvesters. Smallholder and subsistence farmers harvest sorghum by cutting the heads with knives or sickles (Figure 2). Other variations in harvesting are dependent on the use of the other parts of the plant and include cutting the plants with their heads on or digging the whole plant from the roots.

Sorghum stalks remaining can be used as green fodder or cut and stored as hay. The roots and stalks can be used for fuel.

In China, mechanical harvesters are designed for long- and short-stalked sorghum. Most sorghum grown in the USA and other developed countries is combine harvested. Dwarf varieties are suitable for harvest by combine. Harvesting speeds  $(4-5 \text{ km h}^{-1})$ are set to minimize combine header losses. The combine is adjusted in such a way that all grain is threshed and separated from the heads with minimum cracking and as little trash as possible is left in the grain. The grains are normally allowed to dry in the field, to allow reduction of the moisture content of 13% or less if they are going to be stored after harvesting. Varietal differences exist in suitability for harvesting with some types maintaining high moisture levels until the plants are killed by frost. Other types have a genetically conditioned head-drying character resulting in rapid drying of the grain and head after maturity. Combine harvesting can also be conducted when levels of grain moisture contents are at 20-25%, when this is followed by ensiling or artificial drying to a safe storage moisture. Where lodging from storm damage, insects, or diseases presents harvesting problems, special types of combine attachments are available to aid in harvesting the lodged fields. If lodging is excessive, the crop can be cut with a header and the heads swathed to dry before being picked up and threshed by a combine equipped with pickup attachment. Livestock are used to harvest sorghum directly as a last resort if lodging is severe and yields are low. Sorghum heads can be harvested and stored as head silage in some very humid areas where grains remain highly moist and losses to birds and weather can be substantial. The silage stores well at 25–40% moisture if it is finely cut. The grain in the head silage requires no further processing, as it is highly palatable and digestible. The stubble remaining after sorghum grain has been harvested is used as low-quality forage for fall grazing.

## Drying, Threshing, and Cleaning of Sorghum Grain

Sorghum panicles should be thoroughly dried before threshing. Excess moisture is undesirable as it may lead to infestation by insects and fungi, and deterioration of quality. Partially dried heads are also difficult to thresh and too dry heads usually result in huge losses in the field while harvesting. Optimal moisture content is 10–12% for grain and 9–10% for seed. Panicles are either left in the field or transported to storage yards where the grain is left to dry. Grains are



**Figure 3** Sorghum panicles are threshed to release the grains by beating the heads with sticks. Time required for threshing depends on variety and degree of dryness of the grain.



**Figure 4** Grain is cleaned by winnowing so that the light chaff is carried by wind.

dried further, if necessary, after threshing, using natural and mechanical drying practices. Dryers are used commercially. Drying temperatures are limited to ~43°C and 60°C for seed and feed grain, respectively. Airflow rates of 11–28 m³ min<sup>-1</sup> t<sup>-1</sup> are common for feed grain. Sorghum can be dried successfully at up to 94°C and airflows of 110–220 m³ min<sup>-1</sup> t<sup>-1</sup> using batch or continuous flow dryers.

Smallholder and subsistence farmers thresh heads either manually, by beating with sticks (Figure 3), or by using livestock to walk on the layers (~25–30 cm thick) of sorghum heads. The latter method is popular in China where animals pull a stone-roller over sorghum heads. A tractor or any other vehicle can also be driven over the heap of harvested and dried panicles. In all cases, the heads should be turned and inspected so that the action is repeated until all grains are released. Hand-harvested heads can be threshed by a stationary combine. Mechanical threshers are available that both commercial and smallholder farmers can afford. During threshing, care should be exercised to avoid physical damage to the grain. Damaged grains will deteriorate quickly in storage.

After threshing, the grain is cleaned to remove chaff (glumes, broken grain, very light seeds, and any grasses). Among smallholder farmers, women use woven trays to winnow the chaff from the grain. Alternatively, the chaff is removed by dropping the grain in a bucket against the wind (Figure 4). Chaff is also removed from the grain by sifting through a screen or sieve. The grains fall through and the chaff remains. Vibrating screens comprising of a feed hopper, sieves, dust remover, cleaner, and drive equipment are used at a commercial level. Pneumatic cleaning equipment is also available. The principle of pneumatic cleaning is to separate impurities from the grain based upon differences in specific gravities.

#### **Treatment before Storage**

There are several chemicals that are used commercially to treat the grain before storing it. Some organophosphates include pirimiphos-methyl (Actellic), malathion, fenitrothion, and iodophenphos. Traditionally, sorghum grain is treated with wood ash, sand, and mineral powders to prevent insect damage in developing countries. Botanical insecticides such as neem (Azadirachta indica) and pyrethrum (Chrysantheum cinnerariafolium) are also applied before grain is stored for a short-term period.

#### **Storage of Grain**

The objective of storage is to preserve as much as possible the value of the grain for its intended future use. Either the food value of the grain is preserved or a high proportion of viable seeds are retained for planting next season. Some attributes such as a hard endosperm found in some traditional varieties, contribute to reduced pest attack during storage. On the other hand, improved varieties mature early and generally have a soft endosperm, thus, making them more prone to pest attack. Factors leading to loss of viability and nutrients include depredations by pests (insects, birds, and rodents) and also mold damage. Germination of grain causes losses on a smaller scale. Physical factors contributing to losses include moisture content of the grain and the temperature of storage. Losses increase as moisture content and temperature of the grain increase. Minor changes in temperature during grain storage lead to moisture migration and accumulation in certain areas that are cooler than the rest. This often allows microbiological activity to occur in comparatively dry grain, which in turn leads to heat production. Moist areas

in unventilated areas can get so hot that charring can occur resulting in grain destruction. Moisture migration can be prevented by forcing low volumes of air  $(0.11-0.27\,\mathrm{m^3\,min^{-1}\,Mg^{-1}})$  through grain held in silos or tanks. Aeration fans can also be installed to draw cold air down the grain while moving the warm moist air to the outside. Regular inspection of the grain is essential to detect moisture changes, insects, and spoilage during storage.

Methods for storing grain are generally influenced by the value of the crop, quantity stored, and environmental conditions. Grain augers are used commercially to place grain in metal bins. Storage bins are best filled early in the day when air is cool and humidity is often at its lowest. Grain should be packed as tightly as possible to minimize space for insects to move around and to breed. In some developing countries, sand is mixed with grain to further reduce the free space. Very often smallholder farmers store seed without treatment. This often results in the seed getting badly damaged by insects before planting. Table 1 lists the common methods of grain storage based on tradition, cost, and convenience.

#### **Containers for Storage**

Silos, bins, baskets, and other storage containers are made from a number of different materials (**Table 1**). There is not much need for bulk storage for smallholder and subsistence farmers in developing countries. Clay pots are used on the smallest scale. Larger containers are constructed from wood, brick, or stone, or from bamboo made into a basket that is then sealed with mud or dung. Containers can sometimes be left uncovered if kept indoors or covered with

Table 1 Common methods used for sorghum grain storage

Method of storage	Quantities	Construction materials	Cost	Dis- advantages
Bulk silos	Large	Steel, aluminum, concrete	High	Costly to operate
Drums and bins	Medium	Metal	Medium	Poor ventilation
Pots	Small	Clay	Low	Poor ventilation
Pits	Large	Brick, cement, mud walls	Low to medium	Prone to mold growth
Baskets	Medium to large	Stalks, mud, cow dung	Low	Prone to pest damage
Bags	Small to medium	Cloth, sack, jute	Low to medium	Prone to pest damage

either a lid or a thatched roof if kept outdoors. Grain for immediate consumption is usually stored in the house in metal or plastic bins. However, grain can also be packed in suitable material (jute bags, cloth bags, cardboard cartons) for short and long-term storage. The grain is taken out of the bags and dried in the sun by spreading on the floor or on a sheet at regular intervals during the dry season. Exposing grain to the sun and heat at intervals keeps away storage pests. Occasionally, sorghum heads are stored on the ground; usually unthreshed. Panicles from the heaped pile are removed and threshed when grain is needed. It is generally not recommended to store unthreshed grain on the heads.

#### **Storage Structures**

The idea of storage structures is to prevent insect pests and rodents from attacking the grain. Storage structures are many and are varied in the way they are constructed. Bulk silos of heights of 30-50 m are used commercially for centralized storage. In many countries, small granaries are made by weaving plant materials, such as bamboo, stalks, bark, and small branches, and then sealing any gaps with mud or dung. These structures can either be built directly on the ground or raised off the ground on platforms or stilts (Figure 5). The store should be dry, with low humidity. The bags must be stacked on wooden pallets so that bags do not touch the ground. The structures could either be raised on a platform or built on the ground. Traditional storage structures vary according to regions and countries. For example, in southern Africa, a thatched roof is usually placed on top of a granary (Figure 6). In Nigeria, sorghum is stored as unthreshed heads in a solid walled container called a "rumbu." Heads are laid out individually for long-term storage (3-6 years) or in bundle layers for short-term storage (< 3 years). When filled, the rumbu is sealed with clay. In Sudan, pits holding 2-5 t of



**Figure 5** Sorghum panicles are dried while being kept off the ground on raised platforms.

grain are used as underground stores. In India, underground pits may be located underneath the houses or outside. The pit is lined with paddy straw or sorghum straw and then covered with straw and soil when it is full of grain. The top is plastered over with mud for longer storage.

#### **Storage Pests**

Insects are the most serious pests of stored products and can reduce the amount of grain harvested substantially. A good understanding of the important types of insects and their behavior will significantly help in their control. The important insect pests of stored products are either beetles or moths. These insects can be divided into two groups namely, primary and secondary insects. Primary insects are those that are able to attack undamaged grain cereals such as the Angoumois grain moth (Sitotroga cerealella), large grain borer (Prostephanus truncatus), and weevils (Sitophilus spp.). Secondary pests such as flour moths and flour beetles (Tribolium spp.) can only attack stored commodities, which have previously been damaged by either primary pests or through processing (shelling or milling).



Figure 6 Example of granary used for storing sorghum in Zambia. Smallholder farmers store grain threshed or unthreshed.

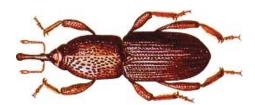


Figure 7 The rice weevil (S. oryzae) infects sorghum grain at moisture contents ranging from 10% to 16%. Soft endosperm types are less resistant than sorghums with hard endosperms.

For sorghum, the most important pests are the rice weevil (Sitophilus oryzae) (Figure 7) and grain moth (S. cerealella) (Figure 8). Infestation by these pests often starts when the crop is drying in the field, but there can also be carry-over from storage. Recently, the larger grain borer (Prostephanus truncatus) has been reported spreading into sorghum-growing areas in the southern African region (Figure 9).



Figure 8 The Angoumois grain moth (S. cerealella) attacks sorghum in the field. It multiplies rapidly if sorghum is stored unthreshed.



Figure 9 The larger grain borer (P. truncatus) has also been reported as a storage pest of sorghum.

#### Weevils (Rice Weevil - S. oryzae)

Both adults and larvae feed on grain, damaging it beyond use. The adult female bores a hole in the kernel and deposits a single egg. A female can lay 300–500 eggs. The egg hatches into a legless white larva (grub) after 3 days. The larva becomes mature in 3–6 days and adults can live for 5–6 months. In most cases, eggs are carried on grain harvested from the field.

#### Moths (Angoumois Grain Moth - S. cerealella)

Angoumois grain moth infestation can begin in the field. Attack in storage is confined to the upper layer of the grain and surfaces of bags containing the grain. The larvae bore into grain, where they feed, pupate, and finally emerge as adults, leaving round holes in the grain. The infested grain is completely hollowed out and filled with larval excreta and webbing. The larval period is 2–3 weeks and the pupal period 1 week. The adult moth does not eat the grain.

#### **Control of Insect Damage**

Insect damage can be greatly reduced by preventive action. The following practices can limit the initial infestation of the stored grain.

- 1. To avoid infestation of the new crop, the old grain must be removed preferably before the new crop is mature, but certainly before the mature crop is brought to the homestead. Old grain from the previous season is often a major source of insects.
- 2. The new crop must be rapidly dried to attain safe moisture content levels and threshed as soon as it is dry. The grain is then treated and put into store before any significant damage has occurred.
- 3. Before the new crop is brought in, the storage structure and its surrounding should be well cleaned and maintained.
- 4. The product going into the store should be: (a) well dried, (b) well cleaned, and (c) undamaged (sound). Any damaged grain should be removed and consumed first.
- 5. The storage structure should have no entry points at all times to prevent re-infestation with insects.
- 6. Adequate system of inspecting the grain throughout the storage period should be put in place.

If any pests or damage is detected, immediate action should be taken through the following exercises: (1) repairing any damage to the storage structure if necessary, (2) cleaning and drying the grain either by sunning or kilning, (3) treating the grain again with a suitable insecticide such as pirimiphosmethyl (Actellic) or pyrethrum dust, or with wood ash if necessary.

#### Insecticides

Insecticides provide a good protection against insects in storage due to their residual effects. There are several dust insecticides on the market that are recommended for use to control insects in stored grain. In southern Africa, commonly used dust insecticides include Actellic super dust, Chirindamatura Actellic super dust, and Shumba super at application rates of 25 g per 50 kg of threshed sorghum. These are most effective when they are admixed with threshed grain. Aluminum phosphide or phosphine, also known under trade names such as phostoxin, phosphume, phostek, is used for indoor fumigation of grain as a means of controlling storage insects. It is strongly recommended by the Food and Agriculture Organization of the United Nations as it leaves no toxic residues. According to the US Environmental Protection Agency (EPA), tolerances are established at 0.1 ppm for residues of the insecticide phosphine on sorghum grain resulting from postharvest fumigation. In Botswana, phostoxin is the most commonly used fumigant.

Another common practice among farmers is to store the grain with neem leaves. Neem (A. indica) contains pesticidal ingredients for protection of the plant from a multitude of pests. The ingredients can affect more than 200 insect species as well as some mites, nematodes, fungi, bacteria, and even a few viruses. Neem substances have protected stored sorghum and other foods against pests for up to 10 months in some very sophisticated controlled experiments and field trials. The main compounds belong to a general class of natural products called triterpenes or limonoids. Azadirachtin, salannin, meliantriol, and nimbin are the best known among neem limonoids. Azadirachtin repels and disrupts the growth and reproduction of insects; salannin, and meliantriol both inhibit insect feeding; and nimbin manifests antiviral activity. Neem leaves contain other ingredients shown to disrupt the fungi that produce aflatoxin on moldy cereals, legumes, and other foods. Although the fungi remain alive, it switches off their ability to produce aflatoxin, the most powerful carcinogen known.

#### Selection of a Storage Structure

To store grain properly and successfully the storage structure must provide an environment, which prevents grain losses and maintains the grain in good condition until it is consumed or sold. Therefore, the storage structure must be able to:

1. keep the grain dry – the grain must be protected against rain and ground moisture. An increase in

- moisture content encourages germination, mold, and insect development;
- 2. protect the grain against pests a well-constructed storage structure should provide a barrier against rodents and insect re-infestation;
- 3. keep the grain temperature low a low and stable temperature will reduce insect and mold development. Uneven temperatures cause moisture migration in storage; and
- 4. facilitate easy cleaning and inspection grain stored in a clean and tidy storage structure is less prone to deterioration.

Smallholder farmers can use better storage structures such as the improved mud-plastered grain bin for on-farm grain storage.

#### **Fungal Contamination of Stored Sorghum**

Mycotoxins are poisonous chemicals produced by the growth of fungi on grain, oilseeds, and other materials. Mycotoxigenic fungi such as Aspergillus, Alternaria, Fusarium, Curvularia, and Phoma, and mycotoxins such as alternariol, alternariol monomethylether, patulin, trichothecenes, zearalenone, altenuene, altertoxin I, aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>, and T-2 toxin have been reported in sorghum under certain environmental conditions. Storage fungi of the genera Aspergillus and Penicillum can be produced on grain stored with moisture content greater than 13%. Aflatoxin produced by Aspergillus flavus in sorghum is the most carcinogenic of the known mold metabolites. For example, pigs can tolerate only 0.23 ppm in their feed. Aflatoxin has been shown to be hepatotoxic, carcinogenic, mutagenic, and teratogenic. Proper drying and storage would greatly prevent the contamination of food grains with these mycotoxin-producing fungi. Failure to dry grain or prevent accumulation of moisture through condensation favors the growth of molds. Under conditions of 30-40°C, aflatoxin can be produced within 2-6 weeks, and signs of mycotoxicosis of pigs may be noticed within a week of it being introduced into the diet. The total quantity of the food to be consumed and the animal (including humans) for which it is intended will both influence what is considered a "safe" tolerance limit. Young infants are more sensitive than healthy adults. Many grain-importing countries enforce regulations on mycotoxin levels set by the Codex Alimentarius Commission. To avoid or minimize grain contamination by mycotoxins, proper agronomic, storage, handling, and inspection practices should be implemented.

#### **Transport**

Transport of grain is an important component and is varied. An efficient transport mechanism is needed to get the produce from the field to storage warehouses and ultimately to markets. Compared to other cereal crops, sorghum is not widely traded internationally; and within those developing countries where they are grown for human food, there is usually a balance between local production and local demand. Smallholder farmers use oxen-drawn carts or trailers to transport farm requisites and farm produce. Donkeys are also widely used as well. Sorghum heads are harvested in the field and placed on a cart to the threshing area. In many cases, women transport the harvest in baskets on their heads. The produce can also be taken to markets. Intermediate and other larger farmers use either tractors or trucks to get their produce to storage areas and markets.

In developed countries, considerable movement occurs not only within surplus grain areas but into export channels and into feed-deficit areas. Sorghum for animal feed is transported mostly by rail or truck. The former is considered the best for distant shipments whereas the motor truck is used for short shipments. The shipper's choice on both long and short hauls is based upon relative transport rates, services performed, and other costs and convenience factors. The nature of operation is reflected by the ratio of truck to rail movement. Country elevators generally have shorter distance and smaller quantity per haul. Although total shipments are small, since most of their receipts are processed, processing plants are next in distance and quantity. Terminal elevator operations generally have the longest hauls and the largest quantity per haul. Both distance and quantity for each type of handler are generally less for receipts than for shipments. Domestic shipments are also done to a lesser extent by barge. Barge shipment is primarily used for sorghum export. The USA is the largest exporter of sorghum. Argentina and Australia also produce sorghum meant for the domestic and export market. Most sorghum is exported to feed grain-deficit areas and Japan, Mexico, the former USSR, and Venezuela are the main importers. Global standards for sorghum grain trading are specified under the Codex Alimentarius Commission under Codex Standard 172-1989.

#### **Future Prospects**

Sorghum will remain an important global food and feed crop. The largest group of producers, the smallholder and subsistence farmers, can play a greater role in world sorghum production and marketing

provided they have reasonable access to production inputs, improved harvest techniques, better storage facilities, and transport mechanisms for their produce. Sorghum is poised to compete equally with rice, wheat, and maize given its yield potential and the area currently under cultivation. However, inputs similar to those provided for rice, wheat, and maize on genetics, production, and postharvest research are still to be realized.

See also: Animal Feed. Cereals: Overview. Cultural Differences in Processing and Consumption. Grain Crops, Overview. Grain Production and Consumption: Africa; Asia. Sorghum: Breeding and Agronomy; Utilization. Stored Grain: Handling from Farm to Storage Terminal; Invertebrate Pests.

#### **Further Reading**

- Bennett WF, Tucker BB, and Maunder ABM (1990) Harvesting, marketing, and utilization. Modern Grain Sorghum Production, pp. 144-153. Ames, Iowa: Iowa State University Press.
- Chung DS and Devoe CW (1982) Grain Postharvest Technology for Developing Countries. Sorghum in the Eighties, The Proceedings of the International Symposium on Sorghum, vol. 2, pp. 527–532. Patancheru, AP: ICRISAT.
- Davey PM (1965) The susceptibility of sorghum to attack by the weevil Sitotroga oryzea (L). Bulletin of Entomology Research 56: 287–297.
- De Lucia M and Assennato D (1994) Post-harvest Operations and Management of Food Grains. FAO Agricultural Services Bulletin, No. 93. Rome: Food and Agriculture Organization of the United Nations.
- Doggett H (1988) Sorghum, 2nd edn., pp. 329-341. Harlow, England: Longman.
- Hammond C (1987) Grain Sorghum: Harvesting, Drying and Storing, pp. 1-8. Athens: Cooperative Extension Service, University of Georgia, College of Agriculture.
- Hill DS (1987) Agricultural Insect Pests of the Tropics and their Control, 746pp. Cambridge: Cambridge University Press.
- Kramer NW and Ross WM (1970) Production of sorghum. In: Wall JS and Ross WM (eds.) Sorghum Production and Utilization, pp. 167-287. Westport, Connecticut:
- Leuschner K (1990) Sorghum and Millet Entomology in the SADCC Region 1989/90. The Proceedings of the Seventh Regional Workshop on Sorghum and Millets for Southern Africa, 17-21 Sept. 1990, Manzini, Swaziland, pp. 71-84. SADC/ICRISAT, Bulawayo.
- McFarlane JA, John AE, and Marder RC (1995) Storage of sorghum and millets including drying for storage, with particular reference to tropical areas and the mycotoxin problem. In: Dendy DAV (ed.) Sorghum and Millets: Chemistry and Technology, pp. 169-183. St. Paul, MN: American Association of Cereal Chemists.

- National Research Council (1996) Grains. The Lost Crops of Africa, vol. 1, pp. 285-296. Washington, DC: National Academy Press.
- Proctor DL (1994) Grain Storage Techniques: Evolution and Trends in Developing Countries. FAO Agricultural Services Bulletin, No. 109. Rome: Food and Agriculture Organization of the United Nations.
- Rukmini C and Bhat RV (1978) Occurrence of T-2 toxin in Fusarium infected sorghum from India. Journal of Agricultural and Food Chemistry 26: 647-649.
- Scott PM (1973) Mycotoxins in stored grain, feeds, and other cereal products. In: Sinha RN and Muir WE (eds.) Grain Storage: Part of a System, pp. 343-365. Westport CI: AVI.
- Wongo LE and Pedersen JR (1990) Effect of threshing different sorghum cultivars on Sitotroga cerealella (Oliv.) and Sitophilus oryzae (L.) (Lepidoptera:Gelechiidae and Coleoptera:Curculionidae). Journal of Stored *Products Research* 26: 89–96.

#### Relevant Websites

http://www.fao.org - The website gives information on postharvest operations of cereals.

http://www.ams.usda.gov - This website gives information on transportation of grain including sorghum by various transport modes.

#### Utilization

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#### Introduction

Sorghum is utilized as food, feed, and industrial products around the world. About 30-40% of the 62 million metric tons (Mt) produced annually is used in a wide variety of traditional foods mainly in Africa, Asia, and Central America. Industrial production of beer, nonalcoholic beverages (see Fermentation: Foods and Nonalcoholic Beverages), and porridges (see Cultural Differences in Processing and Consumption. Maize: Foods from Maize) occurs in Africa, especially Nigeria, South Africa, and Botswana. Small quantities of sorghum are used for foods in the western hemisphere and in Japan. Production, plant characteristics, and related information on sorghum were presented in Sorghum: Breeding and Agronomy, and Harvest, Storage, and Transport.

The major use for sorghum is as livestock feeds for all species of animals. Sorghum is processed, to improve its feed efficiency, into a wide variety of feeds ranging from floating fish food to steam flakes for ruminants in large feedlots. Sweet sorghums are preferred for syrup and for forage. The forage (fodder) may be more valuable than the grain. The stalks are used for dry fodder for ruminants and some sorghums are grazed, since the stalk remains green after grain maturation. Most of the comments in this article are on utilization of sorghum grain, but forage utilization is also important in drier areas of the world.

#### **Grading and Classification**

Sorghum is marketed according to US grain standards in four classes: sorghum, white sorghum, tannin sorghum, and mixed sorghum. The sorghum class cannot contain more than 3% sorghum with a pigmented testa (undercoat). Tannin sorghums have a pigmented testa beneath the pericarp. The pigmented testa is seen as a dark layer between the light endosperm and the pericarp when the caryopsis is scraped to remove the pericarp. Bleaching using the chlorox bleach test causes the constituents in the pericarp and testa to oxidize and gives a pronounced black color to the bleached kernels while nontannin sorghums have a white appearance. The white class contains sorghum with a white pericarp without a pigmented testa and cannot contain more than 2% of sorghum with pigmented testa or colored pericarp. Mixed sorghum contains a blend of kernels with and without pigmented testa. The US also markets "Food-Grade" white sorghum, a white kernel with tan plant and glume characteristics. The amounts of anthocyanin pigments that darken these grains are less than those from grain with purple or red glumes.

#### **Appearance and Genetics of Sorghum**

Many factors affect grain appearance: pericarp color and thickness, presence of a pigmented testa, endosperm color, secondary plant, and glume colors and damage by insects and molds affect the appearance and quality of sorghum (see Grain, Morphology of Internal Structure. Grain and Plants, Morphology). Pericarp color is genetically controlled by the R and Y genes. The combination of these genes produces white or colorless ( $R_y$ y or rryy), lemon yellow ( $rrY_y$ ), or red ( $R_y$ ) color. The intensifier ( $I_y$ ) gene increases the brightness of the pericarp color in red pericarp sorghums. Sorghums with homozygous recessive (zz) genes have a thick mesocarp containing small starch granules, which causes a chalky

appearance that masks the colors of the testa and endosperm. A pigmented seedcoat (testa) is present when both  $B_{1-}$  and  $B_{2-}$  genes are dominant. Caryopses with a pigmented testa ( $B_{1-}B_{2-}$ ) and a recessive spreader gene (ss; type II) or dominant spreader gene ( $S_{-}$ ; type III) contain condensed tannins and are brown (tannin) sorghums. The tptp genes control testa color, which is brown or purple. Type III sorghums have more tannins than type II sorghums. Type I sorghums do not contain tannins (proanthocyanidins).

Yellow endosperm cultivars contain 8–30 ppm carotenoid pigments. Endosperm color affects appearance, especially in caryopses with a thin pericarp and without a pigmented testa where the grain appears to be yellow. A thick mesocarp and colorless pericarp cause a white or chalky appearance. Heteroyellow endosperm sorghum results when sorghums with yellow and nonyellow endosperm colors are hybridized. Bronze sorghums contain a thin, red pericarp with yellow endosperm color, while cream sorghums contain a thin, white pericarp with yellow endosperm.

Waxy endosperm cultivars contain three genes (wx) in the recessive form. Heterowaxy genotypes contain one or two of these genes in the dominant form whereas normal or nonwaxy endosperm sorghums contain all three genes in the dominant form. Waxy cultivars contain nearly 100% amylopectin and the endosperm looks like candle wax.

The high-lysine (*hl*) sorghum from Ethiopia has a soft, floury endosperm texture, a shriveled kernel structure, and is susceptible to deterioration in humid environments during and postcaryopsis development. The improved protein digestibility sorghum and the chemically induced, high-lysine sorghum have intermediate-soft to soft endosperm textures; they have reduced grain yield and increased deterioration due to molds and weathering.

#### **Grain Structure and Physical Properties**

The sorghum kernel is considered a naked caryopsis, although some African types retain their glumes after threshing (*see* Grain and Plants, Morphology). The kernel weight varies from 3 to 80 mg. The size and shape of the grain varies widely among sorghum races. Commercial sorghum grain has a flattened spherical shape, 4 mm long, 2 mm wide, and 2.5 mm thick, with a kernel weight of 25–35 mg. The volumetric weight and grain density range from 708–760 kg m<sup>-3</sup> and from 1.26–1.38 g cm<sup>-3</sup>, respectively.

The sorghum caryopsis is composed of three anatomical parts: pericarp, endosperm, and germ (Figure 1a). The relative proportion of these

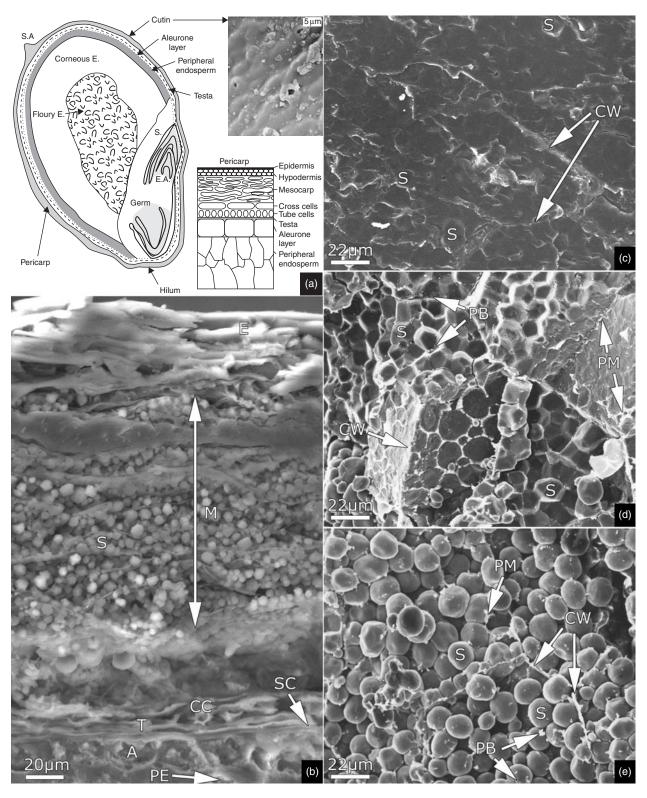


Figure 1 Scanning electron microscopy photos of sorghum microstructure. Note: (a) = cross-section of the sorghum caryopsis; (b) = a thick pericarp; (c) = peripheral endosperm; (d) = corneous endosperm; (e) = floury endosperm. Labels: A = aleurone layer; C = corneous endosperm; CC = cross cells; CW = cell wall; E = epicarp; F = floury endosperm; G = germ; M = mesocarp; PE = peripheral endosperm; P = pericarp; PB = protein body; PM = protein matrix; S = starch granule; SC = seed coat; T = tube cells. (Reproduced with permission from Earp et al. (2004) Journal of Cereal Science 39(1): 21-27.)

structures varies but in most cases is 6%, 84%, and 10%, respectively. The pericarp (Figure 1b) is the fruit coat and is fused to the sorghum seed. It originates from the ovary wall and is subdivided into three distinctive parts: epicarp, mesocarp, and endocarp. The epicarp is the outermost layer and is covered with a waxy film. The mesocarp varies in thickness and thick mesocarps contain starch granules. The endocarp is composed of cross and tube cells and plays a major role in transporting moisture and nutrients during germination and development.

The true seed consists of the seedcoat (testa), endosperm, and germ (see Grain, Morphology of Internal **Structure**). The endosperm tissue is triploid, resulting from the fusion of a male gamete with two female polar nuclei. The testa (seedcoat) is derived from the ovule integuments; in brown sorghums, it is thick and contains condensed tannins. The testa is pigmented when the genes are  $B_1$ \_ $B_2$ \_, The endosperm is composed of the aleurone layer, peripheral, corneous, and floury areas. The aleurone consists of a single layer of rectangular cells adjacent to the tube cells or testa. Aleurone cells contain a thick cell wall, large amounts of proteins (protein bodies) and enzymes, ash (phytic acid bodies), and oil bodies (spherosomes). The peripheral endosperm (Figure 1c) adjacent to the aleurone layer is a hard layer composed of dense cells containing large quantities of protein and small starch granules. These layers affect the processing and nutrient digestibilities of sorghum. Processing of sorghum by steam flaking, micronizing, popping, and reconstitution is designed to disrupt the endosperm structure to improve the digestibility.

The corneous and floury endosperm cells are composed of starch granules, protein matrix, protein bodies, and a thin cell wall rich in  $\beta$ -glucans and hemicellulose. In the corneous endosperm, the protein matrix surrounds the starch granules and protein bodies are embedded in the matrix (**Figure 1d**). The starch granules are shaped polygonally and often contain dents from protein bodies. The appearance is translucent and the texture vitreous. The opaque-floury endosperm is located around the geometric center of the kernel. It has a discontinuous protein phase, air voids, and loosely packaged, round-angular starch granules, and is opaque to transmitted light (**Figure 1e**).

The germ is diploid owing to the sexual union of one male and one female gamete. It is divided into two major parts: the embryonic axis and scutellum. The embryonic axis forms the new plant and is subdivided into a radicle and plumule. The radicle forms primary roots, whereas the plumule forms leaves and stems. The scutellum is the single cotyledon of the sorghum seed. It surrounds the embryonic axis, contains large amounts of oil (spherosomes), protein,

enzymes, and minerals, and separates the endosperm and germ.

## Composition

Sorghum grain composition (**Table 1**) is significantly affected by genetic and environmental factors. Starch (75-79%) is the major component, followed by protein (9.0-14.1%), and oil (1.5-5.0%). The protein content  $(N \times 6.25)$  of sorghum is more variable and usually 1-2% higher than maize. Approximately 80%, 16%, and 3% of the protein is in the endosperm, germ, and pericarp, respectively. Sorghum generally contains 1% less oil and significantly more waxes than maize.

#### Starch

Sorghum starch is composed of 70–80% amylopectin and 20–30% amylose. Waxy sorghums contain starch with 100% amylopectin; their properties and uses are similar to those of waxy maize. The gelatinization temperature of sorghum starch is slightly higher than that of maize starch; however, it can be used interchangeably with maize starch. Sorghum starch from the hard endosperm is difficult to separate from the protein matrix. The wet milling of food-type sorghums produces excellent prime starch. Sorghum endosperm requires a little longer to cook compared to maize endosperm particles. Sorghum endosperm can be easily extruded into a wide variety of snacks and other ready-to-eat products.

**Table 1** Composition<sup>a</sup> (%, unless otherwise stated) of sorghum grain

Component	Value	Range	
Protein (N × 6.25)	11.6	8.1–16.8	
Albumins	5.7	1.6-9.2	
Globulins	7.1	1.9-10.3	
Prolamins	52.7	39.3-72.9	
Glutelins	34.4	23.5-45.0	
Lipid	3.4	1.4-6.2	
Ash	2.2	1.2-7.1	
Nitrogen-free extract Fiber	79.5	65.3-81.0	
Crude	2.7	0.4-7.3	
Dietary, insoluble	7.2	6.5-7.9	
Dietary, soluble Acid detergent	1.1 3.3	1.0-1.2 2.9-3.6	
Sugars	2.1	1.3–2.6	

<sup>&</sup>lt;sup>a</sup> All values are expressed on a dry-matter basis. Nitrogen-free extract was calculated by difference.

Adapted from Rooney LW and Waniska RD (2001) sorghum food and industrial utilization. In: Smith WC and Frederiksen RA (eds.) Sorghum: Origin, History, Technology and Production, pp. 689–725. Wiley.

## **Soluble Sugars**

Mature kernels contain 2.2-3.8% soluble sugars, 0.9–2.5% free reducing sugars, and 1.3–1.4% nonreducing sugars. Glucose and fructose comprise 0.6-1.8% and 0.3-0.7%, respectively. High-lysine and sugary cultivars contain additional simple sugars and amino acids, which accounts for the flavor they develop during roasting.

#### **Fiber**

Most of the fiber is present in the pericarp and cell walls. Sorghum contains 6.5-7.9% insoluble fiber, hemicellulose and cellulose, and 1.1-1.2% soluble fiber,  $\beta$ -glucans and pentosans. Sorghum contains  $\sim 1.3\%$  pentosans, located mainly in the pericarp. Approximately 70% of the pentosans are alkalisoluble, and 30% are water-soluble. Fibers in cell walls of the aleurone and endosperm are associated with ferulic and caffeic acids. Fibers in the pericarp provide structural and protective functions; therefore, fiber content of sorghum products depends on the extent of pericarp removal during milling.

Insoluble dietary fiber increases during food processing due to increased levels of bound protein, mainly kafirins, and enzyme-resistant starch. In tannin sorghum, cooking also forms polyphenol-protein complexes, which increases bulking ability. Sorghum brans do not lower blood cholesterol levels but are excellent bulking agents in rats. Sorghum bran fed to humans increases stool weight, decreases intestinal transit time, and increases the frequency of bowel movement.

#### **Proteins**

Protein content and composition vary due to agronomic conditions (water availability, soil fertility, temperatures, and environmental conditions during grain development) and genotype. Nitrogen fertilization significantly increases kafirin accumulation and protein content. Kafirins (the sorghum prolamin proteins) and glutelins comprise the major protein fractions in sorghum. These fractions are primarily located within the protein bodies and protein matrix of the endosperm, respectively. The alcohol-soluble prolamin fraction comprises 50% of the protein. These proteins are hydrophobic, rich in glutamine, leucine, alanine, and proline, contain little lysine and are primarily located within protein bodies. Kafirins contain cross-linked proteins that slow digestibility of the protein. Sorghums identified with easier to digest proteins have protein bodies with a modified structure and contain less kafirin and less cross-linked protein after cooking. Glutelins are high-molecularweight proteins, mainly located in the protein matrix. The lysine-rich protein fractions, albumins and globulins, predominate in the germ.

Lysine and threonine are the first and second most limiting amino acids in sorghum proteins. Sorghum lysine meets ~40% of the recommended level for infants. High-lysine cultivars contain ~50% more lysine. They are soft, dented, contain lower levels of kafrins and higher levels of glutelins, and salt-soluble proteins.

#### Lipids

The germ contains 80% of the  $\sim 3.5\%$  lipid in the sorghum caryopsis. The fatty acid composition consists mainly of linoleic (49%), oleic (31%), and palmitic (14.3%) acids. Refined sorghum oil is very similar to maize oil in quality and fatty acid content. The reduced oil content of sorghum compared to maize is an advantage for some applications in food products, i.e., extruded whole grains and some brewing operations where whole grains are used with industrial enzymes to produce lager beer without barley.

Waxes form protective films on the surface of the leaves, stalks, and pericarp of the grain of sorghum (see Oil from Rice and Maize). The surface of the pericarp contains up to 0.5% wax, which has properties and composition similar to carnauba wax. The low amount of wax limits commercialization but the economics could improve as a by-product recovered from distillers grains during alcohol production.

#### Vitamins and Minerals

The germ and aleurone are rich in fat-soluble and B-vitamins. Sorghum contains 0.3–0.8 µg per g  $\alpha$ -tocopherols and 9–11.5 µg per g  $\tau$ -tocopherols. Precursors of vitamin A (carotenes) are found in yellow and heteroyellow endosperm sorghums. Yellow endosperm sorghum caryopses contain 1.5-30 ppm of carotenoids: 36.3% zeaxanthin, 28.6% lutein, 24.7% xanthophyll, and 10.4%  $\beta$ -carotene. Weathering causes sorghum to lose 50% of its carotenoids.

Sorghum is an important source of minerals that are located in the pericarp, aleurone layer, and germ. Phosphorus is the mineral found in greatest amounts; its availability is negatively related to the amount bound by phytates. Phytase activity during malting and fermentation significantly increases availability of phosphorus and other minerals as well.

## **Enzymes**

The sorghum aleurone layer is not a major source of endosperm-degrading enzymes. The scutellum of sorghum is where  $\alpha$ -amylase is formed and diffuses into the endosperm. Sorghum does not respond to gibberellins to enhance production of amylases during

malting. The  $\alpha$ -amylase activity in sorghum starts 24–36 h after germination. A rapid increase in dextrinase activity is observed 24 h after germination. Limit dextrinases and proteases are found mainly in the endosperm, whereas, carboxypeptidases are located primarily in the germ. Sorghum malt has high levels of  $\alpha$ -amylase activities but it has reduced  $\beta$ -amylase activities.

#### **Tannins and Phenols**

Condensed tannins (proanthocyanidins) are not present in all sorghums; however, all sorghums contain phenolic acids, and most contain flavonoids. Kernels that contain condensed tannins have a thick, highly pigmented testa. These sorghums were referred to as brown sorghums but are now classified as tannin sorghums. Tannins protect the kernel against preharvest germination and attack by insects, birds, and molds. Birds consume brown sorghums when other food is unavailable. Animals fed tannin sorghum rations eat more feed and produce about the same amount of gain, so feed efficiency is reduced. There are no toxicity problems but feed efficiency is reduced by the condensed tannins. This situation is grossly misstated in the literature, because there have been numerous feeding trials reporting decreased feed efficiency with only a few instances of other problems.

The condensed tannins have a high affinity for prolamin proteins and decrease feed efficiency by 5-15% depending upon the livestock species and processing of the rations. In some areas of Africa, special processes are used to prepare the tannin sorghums to improve their food properties. Tannin sorghums bind enzymes during brewing of sorghum beer and additional malt is required.

The tannin sorghums are potent sources of antioxidants. Bran fractions and extracts from them have significantly higher oxygen radical absorbance capacity (ORAC) levels, a measure of antioxidant strength, than most fruits and vegetables (Table 2). Bakery products containing this bran have increased fiber content, higher antioxidant potential, and attractive natural brown or chocolate color. Tannin sorghums can also be transformed into excellent whole grain snacks by extrusion. The extrusion process significantly reduces the degree of polymerization of tannins, which may be beneficial in human foods.

#### **Uses of the Grain**

#### **Food Uses**

The major categories of traditional foods are fermented and unfermented flat breads, fermented and unfermented thin and thick porridges, steamed and boiled

**Table 2** ORAC<sup>a</sup> levels in sorghum brans and extracts relative to common fruits

Sample	ORAC <sup>a</sup> (DM basis)		
Black sorghum bran	1008		
Sumac sorghum bran	3120		
Tannin sorghum bran	2400		
Blueberries	87-873		
Strawberries	356-400		
Plums	452-600		
Grapes	100		
Watermelon	15		
Orange	80-152		
Extracts			
Sumac sorghum bran	11 200		
Grape skin	6124		
Red wine concentrate	3200		
Vitamin C = reference	5000		

 $^a$  ORAC, oxygen radical absorbance capacity, is a measure of antioxidant potential measured in  $\mu mol\ TE\ g^{-1},$  using fluorescein as a probe.

products, snack foods, and alcoholic and nonalcoholic beverages (Figure 2) (see Cultural Differences in Processing and Consumption. Maize: Foods from Maize). Worldwide, the most popular unfermented flat breads from sorghum are "roti" in India and tortillas in Central America. For roti, a portion of the flour is gelatinized, mixed with more flour and warm water, and kneaded into a dough, which is shaped into a circle, and baked on a hot griddle. For tortilla production, whole sorghum is lime-cooked, steeped overnight, washed, stone ground into "masa," shaped into thin circles, and baked on a hot griddle (Figure 2d). Sorghum and maize blends are often used in Salvador, Nicaragua, Guatemala, and Honduras. Food-type white sorghums (Figure 2a) lighten the color and decrease the off-flavors in many food products.

The most popular fermented breads are "injera," "kisra," and "dosa," consumed in Ethiopia, Sudan, and India, respectively. About 80% of the Ethiopian sorghum is used for production of injera (Figure 2i). The sorghum flour is mixed with water and a yeast starter from a previous batch of injera. After fermentation for 24–48 h, the batter is poured onto a greased pan for baking. The resulting product is a flexible, large diameter pancake-like bread containing uniformly distributed fish eyes (air bubbles). It is moist and retains its flexibility for 2–3 days. Dosa is consumed in India and is produced from a mixture of black gram, sorghum, and rice flour. It is used as a wrap for vegetables, sauces, and other foods.

Porridges are popular foods from sorghum (Figure 2g). The pH of porridges vary from acid to neutral to alkaline depending upon the region or country. "Tô" is an unfermented stiff porridge cooked in alkali in Mali, cooked in acid in Burkina Faso, or

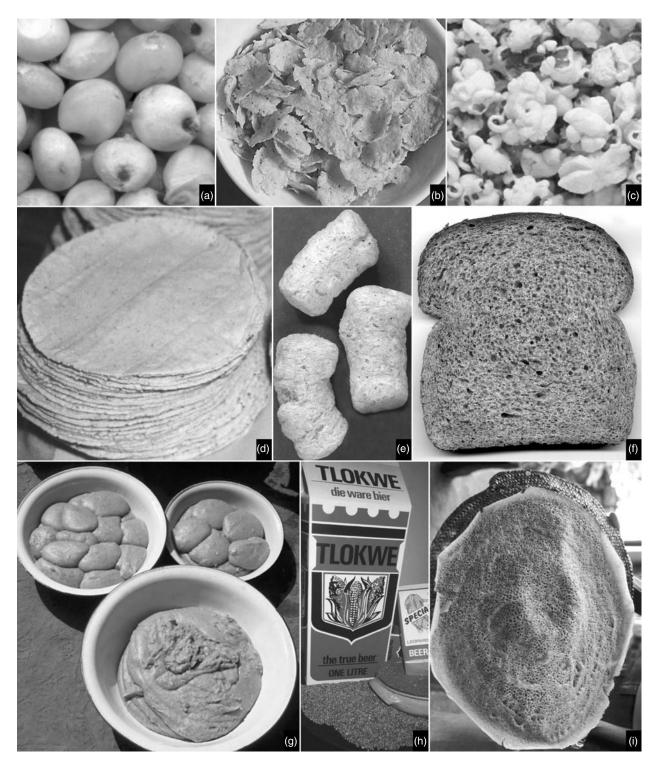


Figure 2 Traditional and processed foods prepared using sorghum. Note: (a) = white, food-type sorghum; (b) = flakes after whole grain was tempered, steamed, rolled, and toasted; (c) = air-popped sorghum; (d) = tortillas after whole grain was nixtamalized, washed, ground, formed into disks and baked; (e) = collets, whole grain was extruded; (f) = wheat bread containing 1.5% tannin sorghum; (g) = stiff porridge from sorghum after de-hulling, milling into flour, and cooking with water; (h) = opaque beer prepared from sorghum and malted sorghum; (i) = "injera" prepared from sorghum after decortication, milling into flour, mixing with water, natural fermentation, preparation of a slurry, and baking on a griddle.

fermented and cooked. Decorticated sorghum flour is cooked in water acidified with tamarind juice or water made alkaline with the leachate of wood ashes (potash). Popular fermented porridges are "ogi" and "nasha," widely consumed in West and East Africa, respectively. Whole sorghum is soaked in water and allowed to ferment for 2–3 days. The wet grain is crushed in a slurry of water and sieved to remove the bran. The fine particles are allowed to ferment longer. Excess water is decanted and the resulting slurry cooked in water or milk to make thin or thick porridges. Porridges are consumed with sauces prepared from vegetables, fish or meat, grain legumes, amaranthus leaves, and other materials.

For couscous production, sorghum flour is kneaded with enough water,  $\sim 30\%$ , to form agglomerates. The wet flour is forced through a coarse screen to form large particles, which are steamed. The cooked product is consumed with a sauce or milk. In some cases, the particles are dried after steaming and used as one of the true convenience foods in the Sahelian zone of Africa. Decorticated sorghums are often cooked like rice.

Alcoholic beverages are produced from malted sorghum (Figure 2h). The high-solids beer is sour, alcoholic, pinkish, and effervescent. The fermentation time is short and the beer is drunk while actively fermenting. The beers vary from sweet to very sour; alcohol and solids contents vary. The most common type in southern Africa, called opaque beer, undergoes souring and yeast fermentation.

Sorghum processing into food is a tedious, time-consuming chore done by the housewives several times per week. There are very few processed foods that meet the convenience, taste, flavor, and texture requirements of urban consumers. Grain obtained in the markets is mixed and usually contaminated with sand, trash, and damaged grains. In some areas, value-added sorghum grain is processed into profitable products for sale in urban areas and local markets. The major challenge is to obtain a consistent supply of good-quality grain for processing.

Sorghum grits, meal, and flour can be used alone or mixed with wheat flour to produce an array of baked goods (Figure 2f). Sorghum does not contain gluten. Thus, the amounts of sorghum flour in the blends depend on the quality of the wheat flour, baking procedure, formulation, and quality of the baked products desired. New food-type sorghums that produce excellent yields of flour with a bland flavor and light color are available. They can be used to extend wheat-based products without affecting flavor. Sorghum can be puffed, popped, shredded, and flaked to produce ready-to-eat breakfast cereals (Figures 2b and 2c). Extrusion of sorghum

produces acceptable snacks (Figure 2e) and precooked products.

#### Milling

For production of most traditional foods, the pericarp is removed by decortication, before it is milled into flour (see Cultural Differences in Processing and Consumption. Maize: Foods from Maize). To decorticate, the grain is usually washed, placed in a wooden mortar, and pounded vigorously with the wooden pestle. The abrasive action separates the pericarp from the kernel at the mesocarp. Grain with thick pericarp, hard endosperm, and spherical shape are preferred because they are easier to mill. A thin pericarp requires 1.5-2 times more time to decorticate. The bran (pericarp) is separated from the grain by washing with water or by winnowing the sun-dried grain. Most sorghums are decorticated to remove 10-30% of the original grain weight. The decorticated kernels are reduced into flour by pounding in the mortar and pestle, with stone mills, or by electric or diesel-powered attrition mills. Flour is sieved to obtain fractions with an acceptable particle size for specific products.

Commercial milling of sorghum is practiced in several countries. Sorghum is decorticated using mechanical decortication with rice-milling equipment or abrasive disks, followed by degermination and subsequent sieving. The decorticated material is then milled, gravity separated and sieved to produce low-fat grits, meal, and flour. In Botswana and South Africa sorghum is milled using abrasive decorticators followed by grinding the grain using hammermills or attrition mills. In India, sorghum is cleaned and milled using a stone mill followed by sifting to remove the bran, 1-3% of the original grain. The coarse flour is used for roti and related products. Milling of sorghum with wheat roller mills produces acceptable flour and other products. Small-industrialscale roller mills produced in South Africa are used extensively to process maize, sorghum, and millet into flour and meal. The yields are quite high and the flour has good characteristics. Also, large sophisticated mills based on degermination and gravity separation of the milling fractions exist and produce highly refined grits, meal, and flour. In many areas of Africa, large-scale milling of sorghum has failed because of poor infrastructure and inadequate supplies of food quality grain.

#### **Malting Sorghum and Brewing**

Nonalcoholic beverages and extracts (see Fermentation: Foods and Nonalcoholic Beverages) are produced from malted sorghum, which has replaced

the malted barley extracts in Nigeria. Maltabella, Morvite, and a shelf stable opaque beer are popular products consumed in South Africa. Some companies produce ground sorghum malt for sale to consumers who prefer to produce traditional opaque beer for special festivals, weddings, and other occasions. In addition, powders containing pregelatinized maize grits, sorghum malt, and yeast allow consumers to produce beer by adding water and storing overnight.

Sorghum opaque beer In southern, eastern, and western Africa, sorghum malt is used for alcoholic and nonalcoholic beverages, weaning foods, and breakfast foods. Sour-opaque beers are produced commercially in southern Africa. Opaque beer is produced following the basic steps of the traditional process, which involves malting the sorghum, converting the cooked sorghum and maize grits into fermentable sugars, souring the mash, and finally fermenting the sugars into alcohol. The beer is packaged in vented cartons (Figure 2h) or transported to beer halls in stainless steel tanker trucks. The beer is drunk while actively fermenting.

Opaque beer was the first large-scale, industrial process of sorghum in Africa. Industrial enzymes replace sorghum malt to convert the cooked, soured grits of maize or sorghum into fermentable sugars. The color of the beer has become lighter, due to a greater use of maize as adjunct, and consumption of opaque beer is decreasing because consumers prefer lager beer.

Large commercial malting operations steep, germinate, and dry the malted sorghum for the breweries. Malting requires 4-5 days and 15-20% of the initial weight is lost in respiration, rootlets, and shoots. Sorghum malt is produced at 23–25°C during steeping and germination. Malting does not modify the cell walls of sorghum. Special varieties are grown for malting that produce malt with higher diastatic and other malt enzymes. Tannin sorghums are treated with formaldehyde and or alkali to limit the effects of tannins on diastatic or malt enzymes.

Lager beer (clear beer) Breweries in Africa and Asia use sorghum grits as an adjunct in brewing lager beer. In Nigeria, sorghum and maize are used to produce lager (clear beer) without barley malt. Nigerian breweries produce clear beer from a combination of malted sorghum, ground whole or decorticated sorghum, and/or maize grits with commercial enzymes to convert the starch to fermentable sugars. The quality of clear beer is good; the taste differs from barley malt beer. The modern industrial brewing of beer in Nigeria uses ground whole sorghum treated with commercial enzymes followed by filtration to remove the solids and then fermentation into alcohol. Sorghum malting is a costly process in terms of time with dry matter (DM) losses of 15% or more. The use of ground whole sorghum is a recent innovation, which requires a more efficient filtration procedure. Sorghum is preferred over maize since it has reduced oil content, a thin pericarp, and bland flavor.

#### **Industrial Uses**

Sweet sorghums contain 20-30% sugar in the juice which is crushed from the stalks, clarified, and concentrated into an amber sorghum syrup (molasses) that is a popular product in the southern US. It has a strong flavor and is sometimes blended with cane syrup.

Sorghum grain and/or sweet sorghum biomass are used for ethanol production. Yields of 182-proof alcohol (3871 t<sup>-1</sup>) from sorghum grain are comparable with maize  $(372 \,\mathrm{l}\,\mathrm{t}^{-1})$ . The commercial technology to ferment sweet sorghum biomass into alcohol has developed in Brazil. Sorghum grain is a good substrate for industrial and beverage alcohol where it competes with maize and other sources of starch. Several alcohol plants in the US, India, and other countries use sorghum as an adjunct for alcohol production depending upon availability and cost. In China, a distilled alcoholic beverage from "kaoliang" (sorghum) is exported. It has a unique flavor and aroma and is high in alcohol content.

Sorghum flour is used in adhesives, building board, ore refining, and metal casting as an inexpensive source of starch. The sorghum is dry-milled to remove the pericarp and sometimes converted into acidmodified dextrins. Binders that strengthen the durability of pellets for livestock feed are made from sorghum.

Sorghum is wet-milled to produce starch in Sudan and India. Sorghum starch has properties similar to maize starch. Commercial wet milling of sorghum in the USA was discontinued in the 1970s due to poor economics.

#### **Nutritional Value**

Sorghum has proximate composition, amino acid contents, and nutritional value similar to that of maize. However, due to its lower fat content, sorghum usually has a slightly lower gross, digestible, and metabolizable energy. Lysine and threonine are the first and second limiting amino acids. The tryptophan content is higher than that in maize. High-lysine cultivars contain  $\sim 50\%$  more lysine and promote better weight gains in weaning rats.

Fermentation, malting, and other processing methods significantly improve its nutritional value.

Proteins in sorghums are 76-82% digestible. This is  $\sim 5\%$  less digestible than proteins from maize and other cereals as determined by *in vivo* studies using swine, poultry, and cattle. Proteins in sorghum were reported to be 20-50% less digestible than maize by the *in vitro* pepsin digestion test. This is based on the reduced digestibility seen when the sorghum was cooked as a gruel without stirring and fed to protein-malnourished, ill children. Extrusion cooking of sorghum eliminated the reduced protein digestibility in these children. Over the centuries, millions of Africans and Indians have been nourished by sorghum foods. Proper processing of sorghum yields foods with high digestibility and nutrient values.

#### **Livestock Feeds**

Sorghum is an excellent feed for livestock and companion animals. The feeding value of sorghum for livestock species is generally considered 95% or more of the feeding value of yellow dent maize. Tannin sorghums have 85–95% the feeding value of maize. Sorghum use can result in savings because it is often less expensive than other grains, even when more grain has to be fed.

Sorghum must be properly processed to enhance its digestibility. Sorghum can be dry rolled, steam rolled, cooked with steam, and flaked into thin flakes, micronized and flaked, roasted, ground, and tempered with water up to 30%, stored under aerobic conditions, or ground or rolled. The two most widely used processes are grinding and steam flaking. Sorghum is hammermilled or attrition-milled into meal and coarse particles. Steam flaking is used in large feedlots where grain is a major part of the rations.

Grinding, steam flaking, micronizing, reconstitution, and popping are used to prepare sorghum grain in beef cattle feedlot rations. Grinding, rolling, crushing, and pelleting are used for poultry and swine feeds. Dairy cattle utilize steam flaked or roasted sorghum grain efficiently and protein content of the milk is enhanced. The light color and bland flavor of the white-food sorghums are advantages, when color is important in pet or companion animal feeds.

## **Future Prospects**

Sorghum will for many centuries continue to be utilized as food, feed, and industrial products. This will be driven by sorghum's robust agronomic characteristics and good nutritive, processing, and organoleptic properties. Development of reasonably priced, convenience foods containing sorghum will increase the

demand for food sorghums. An example of a niche market for sorghum is as an nutraceutical ingredient containing antioxidants. However, decreasing consumer preference for sorghum-based foods and beverages is likely to continue for those who have increased purchasing ability.

See also: Cultural Differences in Processing and Consumption. Grain, Morphology of Internal Structure. Grain and Plants, Morphology. Maize: Foods from Maize. Oil from Rice and Maize. Sorghum: Breeding and Agronomy; Harvest, Storage, and Transport.

## **Further Reading**

Chung KT, Wong TY, Wei CI, Huang YW, and Lin Y (1998) Tannins and human health: a review. *Critical Reviews in Food Science and Nutrition* 38: 421–464.

Earp et al. (2004) Journal of Cereal Science 39(1): 21–27. Moyer AR, Humer KE, Finn CE, Frei B, and Wrolstad RE (2002) Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: vaccinium, rubus, and ribers. Journal of Agricultural and Food Chemistry 50(3): 519–525.

Rooney LW and Serna-Saldivar SO (2000) Sorghum. In: Kulp K and Ponte J (eds.) *Handbook of Cereal Science and Technology*, 2nd edn., pp. 149–175. New York: Marcel Dekker.

Rooney LW and Waniska RD (2001) Sorghum food and industrial utilization. In: Smith WC and Frederiksen RA (eds.) Sorghum: Origin, History, Technology and Production, pp. 689–725. New York: Wiley.

Serna-Saldivar SO and Rooney LW (1995) Structure and chemistry of sorghum and millets. In: Dendy D (ed.) Sorghum and Millets: Chemistry and Technology, pp. 69–124. St. Paul, MN: American Association of Cereal Chemists.

Stoskofp NC (1985) Cereal Grain Crops. Reston, VA: Reston Publishing Co.

Taylor JRN, Dewar J, Taylor J, and von Ascheraden RF (1997) Factors affecting the porridge-making quality of South African sorghums. *Journal of the Science of Food and Agriculture* 73(4): 464–470.

Waniska RD and Rooney LW (2001) Structure and chemistry of the sorghum caryopsis. In: Smith CW and Frederiksen RA (eds.) Sorghum: Origin, History, Technology and Production, pp. 649–688. New York: Wiley.

Waniska RD and Rooney LW (2002) Sorghum grain quality for increased utilization. In: Leslie JF (ed.) *Sorghum and Millet Diseases*, pp. 327–335. Ames, IA: Iowa State University Press.

Weaver CA, Hamaker BR, and Axtell JD (1998) Discovery of grain sorghum germplasm with high uncooked and cooked *in vitro* protein digestibilities. *Cereal Chemistry* 75: 665–670.

#### **Relevant Websites**

http://apps.fao.org - FAO (2003) FAOSTAT Database Data website gives details of yearly production database.

http://www.usda.gov - Federal Grain Inspection Service website provides documentation to procedures and policies used to inspect grains.

# SOYBEAN

Contents

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# Germplasm, Breeding, and **Genetics**

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## **Table 1** World soybean production (2001/02)

Country	Production (Mt)
United States	78.6
Brazil	43.5
Argentina	29.5
China	15.1
India	5.4
Paraguay	3.1
Other	8.2
Total	183.4

### Introduction

World soybean production is ∼183.4 million tons (Mt), with the United States accounting for 43% of the total (Table 1). The three largest producers have shown increasing production during the past 25 years (Figure 1). The increased production in Brazil and Argentina within the past five years has been dramatic.

Soybean oil comprises ~30% of the global consumption of vegetable oils and soybean meal accounts for  $\sim$ 70% of the global protein production. Soybean oil consumption has been under considerable pressure from canola oil and olive oil. These latter two oils also claim some health benefits.

## Origin

The genus *Glycine* (Willd.) belongs to the family Fabaceae, subfamily Papilionoideae, tribe Phaseoleae, subtribe Glycininae. The genus Glycine is composed of two subgenera, Glycine (perennials) and Soja (annuals). The proposed origin of the genus Glycine is given in Figure 2.

The linguistic, geographical, and historical evidence suggests that the annual soybean [Glycine max (L.) Merr.] emerged as a domesticated crop during the Zhou dynasty in the eastern half of northern China. An alternative hypothesis, based upon the center of genetic diversity, suggests that the soybean was domesticated in the Yellow River or Yangzi

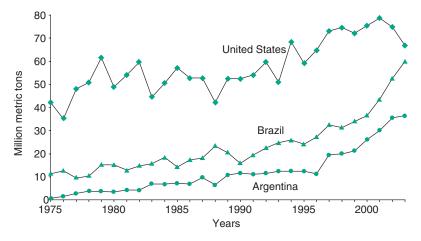
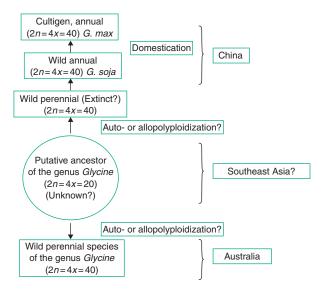


Figure 1 Soybean production of the United States, Brazil, and Argentina from 1975 to 2003.



**Figure 2** The origin of the genus *Glycine*. (Courtesy of Dr. T Hymowitz.)

valleys of central or southern China. Domestication of the soybean took place ~1500–1100 BC, or perhaps earlier. Soybeans were probably grown in the Korean peninsula as well as central and south China by the first century. From about the first century AD to the Age of Discovery (fifteenth to sixteenth century) soybeans were introduced throughout Southeast Asia. Traditional soy food included "miso," "tempeh," "tofu," and soy sauce. Soybean was introduced into Europe before 1737 and was introduced into North America, from China via England, in 1765.

## **Germplasm Collections**

Major *Glycine* germplasm collections exist in Australia, Brazil, China, Germany, India, Indonesia,

Japan, Russia, Republic of Korea, Taiwan, Ukraine, and the United States. Many other smaller but important collections exist throughout Asia, and Europe. The *G. max* germplasm collections are given in **Table 2**, the *G. soja* collections (**Table 3**), and the *Glycine* perennial collections (**Table 4**).

#### **Gene Pools**

The concept of gene pools is useful to plant breeders because it guides them in selecting germplasm to use in hybridizations for plant improvement. The genus *Glycine* has only a primary gene pool and a tertiary gene pool (Figure 3). The primary gene pool consists of *G. max* cultivars, land races, and the wild annual *G. soja*. Cross-pollination within the primary gene pool results in regular chromosome pairing and fertile progeny. The secondary gene pool is not known. The tertiary gene pool consists of the wild perennial *Glycine* species. Cross-pollinations between members of the primary and tertiary gene pools are not possible or require embryo-rescue techniques.

## **Germplasm Diversity**

Within the genus *Glycine* subgenus *Glycine*, there are 22 recognized wild perennial species, and within the subgenus *Soja* are two species (Table 5). These perennial species are diverse morphologically, cytologically, and genomically, and are mostly endemic to Australia (Table 5).

## **Germplasm Utilization**

The practice of germplasm selection and utilization traces to the Chinese farmers who selected desirable phenotypes (plants) to use for seed in the next planting. Desirable changes could have arisen from natural cross-pollinations or mutations.

Table 2 The major Glycine max germplasm collections

Institution	Country	No. of accession
Institute of Crop Germplasm Resources, CAAS <sup>a</sup>	China	23 578
USDA Soybean Germplasm Collection	USA	18 076
Asian Vegetable Research and Development Centre (AVRDC)	Taiwan	12 508
Soybean Research Institute, Nanjing Agricultural University	China	10 000
Department of Genetic Resources I, National Institute of Agrobiological Resourcs	Japan	8630
Institute of Agroecology and Biotechnology	Ukraine	7000
N.I. Vavilov Research Institute of Plant Industry	Russia	6126
Centro Nacional de Pesquisa de Recursos Genéticos e Biotec. (CENARGEN)	Brazil	4693
Soybean Research Institute, Jilin Academy of Agricultural Science	China	4200
All India Coordinated Research Project on Soybean, G B Pant University	India	4015
Centro Nacional de Pesquisa de Soja (CNPSO) EMBRAPA <sup>b</sup>	Brazil	4000
Crop Experiment Stn. Upland Crops Research Div.	Korea, Republic of	3678
Australian Tropical Crops Genetic Res. Centre	Australia	3144
Genebank, Institute for Plant Genetics and Crop Plant Res. (IPK)	Germany	3063
Regional Station National Bureau of Plant Genetic Resources (NBPGR)	India	2808
Taiwan Agricultural Research Institute (TARI)	Taiwan	2699
National Research Centre for Soybean	India	2500
Crop Breeding Institute DR and SS <sup>c</sup>	Zimbabwe	2236
Sukamandi Research Institute for Food Crops (SURIF)	Indonesia	2194
Instituto Agronômico de Campinas (I.A.C.)	Brazil	2000
National Plant Genetic Resources Lab. IPB/UPLB <sup>d</sup>	Philippines	1764
CSIRO, Division of Tropical Crops and Pastures <sup>e</sup>	Australia	1600
Genetic Resources Department – Research Institute for Cereals and Ind. Crops	Romania	1600
G.I.E. Amelioration Fourragere	France	1582
Soyabean Research Institute, Heilongjiang Academy of Agricultural Science	China	1558
Institute of Oil Crops Research, CAAS <sup>a</sup>	China	1529
Institute of Plant Breeding, College of Agriculture, UPLB <sup>f</sup>	Philippines	1508
Instituto Nacional de Investigaciones Agricolas, Station de Iguala	Mexico	1500
Stat. De Genetique et Amelioration des Plantes INRA C.R. Montpellier <sup>g</sup>	France	1404
Kariwano Lab., Tohoku National Agricultural Experiment Station	Japan	1400
Hokkaido Agricultural Experiment Station	Japan	1383
International Institute of Tropical Agriculture	Nigeria	1358
Centro de Investigación La Selva (CORPOICA)	Colombia	1219
Institute of Crop Breeding and Cultivation CAAS <sup>a</sup>	China	1200
Institute for Field and Vegetable Crops	Yugoslavia	1200
Institute of Industrial Crops, Jiangsu Academy of Agric. Sci.	China	1199
Corporacion Colombiana de Investigacion Agropecuaria (CORPOICA)	Colombia	1170
Genebank Cereal and Oil Crops Inst., Hebei Academy of Agricultural Sciences	China	1154
Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias (INIFAP)	Mexico	1124
Maharashtra Association for the Cultivation of Science	India	1081
Total		156 849
IUIAI		100 049

<sup>&</sup>lt;sup>a</sup>CAAS = Chinese Academy of Agricultural Science.

The data in this table were gathered from the database maintained by the International Plant Genetic Resources Institute (http://www.ipgri.org/) (verified 25 Nov. 2002). Some numbers were updated *via* direct contact with the holding institutions.

Plant breeders made sexual pollinations in soybean as early as 1927 in China and in 1902 in the United States by E E Evans. By year 2000, ~800 public cultivars in China, 100 public cultivars in Japan, and more than 600 public and 2000 proprietary cultivars in North America had been released. In addition, plant breeders in South America, Korea, and India had released a total of more than 400 cultivars.

The cultivated soybean is considered a quantitative short-day plant. This photoperiod sensitivity defines the area of adaptation as delimited by latitude. For convenience, in North America, cultivars have been classified into maturity groups based upon their responsiveness to photoperiod. This relatively narrow band of latitude, in which a soybean genotype is adapted, results in 13 maturity group

<sup>&</sup>lt;sup>b</sup>EMBRAPA = Empresa Brasileira de Pesquisa Agropecuaria.

<sup>&</sup>lt;sup>c</sup>DR and SS = Department of Research and Specialist Services

<sup>&</sup>lt;sup>d</sup>IPB/UPLB = Institute of Plant Breeding/University of the Philippines at Los Baños.

<sup>&</sup>lt;sup>e</sup>CSIRO = Commonwealth Scientific and Industrial Research Organization.

<sup>&</sup>lt;sup>f</sup>UPLB = University of the Philippines at Los Baños.

 $<sup>^</sup>g {\sf INRA} = {\sf Institut}$  National de la Recherche Agronomique.

Table 3 The major Glycine soja germplasm collections

Institution	Country	No. of accession
Institute of Crop Germplasm Resources, CAAS <sup>a</sup>	China	6172
USDA Soybean Germplasm Collection	United States	1114
Soybean Research Institute, Nanjing Agricultural Univ.	China	1000
Soybean Research Institute, Jilin Academy of Agric. Sci.	China	600
Soyabean Research Institute, Heilongjiang Academy of Agricultural Sciences	China	400
Crop Experiment Station Upland Crops Research Division	Korea, Republic of	342
Asian Vegetable Research and Development Centre (AVRDC)	Taiwan	339
N.I. Vavilov Research Institute of Plant Industry	Russia	310
Breeding Laboratory Faculty of Agriculture, Iwate University	Japan	151
CSIRO, Division of Tropical Crops and Pastures <sup>b</sup>	Australia	60
Taiwan Agricultural Research Institute (TARI)	Taiwan	46
Hunan Academy of Agriculture Sciences	China	45
Tieling District Agricultural Research Institute	China	29
Department of Agronomy, National Chung Hsing University	Taiwan	20
Eastern Cereal and Oilseed Research, Centre Sasketoon Research Centre	Canada	18
Soybean Breeding Laboratory, Tokac. Agricultural Experiment Station	Japan	15
Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP)	Mexico	9
All India Coordinated Res. Project on Soybean G B Pant University	India	7
Maharashtra Association for the Cultivation of Science	India	6
Sukamandi Research Institute for Food Crops (SURIF)	Indonesia	4
Research Institute for Food Crops Biotechnology (RIFCB)	Indonesia	4
Kariwano Laboratory Tohoku National Agricultural Experiment Station	Japan	3
Genebank Institute for Plant Genetics and Crop Plant Research (IPK)	Germany	2
S.K. University of Agricultural and Technology	India	1
Total		10 697

<sup>&</sup>lt;sup>a</sup>CAAS = Chinese Academy of Agricultural Science.

The data in this table were gathered from the database maintained by the International Plant Genetic Resources Institute (http://www.ipgri.org/) (verified 25 Nov. 2002). Some numbers were updated *via* direct contacts with the holding institutions.

designations: 000, 00, 0, and Roman numerals I–X, from North to South. The few photoperiod insensitive cultivars that have been released have not been agronomically competitive with photoperiod-sensitive cultivars.

#### **Germplasm Utilization – Perennials**

The 22 wild perennial soybean species represent a reservoir of useful genes to improve the cultivated soybean. This includes resistance to soybean rust (Phakopsora pachyrhizi Sydow), soybean brown spot (Septoria glycines Hemmi.), powdery mildew (Microsphaera diffusa Cke. and Pk.), phytophthora root rot (Phytophthora sojae H J Kaufmann and I W Gerdemann), white mold (Sclerotinia sclerotiorum (Lib. De Bary)), sudden death syndrome (Fusarium solani (Mart.) Sacc.), tobacco ring spot (G L Hartman, personal communication), yellow mosaic virus, alfalfa mosaic virus, soybean cyst nematode (Heterodera glycines Ichinohe), and tolerance to certain herbicides and to salt, and are more amenable to tissue culture regeneration. Exploitation of the wild perennial species for soybean improvement has received renewed interest in recent years. The major impediment is the extremely low crossability and the need to use embryo-rescue techniques to obtain hybrid plants. Many interspecific hybrid combinations give weak, slow growing plants that are completely sterile.

#### Germplasm Utilization - G. soja

The *G. soja* accessions harbor many undesirable genetic traits, e.g., lodging, vining growth habit, susceptibility to biotic and abiotic stresses, lack of complete leaf abscission, pod shattering, and black seedcoat. Limited numbers of interspecific crosses between *G. max* and *G. soja* have been made in attempts to broaden the genetic base of *G. max* cultivars. Generally, introgression of favorable traits has not been successful. However, small-seeded cultivars, that have *G. soja* as one parent, have been released for sprouts or the fermented Japanese product "natto." One Russian cultivar that has a higher percentage germination under cooler temperatures has been released. The wild annual soybean is high in seed protein but low in seed oil.

#### Germplasm Utilization (North America) - G. max

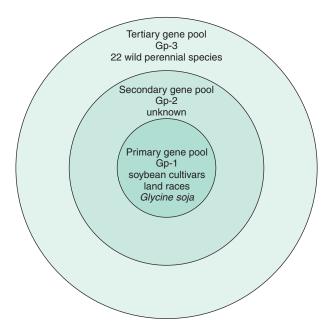
The G. max accessions form the basis for modern soybean cultivar improvement programs. Ten plant

<sup>&</sup>lt;sup>b</sup>CSIRO = Commonwealth Scientific and Industrial Research Organization.

Table 4 The major perennial Glycine collections

Species Australia				Country				Total
	USA	South Africa	Taiwan	Russia	Japan	United Kingdom		
G. albicans	5							5
G. aphyonota	1							1
G. arenaria	6	3						9
G. argyrea	16	12						28
G. canescens	222	119	1	2	3		1	348
G. clandestina	411	116	7	3	6	5		548
G. curvata	10	6			1			17
G. cyrtoloba	51	44			1			96
G. falcata	54	26		2		1		83
G. hirticaulis	8							8
G. lactovirens	10							10
G. latifolia	120	43	21		2			186
G. latrobeana	19	6			1			26
G. microphylla	211	34	50					295
G. peratosa	1							1
G. pindanica	5	1						6
G. pullenii	4							4
G. rubiginosa	53							53
G. stenophita	42							42
G. tabacina	303	229	111	4	13	15		675
G. tomentella	493	279	113	5	4	6		900
Glycine spp.	139	1	1	53				194
Total	2184	919	304	69	31	27	1	3535

The data in this table were gathered from the database maintained by the International Plant Genetic Resources Institute (http://www.ipgri.org/) (verified 22 Nov. 2002). Some numbers were updated *via* direct contacts with the holding institutions. Number of accessions is reported by country and in some cases there may be more than one collection per country.



**Figure 3** The gene pools of the soybean. (Courtesy of Dr. T Hymowitz.)

introductions (accessions) have contributed more than 80% of the North American maturity gene pool, and seven introductions contributed more than 80% of the southern United States maturity groups. Accessions have contributed the most to pest resistance. The most common method of gene transfer has been through backcrossing. For example, resistance to downy mildew (caused by Peronospora manshurica), to root rot (caused by Phytophthora sojae (Kaufmann and Gerdemann)), to bacterial pustule (caused by Xanthomonas campestris pv. glycines (Nakano) Dye), and root knot (caused by Meloidogyne spp.) has been incorporated into susceptible cultivars to develop near-isogenic resistant cultivars. Recently, accessions have played a prominent role as sources of resistance to brown stem rot (caused by Phialophora gregata Allington and Chamberlin) W. Gams and to cyst nematode. Another example includes the cultivar Kunitz; which is missing the antinutritional factor Kunitz trypsin inhibitor SBTI-A2 derived from a G. max accession (PI 157440) from South Korea.

The selection of parents used for soybean cultivar development is determined largely by the breeding objective and the germplasm available to use as parents. Direct cross-pollination of cultivars with germplasm accessions, followed by inbreeding and selection, generally has not contributed to the development of modern high-yielding cultivars. A topic of much discussion among soybean breeders is whether continued advances in cultivar development for seed yield, beyond the level possible with the domestic gene pool

Table 5 List of species in the genus Glycine (Willd.)

	2 <i>n</i>	Distribution
Subgenus Glycine		
1. G. albicans Tind. and Craven	40	Australia
2. G. aphyonota B. Pfeil	40	Australia
3. G. arenaria Tind.	40	Australia
4. G. argyrea Tind.	40	Australia
5. G. canescens F.J. Herm.	40	Australia
6. G. clandestina Wendl.	40	Australia
7. G. curvata Tind.	40	Australia
8. G. cyrtoloba Tind.	40	Australia
9. G. dolichocarpa Tateishi and Ohashi	80	Taiwan
10. G. falcata Benth.	40	Australia
11. G. hirticaulis Tind. and Craven	40	Australia
	80	Australia
12. G. lactovirens Tind. and Craven	40	Australia
13. G. latifolia (Benth.) Newell and Hymowitz	40	Australia
14. G. latrobeana (Meissn.) Benth.	40	Australia
15. G. microphylla (Benth.) Tind.	40	Australia
16. G. peratosa B. Pfeil and Tind.	40	Australia
17. G. pindanica Tind. and Craven	40	Australia
18. G. pullenii B. Pfeil, Tind. and Craven	40	Australia
19. G. rubiginosa Tind. and B. Pfeil	40	Australia
20. G. stenophita B. Pfeil and Tind.	40	Australia
21. G. tabacina (Labill.) Benth.	40	Australia
	80	Australia, West Central and South Pacific Islands
22. G. tomentella Hayata	38	Australia
	40	Australia, Papua New Guinea
	78	Australia, Papua New Guinea
	80	Australia, Papua New Guinea
		Indonesia, Philippines, Taiwan
Subgenus Soja (Moench) F.J. Herm.		
23. G. soja Sieb. and Zucc.	40	China, Russia, Taiwan, Japan, Korea (wild soybean)
24. <i>G. max</i> (L.) Merr.	40	Cultigen (soybean)

of elite breeding lines and cultivars, can be achieved. The utilization of accessions in cultivar development might be successful if selection systems were known that could identify introductions that have good combining abilities with adapted genotypes. Two-parent crosses of "good" by "good" cultivars or elite experimental breeding lines, have been the most extensively used by breeders because of the higher probability of obtaining high-yielding cultivars.

# Germplasm Utilization (Other Countries) - *G. max*

Unlike the North American genetic bases, the Chinese genetic base encompasses 190 ancestors that have contributed 80% to the parentage of the Chinese soybean cultivars. The genetic base of the Japanese cultivars has shifted over time. Before 1945, Chinese accessions were important as parents in Japanese cultivars. In the late 1950s, increasing numbers of North American cultivars and breeding lines were used as parents.

South Americans have relied extensively upon North American cultivars for direct release to farmers. In recent years, the South American breeding programs have released cultivars that are competitive with those introduced in North America.

# **Breeding**

The first cultivar that originated as a selection made among progenies of controlled hybridizations was the cultivar Ogemaw released in 1902. Cultivar development in the United States initially was conducted by the USDA and state agricultural experiment stations. Now, in the main soybean-producing regions, private companies develop and release most of the cultivars.

Conventional soybean breeders follow a cycle of "crossing of parents" followed by "progeny evaluation and selection of parents" and "development of progeny for testing."

## **Breeding Objectives**

The first step in cultivar development is to identify the characters that are important for commercial soybean production whether it is for feed, food, or industrial uses. Yield is the character of prime importance to the

farmer. Data from 1941 to 2000 of North American maturity groups 00-IV show that soybean breeders have increased seed yield  $\sim 1\%$  per year. In China yield gains for soybean breeding programs were low before 1970. Since the 1980s yield gains between 1% and 2% per year have been realized.

In soybean, diseases, nematodes, and insects can reduce yields substantially. Generally in soybean breeding, tolerance to pests is not the objective, rather the emphasis is breeding for resistance.

Lodging resistance or standability is important for mechanical harvest, and plants that are not upright have decreased yield. Most earlier maturing cultivars are of indeterminate growth habit, while later maturing cultivars are of determinate growth habit. The short, determinate cultivars have better lodging resistance.

Pest resistance is of paramount importance in soybean-growing areas where diseases, nematodes, or insects are major problems. Most major soybean-1growing areas have one or more major pest problems. Breeding for pest resistance is dependent upon the prevalence and regularity of economic loss in each area.

Shattering resistance refers to the ability of a plant to retain its seed when mature. All modern cultivars have adequate resistance to shattering.

#### **Seed Composition**

Seed composition is becoming an important factor to consider in soybean breeding. Cultivars generally have ~40% seed protein and 20% seed oil. In most environments, if seed protein increases, seed oil decreases, and vice versa. Both food uses and industrial uses are creating greater demands for specialty use soybean such as soy milk and soy ink for printing. A number of breeding programs now focus on breeding specialty beans.

Soybean seed oil is the major vegetable oil among oil seed crops worldwide and modification of seed oil has received the most focus. Oil synthesis in the seed and seed fatty acid composition are largely determined by the maternal parent. The fatty acid content of conventional soybean cultivars is  $\sim 11\%$  palmitate, 4% stearate, 24% oleate, 54% linoleate, and 7% linolenate. The desired variation in seed oil fatty acid composition was not found in the G. max or G. soja germplasm collections. Mutagenesis, both chemical and ionizing radiation were used to create a number of highly desirable mutations with modifications in individual fatty acids (Table 6). The modification of soybean oil and selection for superior agronomic traits has resulted in cultivar releases, e.g., Satelite, which has lower saturated fatty acids and lower linolenic acid.

**Table 6** Mutations that either elevate or reduce soybean seed oil composition

Fatty acid	Number of mutants			
	Elevate	Reduce		
16:0 (palmitate)	6	8		
18:0 (stearate)	5	0		
18:1 (oleate)	2	2		
18:2 (linoleate)	0	1		
18:3 (linolenate)	2	5		

Industrial uses include soy ink for both black/ white and color newsprint and as a major component of lubricants and gasoline for automobiles, trucks, buses, and marine engines.

Several vegetable-bean cultivars, known as "edamame," with improved agronomic qualities are available. Natto, a fermented product, soymilk, and soy protein isolates are important food uses of soybean. Tofu, a cheese-like curd for human consumption, has a high percentage of protein.

Other traits that are important in breeding cultivars include maturity, seed quality, seed emergence, herbicide tolerance, plant height, and resistance to mineral deficiencies and toxicities.

#### **Specialty Cultivars**

Breeding of specialty cultivars, especially cultivars with value-added traits, has been given increased emphasis in recent years. These include cultivars with altered seed composition for oil and protein quality and quantity, seed size and color, and growth habit, e.g., forage soybean. Specialty cultivars are used for soyfoods, industrial applications, and for feed. Parents used to breed specialty cultivars have added genetic diversity to the soybean gene pool.

#### **Breeding Methodology**

Soybean is a highly self-pollinated species and all cultivars are either pure lines (inbred lines) or mixtures of pure lines. All cultivars released today in the major soybean-growing areas of the world are the result of sexual hybridization followed by selection. The cyclic process of hybridization, inbreeding, and evaluation/selection form the basis for soybean cultivar development.

Breeding methods include pedigree, bulk, mass selection, single-seed descent, and backcross. Single-seed descent, also known as a modified pedigree method is favored by many soybean breeders, because it minimizes the number of years for cultivar development. Single-seed descent is well suited for generation advances (inbreeding) in off-season nurseries,

Season	Procedure Plants					
1	Make F <sub>1</sub> seed	Α	В	С	D	Е
2	Grow F <sub>1</sub> plants Harvest F <sub>2</sub> seed from each plant separately					
3	Grow F <sub>2</sub> plants Harvest F <sub>3</sub> seed from each plant separately					<b>↓</b>
4	Grow individual hills Harvest $F_4$ seed from each hill separately		<b> </b>	<b> </b>	<b> </b>	<b> </b>
5	Grow individual hills Harvest F <sub>5</sub> seed from each hill separately	<b>1</b>	<b>1</b>	<b>+</b>	<b>\</b>	<b>\</b>
6	Grow individual hills Harvest one $F_5$ plant from each hill			•		•
7	Grow individual progeny rows Harvest selected rows in bulk	<u> </u>	<b>↓</b> D	iscard	Disca	rd 🔻
8	Extensive testing of F <sub>5</sub> -derived lines					

**Figure 4** Example of a single-seed descent method. Variations include harvesting one pod, or only one seed to advance to the next generation. Harvesting one plant insures that all F<sub>2</sub> plants are represented in the final selection process.

e.g., Puerto Rico, Hawaii, Hainan Island (China) or South America, or in glasshouses (Figure 4).

The pedigree method is based upon selection among plants during inbreeding. The inability to make selections in off-season nurseries and glasshouses for important agronomic traits is a severe limitation.

The bulk method entails growing a population of plants, usually  $F_2$  generation, harvesting all the seed in bulk, and planting a seed sample from the bulk harvest the next season. This procedure is repeated until the desired level of inbreeding (homozygosity) is achieved, then selection is initiated.

The mass selection method involves inbreeding plants selected on the basis of phenotype, compositing the seed without progeny testing, and inbreeding until the desired level of homozygosity is attained.

Early generation testing has been used to produce soybean cultivars. This method involves identifying desirable heterogeneous populations or lines from which superior homozygous individuals can be selected.

In North America, the USDA coordinates the regional evaluation of advanced breeding lines submitted by USDA scientists, Agriculture and Agri-Food Canada scientists, and state (United States) or provincial (Canada) agricultural experiment stations. These elite breeding lines are advanced to the Preliminary

and Regional Tests. This testing program, from maturity groups 00 through VIII, enables breeders to evaluate breeding lines under a wide range of environmental conditions. Lines are usually entered only once in the Preliminary Tests, and then are either deleted or advanced to the Uniform Tests for a maximum of three years, if agronomic performance warrants further testing. The best publicly derived cultivars in each maturity group are used as check (control) cultivars in which to compare the elite breeding lines for seed yield, seed chemical composition, maturity, height, lodging, seed quality, and reaction to diseases and nematodes.

#### **Hybrid Soybean**

Soybean male sterility was reported in 1971. Since that time at least eight international patents on soybean sterility systems and hybrid seed production have been granted. There are five components that are crucial for the successful development of commercial hybrid soybean:

- Parental combinations that produce heterosis levels superior to the best pure-lines cultivars.
- A stable male-sterile, female-fertile sterility system.
- A selection system to obtain 100% female (pod parent) plants that set seed normally and can be harvested mechanically.

- An efficient pollen transfer mechanism from pollen parent to pod parent.
- An economical level of seed increase for the seedsman and growers that ultimately benefits the consumer.

Research results show that F<sub>1</sub> hybrid vigor (heterosis) does exist in soybean with the better hybrids yielding between 10% and 20% above the high parent. The major obstacle to commercialization of hybrid soybean is pollen transfer from the male parent to the pod parent. Considerable advances in insectmediated cross-pollination are necessary before large numbers of hybrid seed can be obtained. Then hybrid combinations can be tested in multiple environments to assess hybrid vigor and to determine the potential for commercialization. Perhaps equally important for the commercialization of hybrid soybean would be the benefit of a technology protection system. Such a system when combined with "trait stacking" would benefit the seedsman, growers, and ultimately the consumer.

#### Genetics

Soybean is expected to have 20 linkage groups (2n = 4x = 40 chromosomes). The diploid progenitor of soybean has not been identified. The small size, large number, and similar morphology of the chromosomes have been deterrents to progress in soybean cytogenetics and genetics. However, a chromosome map based upon pachytene chromosome analysis has been completed.

#### Cytogenetics

Haploids can be produced in soybean, but the difficulty in production, e.g., unique mutants, and the low frequency has precluded their use in breeding. Polyploids rarely occur spontaneously but can be produced through chemical treatment, but are not suitable agronomically.

Addition aneuploids, especially primary trisomics, have been very useful to locate genes to specific chromosomes and all 20 unique primary trisomics are known. Soybean can tolerate addition aneuploids but surprisingly the occurrence of deficiency aneuploid plants has been low and they cannot be maintained.

Chromosome interchanges (translocations) have been studied cytologically and have been used to locate genes to specific chromosome arms. Chromosome inversions, both pericentric and paracentric, are found in accessions in *Glycine*. Inversions have not been used in linkage studies.

Extensive cytogenetic studies in the genus *Glycine* have been done with the perennial species. Because of the reservoir of desirable traits in the perennial species, active gene transfer programs from wild perennial species to the cultivated species are of paramount importance.

Soybean has been suggested to be an ancient polyploid based upon chromosome number, genome size, the identification of duplicate loci, high fertility levels (male and female) of primary trisomic plants, and the high levels of transmission of the extra chromosome through both male and female gametes. The duplicated genome(s) and duplicated genes in soybean have been reorganized through processes such as loss of duplicated segments, single-gene duplication via unequal crossing over, chromosome re-patterning, transposition, gene conversion, recombination, divergent evolution, and gene silencing. Collectively, the cytogenetic and genetic evidence supports the view that the cultivated soybean is a diploidized polyploid.

#### **Qualitative Genetics**

The soybean *Genetic Type Collection* maintained by the USDA is a comprehensive tabulation of qualitative genetic traits with gene symbols, phenotypic descriptions, source of mutants, and reference citations. Genetic mutations include resistance to fungi, bacteria, viruses, nematodes, insects, resistance or tolerance to herbicides, response to *Rhizobium*, time of flowering and maturity. Plant growth traits include stem, petiole, inflorescence, leaf form, pubescence type, and seed-coat structure. Genes are known that cause sterility, either complete or partial, of male and/or female gametes. Genes for response to nutritional factors, to pigmentation (seed, leaves, flowers, pod, and pubescence) are known. Many mutations that affect isoenzymes and proteins are documented. Qualitative genetics have played a major role in plant breeding programs for genes for pest resistance.

#### **Molecular Genetics**

The development of a soybean molecular genetic map based upon DNA sequence polymorphisms initially relied upon restriction fragment length polymorphisms (RFLP). Additional classes of DNA markers were developed after the advent of the polymerase chain reaction (PCR) technology. They include simple sequence repeat (SSR) markers, random amplified polymorphic DNA (RAPD) or arbitrary primer PCR (AP-PCR) markers, DNA amplification finger-printing (DAF) markers, amplification fragment length polymorphism (AFLP) markers, and single-nucleotide polymorphism (SNP) markers. The integration of the classical genetic map into the molecular

genetic map, and the association of the markers to their respective chromosome is under development.

The use of molecular techniques in soybean to locate quantitative trait loci (QTL) has received much attention. This type of marker-assisted selection (MAS) has been used to locate seed composition traits, reproductive traits, time of flowering and maturity traits, response to nutritional factors, etc.

#### **Transformation**

Foreign genes can be delivered into soybean via *Agrobacterium*, particle bombardment, and electroporation. The *Agrobacterium*-mediated floral dipping method and the pollen-tube pathway transformation method have not been successful in soybean. Most of the gene transfer methods are tissue-culture based, requiring regeneration of whole plants from transformed cells. Because of the lack of efficient regeneration and transformation procedures, soybean remains recalcitrant for genetic transformation. In addition, the use of *Agrobacterium*-mediated transformation systems shows considerable genotype specificity.

A commercial success with transformed soybean has been with glyphosate herbicide (Roundup<sup>®</sup>) tolerance. Roundup Ready<sup>®</sup> soybean cultivars were grown on  $\sim$ 70% of the acreage in 2002 in the United States and Argentina and have provided the farmer with a new weed control system. At present, Roundup Ready<sup>®</sup> cultivars cannot legally be grown in Brazil.

## **Future Prospects**

Germplasm collections have continued to expand. Many acquisitions are land races or local cultivars, that are being replaced by newly released cultivars. The addition of new wild perennial species continues and the "discovery" of new species seems likely.

Qualitative genetic mutants are continually being added to the USDA's *Genetic Type Collection* and are available to all soybean scientists. Some are of spontaneous origin but many are from mutation programs, e.g., seed fatty acid mutants. A few transgenes (from genetic engineering research) are available.

Gene discovery, through the use of expressed sequence tags (EST) will permit the identification of differential patterns of gene expression. Genome sequencing is the cornerstone of functional analyses. Functional genomics is the process of generating, integrating, and using information from genomics, gene expression profiles (microarrays and chips), and proteomics. This will permit large-scale genotyping of plants in order to understand gene function. The end result will be improved soybean cultivars for food, feed, and industrial uses.

See also: Genetically Modified Grains and the Consumer. Grains Other than Cereals, Nonstarch Polysaccharides. Nitrogen in Grain Production Systems. Soybean: Agronomy; Processing; Soy Concentrates and Isolates; Soy-Based Fermented Foods; Soymilk, Tofu, and Okara.

## **Further Reading**

Birt DF, Hendrich S, Alekel DL, and Anthony M (2004) Soybean and the prevention of chronic human disease. In: Boerma HR and Specht JE (eds.) *Soybeans, Improvement, Production, and Uses*, Agron. Monogr. 16, 3rd edn., pp. 1047–1117. WI: ASA, CSSA, and SSSA.

Burton JW (1987) Quantitative genetics: results relevant to soybean breeding. In: Wilcox JR (ed.) *Soybeans: Improvement, Production, and Uses*, Agron. Monogr. 16, 2nd edn., pp. 211–247. Wisconsin, USA: ASA, CSSA, and SSSA.

Carter TE, Jr, Gizlice Z, and Burton JW (1993) Coefficientof-parentage and genetic-similarity estimates for 258 North American soybean cultivars released by public agencies during 1945–88. USDA Technical Bulletin Number 1814, 169pp.

Carter TE, Jr, Nelson RL, Sneller CH, and Cui Z (2004) Genetic diversity in soybean. In: Boerma HR and Specht JE (eds.) *Soybeans: Improvement, Production, and Uses*, Agron. Monogr. 16, 3rd edn., pp. 303–416. Wisconsin, USA: ASA, CSSA, and SSSA.

Fehr WR (1987) Breeding methods for cultivar development. In: Wilcox JR (ed.) Soybeans: Improvement, Production, and Uses, Agron. Monogr. 16, 2nd edn., pp. 249–293. Wisconsin, USA: ASA, CSSA, and SSSA.

Hymowitz T (2004) Speciation and cytogenetics. In: Boerma HR and Specht JE (eds.) *Soybeans: Improvement, Production, and Uses*, Agron. Monogr. 16, 3rd edn., pp. 97–136. Wisconsin, USA: ASA, CSSA, and SSSA.

Hymowitz T and Bernard RL (1991) Origin of the soybean and germ plasm introduction and development in North America. In: Shands HL and Wiesner LE (eds.) pp. 148–164. Special Publication No. 17. Wisconsin, USA: CSSA.

Hymowitz T and Singh RJ (1987) Taxonomy and speciation. In: Wilcox JR (ed.) *Soybeans: Improvement, Production, and Uses*, Agron. Monogr. 16, 2nd edn., pp. 23–48. Wisconsin, USA: ASA, CSSA, and SSSA.

Lewers KS and Palmer RG (1997) Recurrent selection in soybean. *Plant Breeding Reviews* 16: 275–313.

Lusas EW (2004) Soybean processing. In: Boerma HR and Specht JE (eds.) *Soybeans, Improvement, Production, and Uses*, Agron. Monogr. 16, 3rd edn., pp. 949–1045. WI: ASA, CSSA, and SSSA.

Orf JH, Diers BW, and Boerma HR (2004) Genetic improvement: conventional and molecular-based strategies. In: Boerma HR and Specht JE (eds.) *Soybeans, Improvement, Production, and Uses*, Agron. Monogr. 16, 3rd edn., pp. 949–1045. WI: ASA, CSSA, and SSSA.

Palmer RG and Kilen TC (1987) Qualitative genetics and cytogenetics. In: Wilcox JR (ed.) Soybeans: Improvement, Production, and Uses, Agron. Monogr. 16, 2nd edn., pp. 135–209. Wisconsin, USA: ASA, CSSA, and SSSA.

Palmer RG, Hymowitz T, and Nelson RL (1995) Germplasm diversity within soybean. In: Verma DPS and Shoemaker RC (eds.) *Soybean Biotechnology*, pp. 1–35. London: CAB International.

Palmer RG, Gai J, Sun H, and Burton JW (2001) Production and evaluation of hybrid soybean. *Plant Breeding Reviews* 21: 263–307.

Palmer RG, Pfeiffer TW, Buss GR, and Kilen TC (2004) Qualitative genetics. In: Boerma HR and Specht JE (eds.) Soybeans: Improvement, Production, and Uses, Agron. Monogr. 16, 3rd edn., pp. 137–233. Wisconsin, USA: ASA, CSSA, and SSSA.

Parrott WA and Clemente TE (2004) Transgenic soybean. In: Boerma HR and Specht JE (eds.) *Soybeans, Improvement, Production, and Uses*, Agron. Monogr. 16, 3rd edn., pp. 949–1045. WI: ASA, CSSA, and SSSA.

Shoemaker RC, Cregan PB, and Vodkin LO (2004) Soybean genomics. In: Boerma HR and Specht JE (eds.) Soybeans, Improvement, Production, and Uses, Agron. Monogr. 16, 3rd edn., pp. 949–1045. WI: ASA, CSSA, and SSSA.

Singh RJ and Hymowitz T (1999) Soybean genetic resources and crop improvement. *Genome* 42: 605–616.

Sonka ST, Bender KL, and Fisher DK (2004) Economics and marketing. In: Boerma HR and Specht JE (eds.) Soybeans, Improvement, Production, and Uses, Agron. Monogr. 16, 3rd edn., pp. 949–1045. WI: ASA, CSSA, and SSSA.

Wilcox JR (2004) World distribution and trade of soybean. In: Boerma HR and Specht JE (eds.) *Soybeans, Improvement, Production, and Uses*, Agron. Monogr. 16, 3rd edn., pp. 949–1045. WI: ASA, CSSA, and SSSA.

Wilson RF (2004) Seed composition. In: Boerma HR and Specht JE (eds.) Soybeans, Improvement, Production, and Uses, Agron. Monogr. 16, 3rd edn., pp. 949–1045. WI: ASA, CSSA, and SSSA.

Wilson RF, Burton JW, Novitzky WP, and Dewey RE (2001) Current and future innovations in soybean (*Glycine max* L. Merr.) oil composition. *Journal of Oleo Science* 50: 353–358.

#### **Relevant Websites**

http://soybase.org — Soy Base home page. USDA-ARS soybean genetics and genome database. This website gives quick access to the latest news about databases, links to other soybean and legume sites and many other items of interest to soybean researchers.

http://www.soygenetics.org — Soybean Genetics Newsletter. The information in the articles is to stimulate thought and to exchange ideas among soybean scientists.

http://www.comparative-legumes.org — Legume Information System (LIS). This website gives quick access to other legume databases and contains comparative information.

http://www.legumes.org – Legumes. This website links to many legume databases.

# **Agronomy**

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Soybean (*Glycine max* (L.) Merr.) is the world's most important oilseed crop. Cultivated soybean originated in China ~5000 years ago, and was originally used as a forage crop when imported into the United States ~80 years ago. During the late 1940s and 1950s soybean became more valuable as an oilseed crop in the Midwest when oil extraction facilities were built to separate the oil from the meal in the seed. Soybeans now are typically grown as an annual summer crop with the ability to fix nitrogen in association with the bacteria *Bradyrhizobium japonicum*. This nitrogen replaces the need to use nitrogen fertilizer on the crop and, therefore, reduces the cost of production.

Soybean production in the United States has been at or near record levels in recent years. The upper Midwest produces 70–90% of the soybeans in the United States. Iowa and Illinois are the leading producers, but production spans 29 states east to west from Delaware to Nebraska, and north to south from the Dakotas to Texas. In 2002, the United States accounted for ~38% of the world's soybean production annually, followed by Brazil (28%), Argentina (17%), and China (9%). This article considers how soybean growers are managing the crop to produce high yields, how lack of soil moisture limits productivity, and how genetic and environmental factors affect the chemical composition of the soybean seed, which determines its value in the marketplace.

# **Managing the Crop for High Yield**

#### **Planting Options**

Soybean crop management includes all activities of crop production that are controlled by the producer.

These activities include everything from soil tillage prior to planting through harvesting and storage. Today about one-fourth of the soybeans in the United States are planted without prior soil tillage (no till). The majority of the soybean crop is planted into soil that has received minimum tillage, which leaves variable amounts of residue on the soil surface to reduce soil erosion by rainfall. Studies have shown that soybean grain yield differences are minimal when no-till and minimum tillage systems have been compared.

Soybean is usually grown as a sole crop (one crop per year on the same field) and is planted in the spring of the year in temperate areas. Temperature of the soil determines the planting date of soybean. Soil temperature near the seed must be 8-10°C before soybean seed will germinate. Therefore, soybean is planted between April and June in the Midwestern United States, and most other northern latitude production areas in the world. In temperate areas of the southern hemisphere, soybean is planted between October and December. Tropical and subtropical production areas are able to plant any month of the year if water is available from rainfall or irrigation, because soil temperatures in these regions usually do not limit soybean plant growth.

Soybean yield responds to the date of planting and row spacing. Early planting from late April to mid-May results in the highest grain yield of soybeans grown in Iowa. June plantings result in lower yields because of a shorter growing season. The machine used and method of planting control row spacing of the soybean crop. A grain drill can plant seeds in narrow rows, 25 cm or less apart. A typical row crop planter can range from 25 cm to more than 100 cm between rows. In Iowa, intermediate row spacings (25-75 cm) produce the highest soybean yields. If the seed is planted by hand, row spacing may be determined by the previous crop. In Thailand, for example, soybean is frequently planted following rice harvest. So the soybean is hand-planted adjacent to the rice plant stubble.

Seeding rate also is an important management variable for high yield. Seeding rate is the number of seeds sown per area of land. The loss of plants during germination or later in the growing season due to insects, diseases, weed competition, or other stresses can amount to 15-25% of the seeding rate. Harvest population is the most important plant density measurement, since this represents the plant stand that produces the grain yield. If moisture and fertility are adequate, and the stand loss is normal, the optimum seeding rate is ~400 000 plants per hectare. However, soybean is very adaptable to different growing conditions and can produce similar grain yields across a wide range of seeding rates. In Iowa studies, soybean yield was not significantly different for harvest populations between 280 000 and 500 000 plants per hectare.

Planting depth of the seed is also an important management consideration. The recommended planting depth is 3-5 cm below the soil surface. However, exceptions occur with different soil types, moisture content of the soil, and soil temperature at seeding depth. Cool, light-colored soils are usually slow to warm to the desired temperature for germination, so planting the seed closer to the surface is desirable. Seeds planted less than 3 cm from the soil surface, however, might not obtain sufficient moisture to germinate. Darker-colored soils tend to warm more rapidly than light-colored soils. When soybean seed is planted into no-till environments, the soil is frequently covered with light-colored crop residue, which reflects radiation and slows soil warming. In such conditions, emergence may be delayed and plant stands reduced due to insect or disease damage to the seed or seedling. Deeper planting might be justified to place the seed closer to moisture, if the upper 5 cm do not contain sufficient moisture for seed germination. Planting soybeans more than 5 cm below the soil surface, however, may lead to poor stands.

# **Pest Management**

Pest control during the growing season is critical to achieve maximum grain yield. Weed control may be achieved by crop rotation, mechanical cultivation, or herbicide application. Herbicides have been the most popular method of weed control for many years. Recent developments in molecular genetics have resulted in the development of herbicide-resistant soybean varieties that allow the soybean plants to survive applications of the herbicide glyphosate. This development allows the producer to apply glyphosate over the soybean crop and control most weed species without damaging the soybean plants. This technology has provided the producer with a simple way to control weeds, and  $\sim 80\%$  of the soybean area in the United States is sown with glyphosate-resistant soybean varieties. In Argentina, between 90% and 95% of the area is sown with cultivars that are resistant to glyphosate herbicide.

Disease control in soybean also is essential and can be achieved with a combination of genetic resistance, crop rotation, pesticides, or biological control. A number of disease organisms can attack the soybean plant during the growing season. The damage caused by diseases may affect the ability of roots to absorb moisture and nutrients, or limit leaf area development, resulting in reduced yield potential. Genetic

resistance is the preferred method to control the impact of disease infestations, since chemical methods must be timely and often repeated.

Insects may also cause damage to the soybean crop. The severity of insect damage depends on the stage of plant growth when damage occurs and the intensity of the infestation. Damage to the roots, leaves, stems, and pods are common and may reduce the ability of the plant to function properly. Mechanical damage may open avenues into the plant for disease pathogens to enter and cause additional plant stress. The intensity of insect damage to a soybean crop varies from year to year due to differences in environmental conditions, the reproductive capability of the insect, and whether the insect is a vector for viruses such as soybean mosaic virus or bean pod mottle virus.

The soybean cyst nematode is the most severe pest to the soybean yields in the upper Midwestern United States. Other nematode species attack soybeans in other production areas of the country. Visual symptoms of soybean cyst nematode feeding usually do not occur until late in the growing season when leaves turn yellow prematurely. Unless a soil sample is tested for nematode eggs, the producer might not be aware of a nematode problem. If the nematode egg population in the soil is high, the yield loss may be 50% or greater. Crop rotation will interrupt the multiplication of cysts, but genetic resistance is the most successful control for this damaging pest.

### **Harvesting Considerations**

Soybean harvesting and storage are also management practices, which must be carefully controlled to ensure high yields. Unless care is taken to adjust the harvesting combine correctly for the environmental conditions and seed size, seed will be lost unnecessarily during the harvesting process. Slower ground speed of the combine will reduce harvest losses. Many producers store the soybean grain after harvest for sale at a later date. In such cases, yield can be lost during storage due to seed respiration, physical deterioration, or diseases. Therefore, care must be taken to store the grain in clean dry conditions so the seed quality does not deteriorate, and quantity remains stable until the moment of sale.

Soybean crop management for high yield is very complex and these management practices challenge the producer, but they must be integrated into a production system to produce the maximum potential genetic yield of each variety grown. Failure to carry out all the recommended crop management practices may result in reduced yield and profit for the producer.

## **Managing Water Use for High Yield**

Despite proper soil testing, seedbed preparation, cultivar selection, weed control, and other management options, biotic, and abiotic stresses can result in partial or near-total failure of a soybean crop. On a worldwide basis, an adequate and timely water supply is the condition most likely to limit yield of soybean or other crops. In this section, we will review the importance of water supply to soybean growth and yield, and discuss management options for ameliorating yield loss due to drought.

A useful framework of evaluating soybean grain yield  $(kg ha^{-1})$  in water-limited environments is that grain yield  $(Y, g m^{-2})$  is the product of three largely independent entities according to eqn [1]:

$$Y = T \times WUE \times HI$$
 [1]

where T is cumulative crop transpiration from emergence to physiological maturity  $(l\,m^{-2})$ , WUE is the water use efficiency (g plant mass  $l^{-1}$  water transpired), and HI is the harvest index  $(g\,g^{-1})$  (proportion of grain mass to total shoot mass at maturity). Management strategies or genetic traits that increase any of these three determinants of grain yield likely will increase the yield under drought conditions. Conversely, if transpiration, WUE, or harvest index are not affected by management strategies or by a genetic trait, the trait is unlikely to have an impact on yield in water-limited environments. Each of these determinants will be examined for ways in which they may be improved, through either genetic traits or crop management.

#### **Increasing Water Use for Transpiration**

Equation [1] indicates that yield is proportional to the amount of water transpired by the crop, provided that WUE and harvest index remain fairly constant. Several management strategies can play important roles in increasing the amount of water available for crop transpiration. For some soils, soil-water storage and infiltration can be increased by deep tillage in the fall and by residue management. Old root channels and crop residue from no-till systems increase water infiltration and water-holding capacity of soil and function as a mulch for the soil surface, which decreases evaporative losses. Decreasing tillage by sowing soybean in a stale seedbed conserves a considerable amount of soil moisture that can be utilized during later stages of crop development. In Iowa, an estimated 20-30 mm of soil moisture is lost from the seed zone during traditional spring cultivations compared to less than 6 mm lost from a no-till system. Evaporation of water from the soil surface may account for up to one-half of the total amount of evapotranspiration for a soybean crop. Rapid canopy closure, by using narrow rows and high population densities, decreases evaporative losses from the soil surface, which increases the amount of water available for crop growth and yield.

One determinant of the quantity of water available to a crop is rooting depth. If the rooting depth (D) is known for a particular site, then the total amount of available soil water can be estimated as  $D \times 0.13$ . The value of 0.13 was derived from an extensive survey of soils across the US, and represents the difference in the volumetric water content of soil at field capacity and when the soil is very dry and plants are dead or dormant. This relationship was similar across all soil textural classes except those in which the sand content was greater than 0.55. For sandy soils, the difference between the volumetric water content at field capacity and when plants were dead and dormant was considerably less than 0.13.

As a soil begins to dry due to evapotranspiration, crop photosynthesis and other physiological processes are generally unaffected until  $\sim$ 0.65 of the available soil water is depleted. A critical water deficit, defined as the volumetric water content at which physiological processes and yield begin to decrease, can be estimated from eqn [2]:

$$CWD = D \times 0.13 \times 0.65$$
 [2]

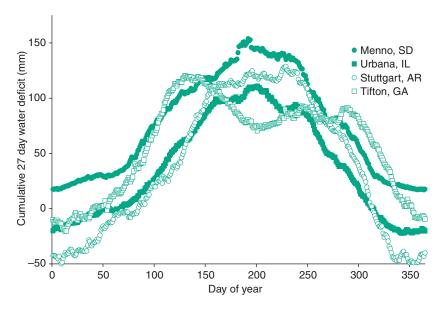
where CWD is the critical water deficit (mm) and *D* is the rooting depth (m) of the crop. The difference between rainfall and potential evapotranspiration is used to estimate the crop water deficit for each day.

By summing the daily water deficits, one can evaluate when water deficit approaches a critical value for a specific site.

Figure 1 shows the long-term averages of the 27 day running sum of water deficit for each day of soybean production at four sites in the United States. Although these sites are geographically and climatologically distinct, the average water deficit exceeds 110 mm in each case. Avoiding a critical water deficit in an "average year" at these sites would require a rooting depth of at least 1300 mm (eqn [2]). For many sites, roots are unable to reach such depths. For example, in the mid-southern US, irrigation is generally scheduled when the critical water deficit reaches 37–50 mm, which corresponds to a rooting depth of 438–592 mm.

Genetic differences among soybean lines offer an important means of increasing rooting depth in specific environments. Variation has been found in soybean for deeper root penetration in soils with high aluminum content. High soil aluminum is generally associated with low soil pH and estimated to affect  $\sim$ 40% of the arable land worldwide. Although the upper 15–20 cm of a soil may be limed to decrease soil aluminum, subsurface pH is generally unaffected by liming and often restricts soybean rooting depth.

In screening for drought tolerance among soybean plant introduction lines, scientists from the USDA discovered that line PI416937 had delayed wilting when grown on a sandy soil under nonirrigated conditions. The ability of PI416937 to maintain leaf turgor during drought was partially due to its tolerance for high soil aluminum. Genetic studies



**Figure 1** Cumulative 27 day water deficit for each day of year based upon long-term weather data at four sites in the US. Water deficit was calculated as the difference between precipitation and potential evapotranspiration using a centered 27 day running sum.

show that aluminum tolerance is a quantitative trait in soybean, and there are numerous genes associated with aluminum tolerance.

In addition to its greater tolerance to aluminum, PI416937 has a taproot that penetrates compacted soil layers more readily than does the taproot of the aluminum-sensitive cultivar "Weber." The basal roots of Weber, however, penetrated compacted soil layer more easily than did those of PI416937. To realize the full benefit of deep rooting, it would be desirable to combine the ability of both tap and basal roots to penetrate compacted soil layers. In rice, regions of the genome have been identified that are associated with the ability of roots to penetrate an impervious soil layer. Given the genetic diversity in soybean for this trait, it might be possible to use genetic marker techniques to select plants with enhanced capacity for root penetration.

#### **Increasing WUE**

Open stomata on the leaf surfaces allow CO<sub>2</sub> to diffuse from the atmosphere to the sites of carboxylation in the chloroplasts. These same stomata allow the diffusion of water out of the leaf (transpiration). Thus, transpiration is inseparably linked with photosynthesis; the ratio of these two processes is a measure of water use efficiency. Because of the close coupling between water use via transpiration and carbon gain via photosynthesis, even small improvements in WUE could have large effects on the drought tolerance of sovbean.

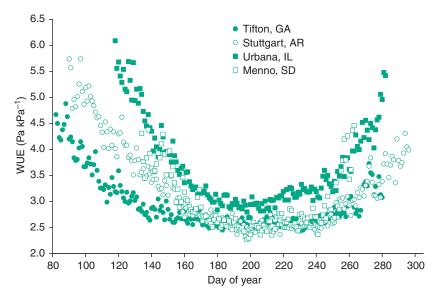
WUE can be roughly divided into a biological component and a meteorological component. The biological component regulates the stomatal conductance for gas exchange between the atmosphere and the leaf, and it determines the CO<sub>2</sub>-diffusion gradient from the atmosphere to the chloroplast. Intuitively, if the photosynthetic efficiency of soybean is increased in the chloroplast, the CO2 concentration in the chloroplast will decrease, resulting in a greater CO<sub>2</sub>concentration gradient from the atmosphere to the chloroplast and an increase in WUE. It should be noted that leaf photosynthesis would increase if stomatal conductance increases or remains constant with a decrease in the CO<sub>2</sub> concentration inside the leaf. It is precisely this mechanism that provides species having the C4 photosynthetic pathway (e.g., maize, sugarcane, and millet) greater WUE than species having the C3 photosynthetic pathway (such as soybean). If a decreased CO<sub>2</sub> concentration inside the leaf is associated with a substantial decrease in stomatal conductance, WUE will be increased but leaf photosynthesis may remain unchanged or could even decrease.

There are differences among soybean genotypes in this biological component of WUE. For example, the efficiency of the cultivar Young is  $\sim 25\%$  greater than that of the aluminum-tolerant line PI416937 mentioned earlier. Crosses between parents differing in WUE have been used to identify molecular markers associated with this trait. A large-scale breeding program in Australia has led to the commercial release of drought-tolerant wheat with increased WUE. Although a comparable effort has not been undertaken in soybean to increase yield per unit of water used, the success of the wheat breeding program in Australia gives clear validity for this approach and encouragement that a similar improvement can be made in soybean.

The meteorological component of WUE is determined by the concentration gradient of water vapor from inside the leaf to the atmosphere. Water vapor concentration inside a leaf is saturated at any given temperature; this saturated water-vapor pressure increases exponentially as temperature increases. Therefore, WUE at the leaf level will be higher under relatively cool and humid growing conditions. Figure 2 illustrates the daily pattern of WUE for soybean leaves on plants grown at four locations. The values are calculated using long-term weather data and assuming the biological component was constant. This figure illustrates that WUE is approximately twice as great early in the growing season relative to the middle of summer. Thus, for locations where drought is common during the later portion of the growing season, early sowing of early maturing cultivars would not only avoid the drought but it also increases WUE due to the cooler temperatures early in the season.

# **Increasing Harvest Index**

Drought during different stages of sovbean development affects yield and yield components differently. As such, drought can increase, decrease, or not affect the harvest index, depending on when it occurs. Severe drought during vegetative stages that is relieved during reproductive stages may decrease the total amount of plant mass produced and also decrease the yield, but may have little impact on harvest index. Drought during flowering and early stages of pod formation that is relieved during pod filling will decrease the number of seeds produced, but the decrease in seed number is often compensated (at least partially) by an increase in the mass of individual seeds. Under these conditions, harvest index often increases. Plants exposed to drought during seed filling generally produce a large number of seeds, but the crop matures prematurely,



**Figure 2** Average WUE vs. day of year during the growing season for Tifton – GA, Stuttgart – AR, Urbana – IL, and Menno – SD. (Adapted from Purcell *et al.* (2003) Drought avoidance assessment for summer annual crops using long-term weather data. *Agronomy Journal*, Nov/Dec issue.)

resulting in smaller seeds and a decrease in harvest index.

Equation [1] indicates that yield under drought conditions will be increased by maintaining a high HI. Practices such as minimum tillage, disruption of hard pans, decreasing evaporation from the soil surface by high population density and residue management, and increased soil—water infiltration are effective because they increase the amount of water available during seed growth and prevent the premature senescence associated with drought. In these cases, water conserved during vegetative development results in an increased water supply during seed filling, which results in increased harvest index.

Direct selection for high harvest index has generally not been used as a means to increase yield of rain-fed soybean. There are uncertainties in quantifying plant biomass at the end of the season. Considerable additional effort is required to determine harvest index for each breeding line — its values can be greatly affected by the stage of development at which drought occurs. Nevertheless, if variations exist among soybean lines for their capacity to remobilize vegetative dry matter into seed mass during drought, then selection for higher harvest index might be useful in identifying lines with improved drought tolerance.

#### **Nitrogen Nutrition and Drought Response**

To produce a grain yield of  $4000 \text{ kg ha}^{-1}$  requires  $\sim 260 \text{ kg}$  of elemental N ha<sup>-1</sup> for grain and approximately 40 additional kg N ha<sup>-1</sup> for the rest of the plant. Depending upon the soil organic matter and

previous cropping history, nitrogen fixation will supply from 40% to 90% of the crop's nitrogen requirement. As such, application of high rates of nitrogen fertilizer is rarely a viable economic option. Nevertheless, increasing the drought tolerance of nitrogen fixation to the same extent as inorganic nitrogen uptake and assimilation would be expected to increase yields. This response likely occurs because nitrogen fixation is more sensitive to drought than is photosynthesis and many other physiological processes. Studies conducted under dryland conditions, for example, show that soybean yield increased from  $2373 \text{ kg ha}^{-1}$  in the absence of nitrogen fertilizer to  $2798 \text{ kg ha}^{-1}$  when fertilized with  $336 \text{ kg N ha}^{-1}$ . Yield of the well-irrigated treatments did not respond to the additional nitrogen fertilizer. Other field experiments have shown that nitrogen accumulation decreases more than plant mass accumulation under drought stress, confirming the greater sensitivity of nitrogen fixation than photosynthesis to drought.

Fortunately, there is genetic variability in soybean for the sensitivity of nitrogen fixation to drought. "Jackson" was the first cultivar to exhibit prolonged nitrogen fixation during drought compared to several other cultivars of the time. Since then, eight additional plant introduction lines have shown the capacity for prolonged nitrogen fixation under drought conditions. These genetic lines could provide an important resource for crop improvement under drought conditions.

In summary, the linkage between photosynthesis and transpiration couples the amount of water available for transpiration to increasing yield under

drought conditions. This may include management and genetic options such as no-till production, high population density and early season vigor, deep tillage, aluminum tolerance, and deep rooting. Similarly, the efficiency with which water is used to produce plant mass and yield (i.e., WUE) could be increased where possible by confining the majority of the cropping period to the cooler portion of the season and by development of cultivars with high efficiency for water use. Increasing the amount of water available for transpiration during grain filling and using water more efficiently prevents the accelerated decline in senescence observed with late-season drought and maintains a high harvest index. Finally, genetic diversity in the tolerance of nitrogen fixation to water deficit offers an additional means to slow the senescence of leaves in response to late-season drought, and thereby increase soybean yield.

# **Managing Seed Composition**

The two main commercial products of soybean seed are oil and protein. Accumulation of these components within the seed depends on key metabolic processes that are required for the successful production of the crop. Carbohydrates used for oil production are derived from photosynthesis; nitrogen, which is often the limiting nutrient for protein production, is derived from a combination of inorganic nitrogen sources and symbiotic nitrogen fixation. It is well known that genetic, climatic, and management variables contribute to the observed variability in seed composition and, therefore, the value of the crop for a specific end use. There is limited understanding, however, regarding the interactions between these environmental and genetic factors that determine the levels and quality of oils, proteins, and other seed constituents accumulated by the soybean seed.

#### **Geographic Variation**

The protein concentration of soybean seed produced in the US averages  $\sim$ 40.5%. There is wide variation, however, in seed protein across growing regions and between years within regions. Early studies on the geographic pattern of oil and protein levels of soybean seed in the United States showed that oil increased and protein decreased from the northern to the southern Midwest. Soybeans grown in northern and northwestern states typically have  $\sim 0.5\%$  higher oil and 1.5– 2.0% lower protein percentages than those grown in southern states. Protein content tends to be higher and oil lower in the southeast and Delta states compared to the Midwest. Results from many field studies suggest that temperature during seed filling accounts for much of the variation in seed composition, when other factors are fairly constant. Over large regions, however, factors such as water stress, tillage, soil acidity, soil nitrogen level, and previous crop history may be important determinants of final seed composition.

Breeding studies indicate that an increase in seed oil content and decrease in protein content has accompanied selection for high yield in short-season cultivars typically grown in the upper Midwest. These observations suggest that genetics, in addition to climate, contribute to the geographic variability in seed composition across the US, and may be an important factor determining the lower seed protein concentrations observed in northern growing areas. The differences in composition between geographic areas are large enough to affect pricing and marketing of the crop.

## **Accumulation of Seed Components**

The developmental patterns of the major storage compounds in soybean seeds, protein, and oil are well documented. There is little accumulation of storage components during the initial phase of seed development, often termed the "lag phase," during which cotyledon cell numbers and the metabolic machinery for storage product synthesis increase. During the subsequent linear phase of seed growth, however, they accumulate rapidly in specific organelles called protein and lipid bodies. Cells of the cotyledons continue to accumulate storage compounds as long as their volume can increase. Once at maximum cell volume, the embryo begins to mature, metabolism of seed reserves slows, and the embryo prepares for desiccation.

Protein concentration (g kg<sup>-1</sup> dry weight) typically increases during the early cell division stage of seed development, and then remains fairly stable during the remainder of seed development. Final protein concentrations vary widely among soybean genotypes from 25% to over 50% of the final seed dry weight. Non-protein nitrogen comprises ~20% of the seed nitrogen during early seed development, and decreases rapidly as the seed develops to less than 5% at maturity. The oil concentration is low (3-5%) during early seed growth then increases rapidly to reach its maximum value when seed fill is 50-75% complete. Final oil concentrations vary from 14% to 25% among soybean genotypes.

The developing seeds obtain carbon from current photosynthesis, remobilized carbohydrates stored in vegetative plant parts, and carbon skeleton of nitrogen compounds delivered from the nodules or proteins hydrolyzed during leaf senescence. Likewise, the growing soybean seed can obtain nitrogen from

dinitrogen fixation, nitrogen uptake from the soil, and nitrogen remobilized from vegetative plant parts (i.e., leaves, stem, root, and pod wall). The ability of soybean to use nitrogen from either nitrogen uptake, dinitrogen fixation, or remobilized nitrogen from vegetative tissues gives the plant much flexibility to provide the seed with nitrogen. Seed protein can decrease when these three sources together cannot meet seed demand for nitrogen. Supplying supraoptimal levels of nitrogen in a hydroponics system has been shown to increase seed protein content, even in high protein lines. These increases, however, decreased grain yield. Apparently, the high seed protein trait (>40% of final DW) was not due to greater accumulation of nitrogen in the vegetative plant or greater nitrogen mobilization from vegetative tissues to the seed. Normal and high seed protein lines studied to date do not differ significantly in these attributes. Also, varying soil nitrate levels may alter the proportion of nitrogen in the seed derived from nitrogen fixation and remobilized nitrogen, but the source of nitrogen has no effect on the final nitrogen content or dry weight of the seed. *In vitro* studies using isolated embryos confirm that final seed composition is not simply a function of nitrogen supply to the plant or assimilate supply from the plant to the seed. Final seed protein concentration of high-protein genotypes is routinely greater than that of a normal-protein genotypes, regardless of the concentration of nitrogen supplied in the culture medium. Together, these results imply that the final composition of the seed is regulated to a large extent within the developing seed itself.

#### **Temperature during Seed Filling**

An important problem faced by soybean producers in the upper Midwest US is that the popular, highyielding soybean varieties generally do not accumulate high levels of protein and oil in the seeds. Beans produced in Iowa, for example, contain ~35% protein and 18.5% oil, on average. This combination of protein and oil at the minimum processors can accept to make animal feeds. Wide variation in seed component levels across the region has made it very difficult for processors to meet industry standards for meal quality. As a consequence, soybean producers in the upper Midwest US often are paid less for the grain they deliver to the elevators. This situation will become even more problematic for soybean growers in the region as commodity markets shift to component-based pricing in the future.

Data from state and federal variety trials, and commodity reports often indicated a general correspondence between low seed protein content and low temperatures during seed filling. This correspondence suggests that the problem of poor seed composition in soybean might be a cold-stress response. Recent studies indicating genotypes that typically produce seed with 32-34% protein in the field have the genetic potential to accumulate up to 40% seed protein when grown at above-normal temperatures (35°C) during seed fill. Both rate and duration of dry weight, protein, and oil accumulation were affected by temperature. In general, rate of accumulation was a better predictor of final oil and protein concentration than was duration of filling. For the early maturing variety "Evans," the rate of oil accumulation (g/plant) was greatest when the day/night temperature was 20°C/12°C and decreased linearly with increasing temperature.

The effect of temperature on final seed protein and oil concentration is expressed within the seed itself. The plant supplies the seed with carbon in the form of sugars such as sucrose and amino acids such as glutamine. Because there are no direct vascular connections between the seed and the maternal plant, the embryo is in essence bathed in a solution of nutrients supplied by the plant. Using an *in vitro* system, which mimics this bathing solution, it has been determined that capacity of the developing embryo to take in sucrose and glutamine from this solution does not limit the accumulation of protein at low temperature. As temperature increases, the rate of sucrose and glutamine uptake also increases. But the ratio of sucrose to glutamine uptake remains at about the same maximum value (~2.2 mol sucrose/mol glutamine) regardless of the growth temperature. These results indicated that soybean possesses adequate capacity for sugar and amino acid uptake at low temperature, and that the ratio of uptake of protein and oil precursors did not dictate the final composition of the seeds.

Most of the carbon deposited into protein is derived from sucrose. A significant amount of carbon from amino acids is metabolized into oil. Thus, carbon from glycolysis is available for protein synthesis, and carbon skeletons from amino acids are available for oil synthesis presumably via the Krebs cycle. *In vitro* studies have shown that the temperature during seed filling has a direct impact on the uptake and metabolism of protein and oil precursors supplied from the plant. At low temperature most of the glycolytic carbon is diverted into oil. At higher temperatures, most of the carbon is diverted into protein. Apparently, the response of oil accumulation to temperature during early reproductive growth reflected a shift in metabolic capacity for oil synthesis, and was not simply a direct effect on the rate of oil biosynthesis. This increase in protein synthesis with temperature may reflect a temperature-dependent

#### **Water Supply**

Seed protein concentration increases have been observed in water deficit conditions and soils with root limiting factors such as low subsoil pH or fragipans. The response of seed oil and protein concentration to water deficit, however, varies with the time and duration the water deficit occurs. Early seed-fill water deficit with favorable conditions during the remainder of the season decreases seed protein while increasing oil. High mean temperatures above 25°C, often associated with water deficit, typically increase the final protein concentration in the seed. Water deficit late in seed fill increases protein and decreases oil concentrations. An index method between irrigated and non-irrigated treatments has been used to show that oil content was more sensitive to water stress than protein content. The varied response of seed protein and oil to the timing of water deficit probably explains why, in the literature, there are poor correlations between rainfall and final seed protein and oil concentration.

## **Management Factors**

Seed protein concentration for a given genotype may vary by 8% or more across environments. In addition to the response to climatic conditions, management factors such as soil inoculation, planting date and pattern, and soil fertility also can influence seed composition. Symbiotically fixed nitrogen and inorganic nitrogen are required for maximum yield and high protein seed. A host of soil factors has been reported to affect Bradyrhizobium japonicum and nitrogen fixation: soil pH, soil organic matter content, initial soil nitrate level, soil temperature, and soil moisture supply. High soil nitrogen from either mineralization of organic matter or addition of inorganic fertilizers decreases nodule weight, nodule number, weight per nodule, and rate of nitrogen fixation. Poor nodulation reduces plant and seed nitrogen concentrations and yield. Using "superior rhizobium strains" can improve yield by increasing vegetative growth and pod number. The benefit to yield formation, however, is realized prior to seed fill. Dinitrogen fixation rates during seed fill reportedly are similar between the superior and indigenous strains, suggesting that dinitrogen fixation during seed fill was limited by factors other than rhizobia strain. While inoculating seed with rhizobia does not always increase grain yield, stimulating indigenous rhizobia to initiate nodule formation in cool soils has proven to be an effective means to increase yield and seed protein content. By inoculating seeds with rhizobia incubated with the soybean isoflavone, genestein, researchers in Canada increased nodule formation, total seasonal nitrogen fixation, and seed protein content on field-grown plants. These results clearly indicate the benefits of establishing nitrogen-fixing activity earlier in the season, but a cost-effective method for doing so is not currently available.

Late planting dates shift the period of seed fill later into the fall when temperatures may be lower and day length shorter. Lower temperatures during early seed development can decrease seed value as total protein plus oil decrease with temperature. Delayed planting reportedly increases seed protein and linolenic acid levels while decreasing the oil and oleic acid levels. Recent reports suggest that the low linolenic acid trait might be amplified by planting these cultivars early (April) in the warmer temperatures of the Southeast. Early planting dates would place flowering and early seed development during warmer periods when the probability of high temperature and water deficit is high.

Soybean seedlings depend on soil nitrogen during the first few weeks of development before nodules develop and dinitrogen fixation commences. As such, "starter" fertilizers can improve early seedling establishment and increase plant growth, which can increase yield if canopy development is otherwise limited. In northern growing areas, responses to starter fertilizer often have been associated with restricted root growth or cool soil temperatures. In southern states, response to starter fertilizer has been evaluated for late July planting of soybean in double-crop systems, which are prone to water deficits. A starter fertilizer of 50 kg ha<sup>-1</sup> on soybean planted late in July, for example, decreased dinitrogen fixation but increased plant dry weight and nitrogen concentration. A vield increase from this starter fertilizer was associated with an increase in seed number per unit area, probably as a result of greater light interception and higher rates of canopy photosynthesis. Yield increases associated with larger seed size have been observed for environments receiving irrigation during seed fill. In one study, a determinate cultivar responded to starter fertilizer by increasing seed nitrogen concentration, while an indeterminate isoline responded with an increase in seed number and vield.

There has been considerable interest in postflowering application of nitrogen fertilizer, especially for late plantings on sandy soils where nodulation is poor due to high soil temperatures or water deficit. The addition of nitrogen fertilizer at flowering or beginning seed has been shown to increase seed yield and seed protein concentration in some cases. Yield decreases, however, also have been observed with postflowering foliar application of nitrogen. Soybean responses to nitrogen fertilizer have been inconsistent probably because of the many interacting climatic and soil factors involved. The changes in seed nitrogen concentration are likely related to the interaction of seed protein with temperature (high or low), water supply, and initial soil nitrogen levels.

In summary, increasing soil nitrogen availability and/or nitrogen fixation rates can lead to an increase in seed yield as a result of increased plant growth. Changes in seed number and yield appear to dominate soybean response to supplement nitrogen with only small changes in seed composition. A soybean crop grown on soils high in organic matter or residual nitrogen have the potential to produce seeds with increased protein concentration in response to late-season nitrogen uptake as the capacity for dinitrogen fixations decreases.

See also: Beans. Carbohydrate Metabolism. Genetically Modified Grains and the Consumer. Genome Mapping. Genomics. Grain and Plants, Morphology. Grain Production and Consumption: Overview. Lipid Chemistry. Nitrogen Metabolism. Nutrition: Soy-Based Foods. Oilseeds, Overview. Proteomics. Soybean: Germplasm, Breeding, and Genetics; Agronomy; Grading and Marketing; Processing; Soy Concentrates and Isolates; Soy-Based Fermented Foods.

### **Further Reading**

Bewley JD and Black M (eds.) (1994) Seeds: Physiology of Development and Germination, 2nd edn. New York: Plenum Press.

Boerma HR and Specht JE (eds.) (2003) Soybean: Improvement, Production, and Uses, 3rd edn. Madison, WI: American Society of Agronomy.

Drackley JK (ed.) (2000) Soy in Animal Nutrition. Savoy, IL: Federation of Animal Science Societies.

Heatherly LG and Hodges HF (eds.) Soybean Production in the Midsouth. Boca Raton, FL: CRC Press.

Hoeft RG, et al. (eds.) (2000) Modern Corn and Soybean Production, 1st edn. Champaign, IL: MCSP Publications.

Kauffman HE (ed.) (1999) Proceedings of the World Soybean Research Conference VI. 4-7 Aug. Champaign, IL: Superior Printing. Norman AG (ed.) (1978) Soybean Physiology, Agronomy, and Utilization. New York: Academic Press.

Purcell, *et al.* (2003) Drought avoidance assessment for summer annual crops using long-term weather data. *Agronomy Journal*, Nov/Dec issue.

Wilcox JR (ed.) (1987) Soybeans: Improvement, Production, and Uses, 2nd edn. Madison, WI: Amer. Soc. Agron.

# **Relevant Websites**

http://www.extension.iastate.edu — How a soybean plant develops.

http://www.soybean.org — Soybean.org. www.stratsoy.uiuc.edu — Stratsoy. www.agron.iastate.edu — The Soy Page.

# **Grading and Marketing**

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#### Introduction

Soybeans vary widely in their appearance and composition. The main constituents of soybeans, in descending order, are protein, oil, complex carbohydrates, oligosaccharides, simple sugars, and minerals. Phytochemicals in soybeans have gained prominence since the 1990s especially isoflavones, saponins, and phenolic acids because of their potential health-protective effects. Soy isoflavones in particular have been associated with a variety of other improved physiological states, including reduced cholesterol levels, anticarcinogenicity, and improved bone health.

Most legumes contain 20–25% protein, but soybeans typically contain 30–45% protein (moisture-free basis), and average ~35% at 13% moisture. Protein-content levels as high as 55% (moisture-free basis) have been observed. The oil content typically ranges from 15% to 24% and averages about 20% on a 13% moisture basis. The composition varies with growing area, for example soybeans from northern and mid-western areas of the United States typically contain 1.5–2% less protein and 0.2% more oil than beans grown in southern states. One Iowa soybean processor has begun offering premiums for soybeans containing higher than normal oil contents.

The total crude fiber content of soybeans is  $\sim$ 4.4% at 13% moisture. These materials are predominantly

cellulose, hemicellulose, and pectin. The outer hull, typically 8% by weight of the bean, is especially rich in crude fiber (35%). Fiber is hard to digest and contributes little to the nutrition of swine and poultry, the primary markets for soybean meal.

The total sugar content of soybeans is 4.9–9.5% at 13% moisture. Of the total sugar content, ~60% is sucrose, 10% raffinose, and 30% stachyose. Raffinose and stachyose are oligosaccharides and cause flatulence (intestinal gas) in humans and reduced feedefficiency in livestock. One seed company has recently developed a soybean line that is low in oligosaccharide content and high in sucrose content.

Isoflavone content of soybeans varies from  $800-4000\,\mu g\,g^{-1}$  on aglycon basis. Soybeans contains 12 isoflavones, namely genistein, daidzein and glycitein, as  $\beta$ -glucosides, 6''-O-malonylglucosides, 6''-O-acetylglucosides, and aglycons. Soy saponin concentrations vary in a similar manner to isoflavones ranging from  $2-12\,\mu mol\,g^{-1}$ . Because of the variety of glucoside forms of saponins and isoflavones, their concentrations should be expressed in  $\mu mol$ . The isoflavones and saponins fractionate with the seed protein and are not lipid-soluble. As soybeans are fractionated into food ingredients, the forms of the isoflavones and saponins are rearranged depending on the extent of heat processing and the method of protein fractionation.

Generally, soybean meal is sold on a 44% protein basis and 48% when soybeans are de-hulled (Hi-Pro meal). The average price of soybeans during the 1997–98 crop year was \$6.47 per bushel. Oil sells for ~2 times the value of meal. Typical selling prices for soybean oil were 25.7 cents per pound and for meal (44% protein) were \$197 per ton (t) or 9.9 cents per pound during 1998. In 2002, crop year prices were considerably lower.

Recent developments in breeding and genetic modification of soybeans have achieved: new fatty acid compositions, e.g., <2% linolenic acid and >80% oleic acid to improve oxidative stability; removal of all lipoxygenase isozymes to improve the flavor of soy protein ingredients; elimination of oligosaccharides to eliminate intestinal gas; altered protein and amino acid composition to improve functional and nutritional properties; and inserted vaccines for human and animal health. While genetic engineering holds great promise for the future (healthier foods, reduced environmental impacts, increased yield, lower input costs, etc.), consumers have begun to question the safety of this new technology.

#### **Proteins**

Glycinin and  $\beta$ -conglycinin comprise 65–80% of the protein fraction or 25–35% of the seed weight.

Glycinin is classified as a legumin, which is characterized by molecular weights of  $300-400\,\mathrm{kDa}$  and sedimentation coefficients of  $11S\pm1S$ .  $\beta$ -Conglycinin is a vicilin, which is in the range  $150-250\,\mathrm{kDa}$ , glycosylated and has sedimentation coefficients of  $7S\pm0.5S$ . In soybeans, the major proteins, glycinin and  $\beta$ -conglycinin, are frequently described by their respective sedimentation values, 11S and 7S, but such fractions often are impure. The 7S fraction of soy protein contains, in addition to  $\beta$ -conglycinin, lectins, lipoxygenase, and  $\beta$ -amylase.

 $\beta$ -Conglycinin is a trimer and/or hexamer in solution and probably occurs in both forms in the seed. Two similar peptides,  $\alpha$  and  $\alpha'$  (57 kDa), and a glycosylated  $\beta$ -peptide (42 kDa) are assembled in the mature protein in a nonrandom set of seven forms,  $\alpha'\beta_2$ ,  $\alpha\beta_2$ ,  $\alpha$   $\alpha'\beta$ ,  $\alpha_2\beta$ ,  $\alpha_2$   $\alpha'$ ,  $\alpha_3$ , and  $\beta_3$  with molecular weights of 125–171 kDa. The  $\alpha$  and  $\alpha'$  subunits have 1–2 mol cysteine/mole peptide while the  $\beta$ -peptide has no cysteine. The health benefits of consuming soy protein are attributed primarily to  $\beta$ -conglycinin.

Glycinin is a hexamer, although older literature calls it a dodecamer. It is composed of six non-randomly paired acidic and basic peptides. The acidic peptides have molecular weights of 44, 37, and 10 kDa; the basic peptides are 20 kDa. The acidic—basic (AB) pairs have been identified in the experimental line, CX635-1-1-1, and are shown in Table 1. Seven acidic and eight basic peptides have been identified in 18 cultivars. There appear to be sulfur-rich and -poor AB pairs.

Soybeans contain two main classes of protease inhibitors or trypsin inhibitors (TI), although there appear to be many isogenic variants. The principal two classes are the Kunitz inhibitor with MW of 21 500 and the Bowman-Birk inhibitor with MW of 8000. The Kunitz inhibitor acts only on trypsin while the Bowman-Birk protein inhibits both trypsin and chymotrypsin. Moist heat treatment denatures ~90% of the TI activity with the residual being heat stable. TI affects animals of guinea-pig-size and smaller but has little effect on larger animals,

Table 1 Glycinin acidic-basic complexes from CX635-1-1-1

AB-complex	S-amino acids	Molecular weight (kDa)
$A_{1a}B_2$	14	57
$A_{1b}B_{1b}$	12	57
$A_2B_{1a}$	14	57
$A_3B_4$	9	62
$A_5A_4B_3$	3	67

Data from Nielsen (1985) Structure of soy proteins. In: Altschul AM and Wilcke HL (eds.) *New Protein Foods, vol. 5. Seed Storage Proteins*, pp. 27–64. New York: Academic Press.

except for weanling pigs. Recently, Bowman-Birk TI has been demonstrated to be an anticarcinogen and is currently in Phase II cancer trials. There are reports that TIs exert a carcinogenic effect on rodents. There has been interest in recovering and purifying soybean protease inhibitors to treat AIDS patients.

The Food and Drug Administration of the United States has approved a health claim for soy protein that as part of a heart healthy diet 25 g per day of soy protein will contribute to improved cardiovascular health.

# Lipids

Soybean lipids contain  $\sim 2-5\%$  phospholipids, depending on the growing conditions, and 1.6% unsaponifiables. The balance is chiefly triacylglycerols.

Oleic (18:1), linoleic (18:2), palmitic (16:0), stearic (18:0), and linolenic (18:3) acids are present in soybean oil along with traces (less than 1%) of myristic (14:0), palmitoleic (16:1), heptadecanoic (17:0), eicosenoic (20:1), arachidic (20:0), behenic (22:0), and erucic (22:1) acids. The range of the acyl groups present in soybean oil has been extended by mutation breeding and selection to the values reported in Table 2.

The acyl groups are distributed asymmetrically in the triacylglycerols with all the saturates on the *sn*-1 and -3 positions and linoleate concentrated on the sn-2 position. Generally, the sn-1 contains more palmitate and stearate than sn-3, and the oleate is enriched on sn-3. Phospholipids contain the same acyl groups found in the triacylglycerols, but the concentration of palmitate is generally higher and oleate lower. Phosphatidyl choline (50%), ethanolamine (26%), and inositol (18%) are the chief phospholipid components along with lower concentrations of phosphatidic acid and phosphatidyl serine.

The unsaponifiables contain sterols, hydrocarbons, and tocopherols. The chief sterols (3.5 mg  $g^{-1}$  oil) are β-sitosterol, campesterol, and stigmasterol. The tocopherols ( $\sim$ 1.25 mg g $^{-1}$  oil) are typically more

Table 2 The range of acyl group percentages produced in soybean lipids by mutation breeding and genetic engineering, and the composition of a typical unselected variety

	Range	Typical value		
Palmitate	3.5–30	10		
Stearate	2.5-32	4		
Oleate	8.0-85	26		
Linoleate	2.0-60	52		
Linolenate	1.8–13	8		

Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 5390, Elsevier Ltd.

than 70% gamma with smaller amounts of delta, and alpha.

# **Methods to Measure Composition**

Proximate analyses for moisture, protein, crude free fat, crude fiber, ash, and total carbohydrate have been adopted by the American Oil Chemists' Society, the American Association of Cereal Chemists, and the Association of Analytical Chemists. All composition values for soybeans are reported either on a moisturefree basis or at 13% moisture.

Several methods are acceptable for moisture determination, but the most widely used procedure involves measuring the weight loss when drying the ground sample for 3 h at 130°C.

Protein is estimated from Kjeldahl nitrogen. The nitrogen content is multiplied by a factor of 6.25 to convert to protein values despite the major soy protein, glycinin containing only 17.5% nitrogen (equivalent to a N conversion factor of 5.7).

Oil content is determined as crude fat by continuously extracting dried ground samples with petroleum ether for 5 h. Total fat, which includes bound fat as well as free fat, requires acid hydrolysis of the sample prior to extraction. In recent years, the use of nearinfrared reflectance (NIR) and transmittance (NIT) has become widespread for rapid estimation of grain composition, especially moisture, protein, and oil. These spectrophotometers must be calibrated against the standard wet chemical methods described above. Moisture is also routinely measured by electrical capacitance.

Crude fiber is measured as the weight loss on incineration of the oven-dried residue remaining after digestion of the sample with boiling dilute sulfuric acid followed by boiling dilute sodium hydroxide.

Ash is primarily composed of noncombustible minerals and is determined by heating the ground sample in a muffle furnace for 2 h at 600°C. Soybeans contain ~4.7% ash at 13% moisture. Total carbohydrate is often estimated as the difference after subtracting other constituents. This method does not discriminate between oligosaccharides and other sugars, and it often gives inflated values. Sugars can be extracted with hot aqueous ethanol and quantified by gas or liquid chromatography.

Acyl group composition of soybean lipids is generally determined by gas chromatography. Lipid classes are separated by thin-layer or liquid chromatograms.

Qualitative and quantitative analyses of the individual proteins have been performed by immunoelectrophoresis and/or SDS polyacrylamide electrophoresis.

pediment to accuracy has been the lack of suitable

standards, although most of the isoflavone forms

# **Grading Standards**

are now available.

The Federal Grain Inspection Service (FGIS) for the United States has established grading standards for soybeans (Table 3) to facilitate marketing and trade. Much more detail than can be provided here is available at the FGIS website http://www.usda.gov/ gipsa/reference-library/brochures/soyinspection.pdf. Soybeans are divided into two classes based on color: yellow soybeans and mixed soybeans. Each class is divided into four numerical grades (US No. 1, 2, 3, and 4) and a US Sample Grade those soybeans, which do not meet the requirements of any of the numerical grades. Special grades (e.g., garlicky and infested) are provided to emphasize special qualities affecting the value, and are added to and made part of the grade designation. Six factors are considered in assessing grade designation: test weight, heat damage, total damage, foreign material, splits, and soybeans of other colors. Although moisture, protein, and oil contents are not part of the official grading standards and do not affect numerical grade, they may be specified on contracts in some markets. NIT is used for rapid estimation of moisture, protein, and oil contents.

Test weight, which is the weight in pounds of grain per Winchester bushel (35.2 l), is determined on a 1<sup>1</sup>/<sub>4</sub> quart (1.18 l) sample before removing foreign material using an official test weight apparatus. If the test weight is extremely low, the soybeans may contain less oil. All other factors are measured as percentages of total sample weight. Foreign material is determined by sieving and is all matter, including soybeans and soybean pieces that readily pass through an 8/64-inch

(3.2 mm) round-hole sieve and all matter other than soybeans remaining on the sieve after sieving. Foreign matter (other grains, weed seeds, pods, leaves, stems, etc.) reduces oil and protein contents and storage life. Splits are soybeans with more than one-fourth of the bean removed and which are not damaged, and are determined by sieving a portion of the grain after removing foreign material. Splits, which result from mechanical damage during handling and overdrying, reduce storage life and oil yield, and increase losses during oil refining. Damaged kernels are soybeans and soybean pieces, which are badly damaged by the ground, weather, frost, heat, insects (stinkbug-stung kernels only are counted at one-fourth the actual percentage), mold, or sprouting, and are determined by hand-picking after removing foreign material. Damaged kernels reduce storage life and oil yield, adversely affect oil color, and increase refining loss. Soybeans of other colors are those, which have green, black, brown, or multiple colors. These soybeans may affect oil color by contributing undesirable pigments.

During the 2002 crop year, almost 27 million metric ton (Mt) of soybeans were exported from the United States. Of that amount, 4.8% was US No. 1, 94.6% was US No.2, 0.4% was US No. 3, and 0.1% was US No. 4. By comparison with Brazilian soybeans, US soybeans are typically slightly lower in oil content (6 year average of 1.2% lower oil content) but are lower in foreign matter, damage, free fatty acid (free fatty acid increase oil-refining loss) and moisture contents, and are higher in test weight.

Grading standards are similar between countries of origin, but differ in some details. For instance, Brazil and Argentina have an export grade for soybeans, Grade No. 1, which limits foreign matter to 1%. Brazilian grades specify a maximum of 14% moisture but Argentina, a maximum of 13%. The moisture content normally regarded as safe for long-term storage is 13%. The Mexican system does not consider test weight as a grading factor but does consider oil acid value (a measure of free fatty acid content). Entirely different criteria are used for grading foodgrade soybeans destined for soymilk and tofu.

Table 3 Official grades and grade requirements of the Federal Grain Inspection Service, United States Department of Agriculture

US sample grade	Minimum test	Damaged kernels		Maximum limits		
	weight per bu. (lbs)	Heat damaged (%)	Total (%)	Foreign material (%)	Splits (%)	Soybeans of other colors (%)
US No. 1	56.0	0.2	2.0	1.0	10.0	1.0
US No. 2	54.0	0.5	3.0	2.0	20.0	2.0
US No. 3	52.0	1.0	5.0	3.0	30.0	5.0
US No. 4	49.0	3.0	8.0	5.0	40.0	10.0

Typically, individual purchasers establish their own criteria often including seed size, seedcoat color, number of hard beans (germination or soaking test), total sugar, oil peroxide value or thiobarbituric acid test (measures of oil oxidation), acid value, protein dispersibility or solubility. Organic production or genetically modified free soybeans may also be criteria for food-grade soybeans.

See also: Cereals: Chemistry of Nonstarch Polysaccharides. Lipid Chemistry. Nutrition: Effects of Food Processing; Soy-Based Foods. Pulses, Overview. Soybean: Germplasm, Breeding, and Genetics; Agronomy; Processing; Soy Concentrates and Isolates; Soy-Based Fermented Foods. Appendix: Test Methods for Grain and Grain-Based Products.

# **Further Reading**

Beecher GR, Holden J, Bhagwat S, Haytowitz D, and Murphy PA (1999) USDA-Iowa State University Isoflavone Database. http://www.nal.usda.gov

Brumm TJ and Hurburgh CR (1990) Estimating the processed value of soybeans. *Journal of the American Oil Chemists' Society* 67: 302–307.

Erickson DR (ed.) (1995) *Practical Handbook of Soybean Processing and Utilization*. Champaign, IL: American Oil Chemists Society.

Erickson DR, Pryde EH, Brekke OL, Mounts T, and Falb RA (eds.) (1980) *Handbook of Soy Oil Processing and Utilization*. Champaign, IL: American Oil Chemists Society.

FGIS (1990) Federal Grain Inspection Handbook, Book II. Washington, DC: Federal Grain Inspection Service.

Hammond EG (2000) Genetic alteration of food fats and oils. In: Chow CK (ed.) Fatty Acids in Foods and Their Health Implications, 2nd edn. New York: Marcel Dekker.

Hurburgh CR, Brumm TJ, Quin JM, and Hartwig RA (1990) Protein and oil patterns in the US and world soybean markets. *Journal of the American Oil Chemists' Society* 67: 966–973.

Murphy PA, Song TT, Buseman G, et al. (1999) Isoflavones in retail and institutional soy foods. *Journal of Agricultural and Food Chemistry* 47: 2697–2704.

Nielsen NC (1985) Structure of soy proteins. In: Altschul AM and Wilcke HL (eds.) New Protein Foods, vol. 5. Seed Storage Proteins, pp. 27–64. New York: Academic Press.

Smith AK and Circle SJ (1978) Chemical composition of the seed. In: Smith AK and Circle SJ (eds.) *Soybeans: Chemistry and Technology, vol. 1, Proteins*, pp. 61–92. Westport CN: AVI Publishing.

USDA (1999) US department of agriculture, economic research service. In: Golbitz P (ed.) Soy and Oilseed Bluebook. Bar Harbor, ME: Soytech.

Wang HJ and Murphy PA (1996) Mass balance study of isoflavones during soybean processing. *Journal of Agricultural and Food Chemistry* 44: 2377–2383.

Wu SW, Murphy PA, Johnson LA, Fratzke AR, and Reuber MA (1999) Pilot plant fractionation of soybean glycinin and β-conglycinin. *Journal of the American Oil Chemists' Society* 76: 285–293.

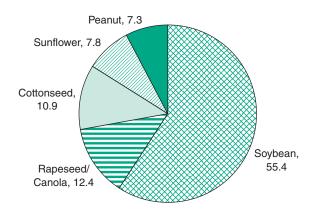
### **Relevant Websites**

http://www.nal.usda.gov — USDA-Iowa State University Isoflavone Database. http://www.usda.gov — US Soybean Inspection.

# **Processing**

**T Wang**, Iowa State University, Ames, IA, USA © 2004, Elsevier Ltd. All Rights Reserved.

Soybean is the dominant oilseed produced in the world due to its favorable agronomic characteristics, its high-quality protein, and its valuable edible oil. It comprises over a half of all oilseeds produced worldwide (Figure 1). The United States ranks number one in soybean production (8.24 million ton (Mt)), followed by Brazil, Argentina, China, and EU-15 (4.28, 3.28, 3.26, and 2.87 Mt, respectively). The production of soybeans and soybean oil is driven by the needs for soy protein meals that are extensively used in commercial feeds for poultry, swine, and cattle. Soybean oil accounted for 80–90% of total edible oil consumption in the United States in 1998 because of its availability and its many desirable characteristics, including compositional and functional properties.



**Figure 1** Percentages of five major oilseeds produced in the world during 2000-01.

This article describes the composition of soybean seed and oil, extraction, refining, and further processing of the oil, and processing and utilization of by-products from oil refining. Oil oxidation and its measurement are also discussed briefly due to the impact of this chemical degradation on oil quality. This article concludes with a general description of the uses of soybean oil as cooking and salad oil, in margarine, shortening, mayonnaise, and salad dressing.

# Composition

## **Seed Composition**

Mature soybeans are oval shaped and their sizes are variety dependent. The seed consists of three major parts: seedcoat or hull, cotyledon, and germ or hypocotyls as shown in Figure 2. Soybean oil is contained in the lipid bodies in the cotyledon cells. The composition of these structure components is shown in Table 1.

## **Oil Composition**

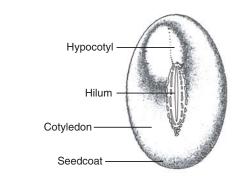
Oil recovered by solvent extraction or mechanical pressing is termed crude soybean oil and it contains various classes of lipids, including neutral lipids (tri-, di-, and mono-acylglycerols), free fatty acids (FFAs), and polar lipids such as phospholipids (PLs). It also contains a minor amount of unsaponifiable matter that includes phytosterols, tocopherols, and hydrocarbons such as squalene. Trace metals are found in soybean oil in ppm concentration. When the oil is refined, concentrations of all minor constituents are reduced. The typical composition of crude and refined soybean oil is shown in Table 2.

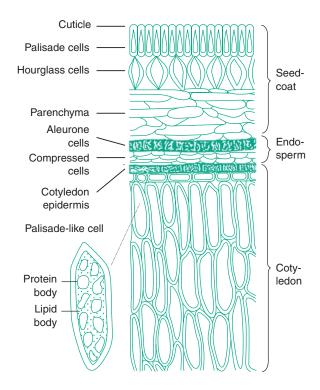
Sphingolipids is an area of research with increasing activity in recent years. Soybeans are a relatively rich source of sphingolipids, a class of polar lipids which are ubiquitous constituents of the cell membrane and are highly bioactive. The hydrolyzed products of sphingolipids are used by cells to regulate growth, differentiation, and apoptosis. There is evidence that these lipids inhibit colon carcinogenesis in experimental animals at a human diet-equivalent concentration. They may reduce colon cancer risk in humans and inhibit skin cancer development. Little is known about how sphingolipid content varies with soybean variety and processing.

### **Fatty Acid Composition**

Typical fatty acid composition of commodity soybean oil, in comparison with the other major vegetable oils, is shown in **Table 3**. Soybean oil has relatively high content of linoleic and linolenic acids. These are both essential fatty acids for humans and therefore of

dietary importance but they are also the reason for the oxidative instability of this oil. Processing techniques such as hydrogenation and lipid composition modification through traditional plant breeding or





**Figure 2** Whole soybean seed and its microscopic structure. (Erickson DR (ed.) (1995) *Practical Handbook of Soybean Processing and Utilization*. Champaign, IL: AOCS Press.)

**Table 1** Composition (wt.%, dry weight basis) of structure components of soybeans

Component	Yield	Protein	Oil	Ash	Carbohydrate
Whole seed	100.0	40.3	21.0	4.9	33.9
Cotyledon	90.3	42.8	22.8	5.0	29.4
Hull	7.3	8.8	1.0	4.3	85.9
Hypocotyl	2.4	40.8	11.4	4.4	43.4

Source: Erickson DR (ed.) (1995) Practical Handbook of Soybean Processing and Utilization. Champaign, IL: AOCS Press.

genetic transformation have been used to modify the fatty acid composition to improve its oxidative or functional properties.

## **Oil Extraction**

The two common processes for soybean oil extraction are solvent extraction and mechanical pressing, but in the United States less than 1% of the soybeans is processed by mechanical means. Solvent extraction with hexane is the standard practice in today's modern processing facilities. There are three major steps in solvent extraction: seed preparation, oil extraction, and desolventizing of the oil and meal. Conventional seed preparation includes drying, cleaning, cracking, optional de-hulling or decortication, conditioning, and flaking of the seeds. The option of expanding after flaking is used to improve oil extraction, percolation,

 Table 2
 Typical composition of crude and refined soybean oil

Component	Crude oil	Refined oil		
Triacylglycerols (%) Phospholipids (%)	95–97 1.5–2.5	>99 0.003-0.045		
Unsaponifiable matter (%) Phytosterols Tocopherols Hydrocarbons	1.6 0.33 0.15–0.21 0.014	0.3 0.13 0.11-0.18 0.01		
Free fatty acids (%)	0.3-0.7	< 0.05		
Trace metals (ppm) Iron Copper	1-3 0.03-0.05	0.1-0.3 0.02-0.06		

Source: Pryde EH (1980) Composition of soybean oil. In: Erickson DR, Pryde EH, Brekke OL, Mounts TL, and Falb RA (eds.) *Handbook of Soy Oil Processing and Utilization*, pp. 13–31. Champaign, IL: AOCS Press.

and solvent drainage, and is accompanied by a doubling of the throughput. The Alcon process is a flake-heating treatment aimed to improve the degumming efficiency of the crude oil. A very low level of PL in degummed oil can be achieved and therefore the oil can be physically refined.

Hexane extraction of soybeans is a diffusion process achieved by immersing solid in solvent or percolating solvent through a bed of solids. Rotary (deep-bed), horizontal belt, and continuous loop extractors are used for soybeans. Solvent, recovered from the miscella (mixture of solvent and extracted oil) by double-effect evaporator and steam stripping and from flake by a desolventizer toaster, is recovered and recycled.

# **Refining of Soybean Oil**

The minor components of soybean oils include PLs, FFAs, chlorophyll pigment, oxidation products, and other unsaponifiable components (tocopherols, sterols, hydrocarbons, etc.). Some of these minor components negatively affect oil quality while others may play a positive role in nutrition and function. The goal of refining is therefore to remove the undesirable components and, at the same time, to maximize retention of the beneficial ones. An overview flow-chart of soybean oil refining is presented in Figure 3.

"Degumming" is a process of removing PLs (gums) from crude soybean oil to improve its physical stability and to facilitate further refining. The water degumming procedure is simple, but its efficacy is influenced by the quality of crude oil. PLs can exist in hydratable form that can be readily removed after hydration, or in nonhydratable form that cannot be removed by this procedure. The nonhydratable phospholipids (NHPs) are probably calcium and

Table 3 Average fatty acid composition (wt.%) of oils from soybean and other oilseeds

Fatty acid		Soybean	Canola	Cottonseed	Sunflower	Peanut
Lauric	12:0				0.5	
Myristic	14:0	0.1		0.9	0.2	0.1
Palmitic	16:0	11.0	3.9	24.7	6.8	11.6
Palmitoleic	16:1	0.1	0.2	0.7	0.1	0.2
Stearic	18:0	4.0	1.9	2.3	4.7	3.1
Oleic	18:1	23.4	64.1	17.6	18.6	46.5
Linoleic	18:2	53.2	18.7	53.3	68.2	31.4
Linolenic	18:3	7.8	9.2	0.3	0.5	
Arachidic	20:0	0.3	0.6	0.1	0.4	1.5
Gadoleic	20:1		1.0			1.4
Eicosadienoic	20:2					0.1
Arachidonic	20:4					
Behenic	22:0	0.1	0.2			3.0
Lignoceric	24:0		0.2			1.0

magnesium salts of phosphatidic acids resulting from enzymatic hydrolysis of the PLs. This degradation results from seed damage during storage and handling or from improper seed preparation. Normal quality soybean oil from conventional solvent extraction has ~90% hydratable PLs and 10% NHPs. Phosphoric acid or citric acid can be used as an aid for more complete removal of NHPs, but their presence in the gum will reduce its quality due to darkening of the gum. Total PLs in crude soybean oils range from 1.1% to 3.2%.

Degumming can be achieved in batch or continuous fashion. In batch degumming, soft water at the same percentage as total PLs is added to heated (70°C) oil and mixed thoroughly for 30–60 min, followed

by settling or centrifuging. In continuous water degumming, heated oil is mixed with water by an in-line proportioning and mixing system and the mixture is held in a retention vessel for 15–30 min before centrifugation. A well-degummed soybean should contain less than 50 ppm of phosphorus, well below the 200 ppm level specified in the National Oilseed Processors Association trading rules for crude degummed soybean oil. Degumming prior to physical refining of soybean oil requires a more complete removal of the phospholipids to prevent darkening during fatty acid distillation.

"Neutralization" is a process of removing FFA, an oil decomposition product, from the oil. The process is also described as de-acidification or caustic

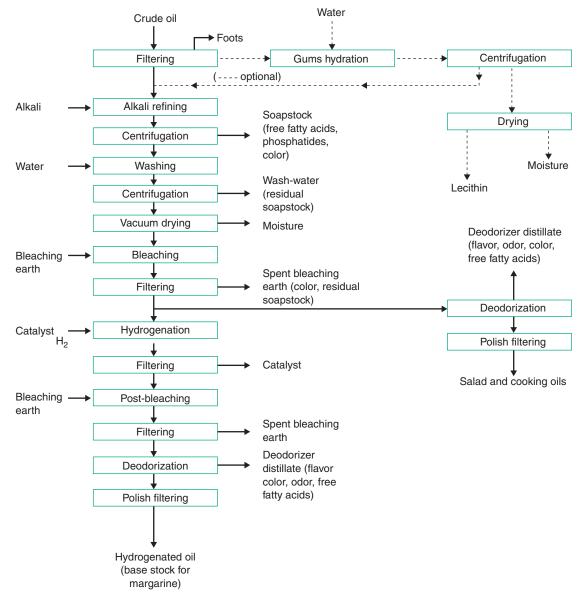


Figure 3 Diagram of soybean oil refining. (Courtesy of Center for Crops Utilization Research, Iowa State University.)

refining. It is achieved by treating the soybean oil with aqueous alkaline solution (generally sodium hydroxide) to neutralize the FFA in a batch or continuous system. The soap formed in the reaction also adsorbs natural pigments, the unhydrated gum, and mucilaginous substances contained in the oil. Settling or centrifugation is used to remove the soap. More details on soybean oil neutralization are discussed by Erickson.

"Bleaching" is a process designed not only to remove the pigment (chlorophyll) but, more importantly, to break down peroxides (primary oxidation products) into lower-molecular-weight carbonyl compounds that can be subsequently removed by deodorization. In soybean oil refining, color reduction occurs at each step of degumming, neutralization, bleaching, hydrogenation, and deodorization. Nevertheless, the most significant reduction of chlorophyll is during bleaching. Acid-activated bleaching clay is most effective in adsorbing chlorophyll and decomposing peroxides. Low levels of phosphorus (5-10 ppm P) and soap (10-30 ppm) in the neutralized oil are required to maximize the bleaching effect. The desired bleaching endpoint is zero peroxide, so the amount of bleaching earth should be adjusted to the quality of oil to be bleached. Earth dosage ranges from 0.3% to 0.6% for typical soybean oil. Successful bleaching can be achieved by atmospheric batch bleaching, vacuum batch bleaching, or continuous vacuum bleaching at temperatures between 100°C and 120°C for 20-30 min. More details of soybean oil bleaching are described by Erickson.

"Deodorization" is usually the last step in oil refining and it is a steam stripping process in which good-quality steam (1–3% of oil), generated from de-aerated and properly treated feed water, is injected into soybean oil under high temperature (252–266°C) and high vacuum (<6 mm Hg). Under these conditions peroxides are decomposed, and the FFA and odorous compounds are vaporized. Heat bleaching is achieved by maintaining the oil for 15–60 min at high temperature to ensure considerable decomposition of carotenoid pigments. During the deodorization process,

many desirable reactions take place but some undesirable ones such as lipid hydrolysis, polymerization, and isomerization also occur. Therefore, deodorization temperature must be carefully controlled to achieve optimum quality finished soybean oil.

There are three types of deodorization operations. The batch process is the least common, due to its low efficiency and inconsistent product quality. Semicontinuous and continuous deodorizers have improved processing efficiency. There are several configurations of the continuous deodorizer, including single-shell cylindrical vessel type, vertically stacked tray type, and the thin-film packed column type. This last one provides excellent fatty acid stripping with minimum use of steam, but it achieves neither desired heat bleaching nor effective deodorization due to the relatively short retention time. Therefore, a retention vessel has to be used after deodorization by column distillation.

Changes in oil quality during refining of soybean oil are shown in Table 4.

Comparing oxidative stability of soybean oil at different stages of refining, crude oil is the most stable while highly purified oil is the least stable. Changes in composition of minor components during refining are shown in Table 5.

#### **Hydrogenation**

The high degree of unsaturation, particularly the significant level of linolenic acid of soybean oil, limits its food application due to its low oxidative stability. Partial hydrogenation is used to increase the melting temperature and, at the same time, to improve the oxidative stability of soybean oil.

When oil is treated with hydrogen gas in the presence of a catalyst (nickel) under appropriate agitation and temperature conditions, it becomes a semisolid or plastic fat suitable for many food applications. Selectivity is often used to describe the relative reaction rates of the fatty acids from the more unsaturated to the more saturated forms. Generally, selectivity increases with increase in temperature and in catalyst

Table 4	Effect of processing	stens on	quality of	sovbean oil
I able T	LITECT OF DIOCESSING	SICPS UII	quality of	30 y D Call Oil

	Phosphorus (ppm)	Iron (ppm)	Chlorophylls (ppm)	Peroxide value (meq/kg)	Tocopherol (ppm)	Free fatty acid
Crude	510	2.9	0.30	2.4	1670	0.74
Degummed	120	0.8	Not available	10.5	1579	0.36
Refined	5	0.6	0.23	8.8	1546	0.02
Bleached	1	0.3	0.08	16.5	1467	0.03
Deodorized	1	0.3	0.00	0.0	1138	0.02

Source: Jung MY, Yoon SH, and Min DB (1989) Effect of processing steps on the contents of minor compounds and oxidation of soybean oil. *Journal of American Oil Chemists' Society* 66:118–120.

concentration and with decrease in hydrogen pressure and in agitation rate.

During hydrogenation various side reactions occur, and some of which have a strong impact on the physical and nutritional properties of the products. Double bond isomerization or trans fatty acid formation is the most important side reaction. The trans double bond is a thermodynamically more stable configuration than its cis counterpart and it is produced in significant quantity during partial hydrogenation. The trans fatty acids have a much higher melting point than their cis isomers; therefore, fat products with considerable trans fatty acids will have elevated melting points, which is desirable in shortening and margarine applications. However, the recently established link between trans fat consumption and health consequences has prompted research to reduce its use in foods.

#### Inter-Esterification

Inter-esterification is a term used to describe reactions in which fatty acid esters react with FFAs (acidolysis), alcohols (alcoholysis), or with other fatty acid esters (*trans*-esterification). In food application, interesterification often refers to the reaction between different oils or fats with their fatty acyl groups rearranging among the molecules.

Inter-esterification is conveniently achieved by an alkali methylate-catalyzed reaction under mild temperature (20–100°C). Microbial lipases are also widely used as biocatalysts in enzymatic interesterification. In contrast to the chemical process, the enzymatic process can be more selective if an enzyme with positional specificity is used, and it is usually much slower and more sensitive to the reaction conditions. The new developments in lipase-catalyzed inter-esterification have resulted in industrial applications of this process. Most inter-esterification reactions are still achieved with a chemical catalyst. Randomization is a special form of inter-esterification

in which acyl groups of a single oil or fat rearrange, resulting ultimately in a change of the natural distribution to a completely random pattern.

Recent reports linking consumption of *trans* fatty acids to the risk of coronary heart disease have generated much interest in producing margarines and shortenings that do not contain *trans* fatty acids. To achieve this, liquid oil and completely hydrogenated hard stock are inter-esterified to give a product with proper plastic property. These products need to have a proper solid fat content (SFC) or solid fat index (SFI) profile so that they maintain good integrity or firmness at room temperature, resist temperature cycling (i.e., repeated room temperature use and refrigeration storage), and melt completely at body temperature.

Various methods of laboratory scale, pilot plant processing, and batch reaction were described by Erickson. List *et al.* pioneered the development of a zero *trans* margarine by inter-esterifying 80% RBD (refined, bleached, and deodorized) soybean oil with 20% fully hydrogenated RBD soybean oil. The resulting product has SFI comparable to the conventional products.

Soybean oils with elevated levels of saturated fatty acids (by genetic modifications) can be randomized to produce margarines with desirable physical properties. A similar study of zero-*trans* margarine from soybean oils with modified fatty acid composition was conducted by List and co-workers.

## **Crystallization and Fractionation**

Fractionation or winterization is a process in which the more saturated molecular species in the oil are solidified during low-temperature treatment and subsequently removed; cold storage stability is thereby increased. When partially hydrogenated soybean oil is fractionated, the more saturated molecular species are removed to produce a clear oil that meets the requirements of a salad oil and a high-stability liquid oil.

Table 5	Effect of processing	on content of tocopherols	s sterols :	and squalene in sovbean oil

Processing step	Tocopherols		Sterols		Squalene	
	ppm	% Loss	ppm	% Loss	ppm	% Loss
Crude	1132		3870		143	
Degummed	1116	1.4	3730	3.6	142	0.7
Neutralized	997	11.9	3010	22.2	140	2.1
Bleached	863	23.8	3050	21.2	137	4.2
Deodorized	726	35.9	2620	32.3	89	37.8

Source: Ramamurthi S, McCurdy AR, and Tyler RT (1998) Deodorizer distillate: a valuable byproduct. In: Koseoglu SS, Rhee KC, and Wilson RF (eds.) *Proc. World Conf. Oilseed Edible Oils Process*, vol. 1, pp. 130–134. Champaign, IL: AOCS Press.

#### Processing and Utilization of Co- or By-Products of Oil Refining

#### Soy Lecithin

Gum obtained from degumming of crude soybean oil is the predominant source of lecithin for pharmaceutical and food industries because of its availability and outstanding functionality. Crude lecithin contains a large amount of neutral oil and it is usually de-oiled to improve its functionality. This separation is based on the solubility difference of neutral and polar lipids in acetone. PLs are precipitated from acetone solution and separated. The three major classes of PLs in soybeans are phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI).

For certain applications, such as in pharmaceutical, nutritional supplement, and cosmetic industries, lecithin product with very high PC content is desired. Alcohol fractionation of deoiled lecithin provides alcohol-soluble and alcohol-insoluble fractions enriched with PC and PI, respectively. The PC-enriched fraction is an excellent oil-in-water emulsifier. The PI-enriched fraction is a good water-in-oil emulsifier often used in the chocolate industry to increase the viscosity of the mass, therefore reducing the need for cocoa butter. The typical composition of these lecithin products is shown in Table 6.

Soybean lecithins can also be chemically altered to modify their emulsifying properties and to improve their dispersibility in aqueous systems. PLs may be hydrolyzed by acid, base, or enzyme (phospholipase A) to achieve better hydrophilic and emulsification properties. Hydroxylation of lecithin improves its oil-in-water emulsification property and water dispersibility. Acetylation creates improved emulsification and water dispersion.

#### Soybean Tocopherols and Phytosterols

Soybean deodorizer distillate (SBDD) is the material collected from the steam distillation of soybean oil.

It is a mixture of FFAs (particularly during physical refining), tocopherols, phytosterols and their esters, hydrocarbons, and secondary lipid oxidation products. The quality and composition of SBDD depends on feedstock oil composition and on processing conditions. Tocopherols and sterols are valuable components that can be further separated from the distillate and used in the nutrition-supplement and pharmaceutical industries. Table 7 shows the composition of deodorizer distillates from soybean and other vegetable oils.

Soybean tocopherols are the major source of natural fat-soluble antioxidants and vitamin E. There are at least four types of tocopherols in soybean oil. The  $\gamma$ -tocopherol is the major tocopherol present in soybean oil with the  $\delta$ -,  $\alpha$ -, and  $\beta$ -compounds present in decreasing quantities (**Table 8**). The table shows tocopherol composition of soybean oils obtained by conventional solvent extraction and mechanical press, in comparison with that of crude wheat germ oil.

Phytosterols are used as raw materials for over 75% of the world's steroid production. The more recent application of phytosterol and phytostanol and their fatty acid esters in margarine and table spreads is related to the cholesterol-lowering effect of these compounds. Hicks and Moreau (2001) have reviewed the recent development of functional foods containing phytosterols.

#### Soap Stock

Soap is recovered from alkaline neutralization of the crude or degummed soybean oil. It can be acidulated to produce acid oil, which contains FFAs, neutral oil, PLs, unsaponifiable matter, proteins, and mucilaginous substances. Soap stock is the lowest-priced by-product from oil processing and is generated at a rate of  $\sim$ 6% of the volume of crude oil refined amounting to as much as 1.8 billion pounds (0.9 Mt) in the United States annually. Most of the soap stock or the acid oil is used as feed currently.

Table 6 Typical composition (%) of commercially refined lecithin products

	Lecithin					
	Oil-free	Alcohol-soluble	Alcohol-insoluble			
Phosphatidylcholine	29	60	4			
Phosphatidylethanolamine	29	30	29			
Phosphatidylinositol and glycolipid	32	2	55			
Neutral oil	3	4	4			
Others	7	4	8			
Emulsion type favored	w/o or o/w	o/w	w/o			

Source: Brekke OL (1980) Oil degumming and soybean lecithin. In: Erickson DR, Pryde EH, Brekke OL, Mounts TL, and Falb RA (eds.) Handbook of Soy Oil Processing and Utilization, pp. 71–88. Champaign, IL: AOCS Press.

Table 7 Composition (wt.%) of deodorizer distillate from various oils

%	Soybean	Sunflower	Cotton	Rapeseed
Unsaponifiable	33.0	39.0	42.0	35.0
Total tocopherol	11.1	9.3	11.4	8.2
α-Tocopherol	0.9	5.7	6.3	1.4
Total sterol	18.0	18.0	20.0	14.8
Stigmasterol	4.4	2.9	0.3	1.8

Source: Winters RL (1990) Deodorizer distillate. In: Erickson DR (ed.) *Proceedings: World Conference Edible Fats and Oils Processing, Basic Principles and Modern Practices*, pp. 402–405. Champaign, IL: AOCS Press.

Table 8 Tocopherol content of crude soybean and wheat germ oils

	Mechanically pressed soybean oil	Solvent extracted soybean oil	Solvent extracted wheat germ oil
Total tocopherol (ppm) $\alpha$ -Tocopherol (%) $\beta$ -Tocopherol (%) $\gamma$ -Tocopherol (%) $\delta$ -Tocopherol (%)	1257 9.3 1.2 62.8 26.7	1370 10.5 1.2 63.5 25.0	2682 67.8 32.2

Soybean oil methyl esters can also be produced from soap stock for biodiesel application.

#### **Oxidative Quality of Soybean Oil**

Soybean oil is a polyunsaturated or linoleic type of oil that is highly susceptible to lipid oxidation. The mechanism of lipid oxidation and lipid hydroperoxide breakdown has been discussed thoroughly by Frankel. Briefly, lipid auto-oxidation is a free radical chain reaction that involves the initiation, propagation, and termination steps. The primary oxidation product is lipid hydroperoxides. Decomposition of the primary product leads to a range of secondary oxidation products, of which some are off-odor and off-flavor compounds.

Oxidative instability limits the use of soybean oil in certain applications, but hydrogenation and other means of composition modification have made soybean oils the most widely used of all vegetable oils. The following analytical methods are frequently used to quantify oxidation of soybean oil.

"Sensory evaluation" provides information most closely associated with the quality of food lipids. Flavor or odor defects may be detected by panelists before they are recognized by chemical or instrumental methods. For example, the "fishy" and "grassy" taste produced in linolenic-acid-containing oils such as soybean oil occurs at very low levels of oxidation that are only detected by sensory analyses. The limitations of this method are poor reproducibility and high cost for panelists and the necessary facilities. The recommended approach is to use more reproducible chemical or instrumental methods to complement or support the sensory analyses.

"Peroxide value (PV)" is the most commonly used measurement of lipid oxidation. The standard iodometric method requires a relatively large sample size (5 g) when the lipid is only slightly oxidized. The ferric thiocyanate method based, on the oxidation of ferrous to ferric ion, involves colorimetric measurement of ferric thiocyanate. This method is more sensitive than the iodometric method and requires a relatively small sample (0.1 g). The PV is a useful measure for samples with low levels of oxidation and when the hydroperoxides are not decomposed. During prolonged oxidation, a maximum PV is reached and the value then begins to decrease due to peroxide degradation.

"Carbonyl compounds" in oxidized lipids are the secondary oxidation products resulting from the decomposition of the hydroperoxides. They can be quantified by the reaction with 2,4-dinitrophenyl-hydrazine and the resulting colored hydrazones are measured spectrophotometrically at 430–460 nm. The carbonyl value is directly related to sensory evaluation, because many of the carbonyl molecules are those responsible for off-flavor in oxidized oil. The anisidine value is a measure of carbonyl compounds that have medium molecular weight and are less volatile. It can be used to discover any prior oxidation or processing history of an oil.

"Conjugated diene hydroperoxides" produced from polyunsaturated lipids can be determined quantitatively by their strong absorption at 234 nm. This is a sensitive method, but it can only apply to the undegraded hydroperoxides.

"Gas chromatographic (GC)" methods have been used for determining volatile oxidation products. Static headspace, dynamic headspace, or direct injection methods are the three commonly used approaches. Each method produces significantly different GC profiles.

"The oxidative stability" of lipids has been evaluated by a variety of methods under a wide range of conditions. Temperature is the most important factor to consider in oxidative stability determination, because the rate of oxidation is exponentially related to temperature increase. Therefore, the shelf life of a lipid decreases logarithmically with increasing temperature. The mechanisms of oxidation and peroxide decomposition are different at different temperatures. Therefore to realistically predict oxidative stability of food lipids, the test conditions should be as close as possible to those under which the lipid is stored.

Storage at ambient conditions or at slightly elevated temperatures and measurement of weight gain, flavor, peroxide value, conjugated diene, or carbonyl compounds are commonly used to study oxidative ability.

#### Food Uses of Soybean Oil

According to Agricultural Statistics 2000, margarine, shortening, and salad/cooking oils accounted for 12%, 31%, and 41%, respectively, of total domestic consumption of oils and fats in the United States in 1998. Soybean oil was used to produce 95% of the total margarine and 83% of the total shortening. Based on 1998/99 soybean utilization in the United States (Golbitz, 2000), 95% of the total soybean oil produced was used in food applications. Among the food uses, 13%, 38%, and 48% of the soybean oil was used in margarine, shortening, and cooking oil, respectively.

#### **Cooking and Salad Oils**

Salad oil is a refined or sometimes fractionated liquid vegetable oil remaining liquid at 4.4°C. An important distinction between salad and cooking oils is the difference in their oxidative and thermal stability. Cooking oil is more stable than salad oil at higher temperatures such as deep-fat frying. Fully refined soybean oil can be directly used as salad oil, whereas other oils, such as sunflower and corn, have to be dewaxed before they can meet the criteria of a salad oil. Because soybean oil contains a relatively high amount of the polyunsaturated and unstable linolenic acid, it is usually partially hydrogenated to produce salad or cooking oils. Synthetic antioxidants - such as butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG), ascorbyl palmitate, and tertiary-butyl hydroquinone (TBHQ) – have been used in cooking oils. Natural antioxidants derived from sage, rosemary, and green tea are increasingly used to meet consumer's preference of natural food ingredients.

New nutrition-oriented salad and cooking oils are being developed. LoSatSoy is a low-saturated fatty acid oil developed at Iowa State University and commercialized as a salad and cooking oil. It has half of the saturated fatty acid compared with commercial soybean oil; therefore, it is believed to have nutritional benefit. Another newly developed oil is low-linolenate (1.2%; 18:3) soybean oil, which has improved oxidative stability in salad and cooking oil applications.

A unique vegetable oil, diacylglycerol oil, developed and successfully marketed in Japan by Kao Corp, is being produced from soybean and/or canola

oil by an Archer Daniels Midland Co. (ADM) – Kao LLC joint venture. This oil is metabolized differently from other oils in that it is not stored as body fat but immediately burned as energy.

#### **Margarine and Shortening**

The traditional margarine is in stick form. Other forms, including spreadable, polyunsaturated, and low-fat margarines, have been developed to satisfy the demands of convenience and nutrition. A significant recent trend is away from margarine (80% fat, as defined by the FDA Standard of Identity) to spreads with less fat.

The most important functional properties of margarines and spreads are spreadability, oiliness, and melting property. Spreadability can be predicted by SFI and penetration measurement. Oil-off refers to the phenomenon when fine fat crystals no longer form a stable network to trap the liquid oil. Melting property depends on fatty acid composition and crystallization form.

Shortening contains 100% fat of vegetable or animal source and is used in frying, cooking, baking, and other confectionary items. It can be in plastic and semisolid or pourable fluid form, or in encapsulated powder, pellet, or flake form. It is produced by formulating a blend, solidifying and plasticizing the blend, and packaging and tempering. The  $\beta'$  form crystals are preferred for both margarine and shortening products. The large number of minute air bubbles incorporated in the shortening improves the leavening of baked foods. A more in-depth discussion of the science and technology of shortening has been presented by Metzroth.

#### **Mayonnaise and Salad Dressing**

The official definition (FDA Standard of Identity) describes mayonnaise as a semisolid food prepared from vegetable oil (no less than 65%), egg yolk, and vinegar. Most mayonnaise in the United States contains 75–82% oil which is usually soybean oil. The production of mayonnaise is partly an art due to the difficulty of producing the o/w emulsion in which the dispersed phase is much more than the continuous phase. Egg solids and processing conditions play critical roles in mayonnaise quality.

Salad dressings were developed as an alternative to mayonnaise. The standard of identity requires that salad dressing contain not less than 30% vegetable oil, vinegar, not less than 4% egg yolk, and is thickened by starch. Pourable salad dressing can be in two different finished forms: one phase or two phases depending upon whether the product is homogenized.

The oils used in salad dressing are selected using the same criteria as for mayonnaise.

The oil used in these products is predominantly soybean oil in the United States. Canada and Europe may use different oils depending on the availability of vegetable oil in that specific region.

See also: Canola: Processing. Soybean: Germplasm, Breeding, and Genetics; Agronomy; Grading and Marketing; Soy Concentrates and Isolates.

# Vesper H, Schmelz EM, Nikolova-Karakashian MN, et al. (1999) Sphigolipids in food and the emerging importance of sphingolipids to nutrition. *Journal of Nutrition* 129: 1239–1250.

Winters RL (1990) Deodorizer distillate. In: Erickson DR (ed.) *Proceedings: World Conference Edible Fats and Oils Processing, Basic Principles and Modern Practices*, pp. 402–405. Champaign, IL: AOCS Press.

#### **Further Reading**

Brekke OL (1980) Oil degumming and soybean lecithin. In: Erickson DR, Pryde EH, Brekke OL, Mounts TL, and Falb RA (eds.) *Handbook of Soy Oil Processing and Utilization*, pp. 71–88. Champaign, IL: AOCS Press.

Erickson DR (ed.) (1995) Practical Handbook of Soybean Processing and Utilization. Champaign, IL: AOCS Press.
Frankel EN (1998) Lipid Oxidation. Dundee, Scotland: The Oily Press Ltd.

Golbitz P (2000) Soya and Oilseed Blue Book. Bar Harbor, ME: Soyatech.

Hicks KB and Moreau RA (2001) Phytosterols and phytostanols: functional food cholesterol busters. *Food Technology* 55: 63–67.

Jung MY, Yoon SH, and Min DB (1989) Effect of processing steps on the contents of minor compounds and oxidation of soybean oil. *Journal of American Oil Chemists' Society* 66: 118–120.

Kok LL, Fehr WR, Hammond EG, and White PJ (1999) Trans-free margarine from highly saturated soybean oil. Journal of American Oil Chemists' Society 76: 1175–1181.

List GR, Emken EA, Kwolek WF, Simpson TD, and Dutton HJ (1977) "Zero trans" margarines: preparation, structure, and properties of interesterified soybean oil-soy trisaturate blends. *Journal of American Oil Chemists' Society* 54: 408–413.

List GR, Pelloso T, Orthoefer F, Warner K, and Neff WE (2001) Soft margarines from high stearic acid soybean oils. *Journal of American Oil Chemists' Society* 78: 103–104.

Matsuo N and Tokimitsu I (2001) Metabolic characteristics of diacylglycerol. *Inform* 12: 1098–1102.

Metzroth DJ (1996) Shortening: science and technology. In: Hui YH (ed.) Bailey's Industrial Oil and Fat Products, Vol 3: Edible Oil and Fat Products: Products and Application Technology, 5th edn., pp. 115–160. New York: Wiley.

Pryde EH (1980) Composition of soybean oil. In: Erickson DR, Pryde EH, Brekke OL, Mounts TL, and Falb RA (eds.) *Handbook of Soy Oil Processing and Utilization*, pp. 13–31. Champaign, IL: AOCS Press.

Ramamurthi S, McCurdy AR, and Tyler RT (1998) Deodorizer distillate: a valuable byproduct. In: Koseoglu SS, Rhee KC, and Wilson RF (eds.) *Proc. World Conf. Oilseed Edible Oils Process*, vol. 1, pp. 130–134. Champaign, IL: AOCS Press.

### Soy Concentrates and Isolates

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#### Introduction

With the continuous escalation in the cost of farmland and feed, food manufacturers are seeking low-cost, efficient protein sources to replace animal proteins. While crops and grains can provide a nutritive source of proteins in the diets of people in many developed and developing countries, the complete or partial replacement of animal protein, particularly in fabricated foods, remains a major challenge. This is due to the fact that the protein ingredient has to provide not only the calorie and nutrients to the products, but also the desirable organoleptic and functional properties required by the consumers. Of all the potential sources of unconventional food proteins, soybeans rank at the top of the list. Soy proteins are found in a large variety of food items, mainly in two categories: traditional soyfoods (e.g., soy milk, tofu, soy sauce), which use whole soybeans as the raw material, and formulated foods in which concentrated forms of soy protein are used as an ingredient. In the second category, a wide range of food items is represented, such as comminuted meat products, meat analogs, whipped toppings, frozen desserts, beverages, coffee whiteners, soups and sauces, pizza toppings and taco fillings, and bakery products.

Soy concentrates and soy isolates are concentrated forms of soy proteins extracted from soybeans after the removal of oils and other non-protein components. They are the by-products of oil processing in which soybeans are separated into oils and meals. The defatted meals have been used mainly as animal feed, but a significant portion of the meals is processed into soy concentrates and isolates for use as food.

#### **Production**

Soy concentrates and isolates, more commonly known as soy-protein concentrates and soy-protein isolates, are produced from de-hulled and de-fatted soy flakes or flours after the extraction of oils and fats. Figure 1 shows the typical manufacturing procedures for producing soy-protein concentrates. Soy-protein concentrates are produced from the de-fatted flakes by removing most of the water-soluble, low-molecularweight components, mainly sugars. Three processes are used commercially to prepare concentrates. In the first process, nonprotein constituents are extracted with aqueous alcohol. In the second procedure, major proteins are insolubilized by dilute acid at pH 4.5 (the isoelectric point of soy proteins). There is some loss of acid-soluble protein in this process. In the third process, the flakes or flours are heated with moisture to denature and insolubilize the proteins, and the low-molecular-weight components are extracted with water.

Soy-protein isolates are produced from de-fatted soybean flakes by removing both the water-soluble sugars and water-insoluble polysaccharides. Figure 2 shows a typical flow diagram for the production of commercial soy-protein isolates. De-fatted flakes are extracted with either water or mild alkali (pH 7–9) at  $50-55^{\circ}$ C. The protein extract is separated from the insoluble residue (polysaccharides and residual protein) by screening, filtering, or centrifugation. By adjusting the pH to  $\sim$ 4.5 with food-grade acid, the major proteins are precipitated. The protein curd can be separated from the solubles (whey) by filtering or centrifugation. Spray-drying of the curd produces

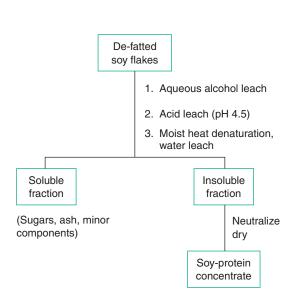
the isoelectric soy protein, whereas neutralization followed by drying yields the soy proteinates, which are preferred due to higher water-dispersibility and hence better functionality as a food ingredient.

Soy-protein concentrates and isolates can also be prepared by membrane processing using ultrafiltration (UF) and reverse osmosis (RO). In a patented procedure, de-fatted soybean flakes are extracted with water adjusted to pH 8-9 by calcium or sodium hydroxide at 43°C for 40 min. The material is centrifuged to remove the fiber and passed through a 70 kDa molecular-weight cutoff membrane. The retentate protein fraction is concentrated by RO and spraydried. The permeate, containing soluble sugars, minerals, and small protein molecules, may also be concentrated by RO and spray-dried. The advantages of membrane processing are the ability to recover soy proteins without alkali solubilization - acid precipitation causing protein damage, the opportunity for removing undesirable components such as phytates, the potential to recover small proteins (12-20 kDa molecular weight) by using smaller-pore-size membrane, and the reduction in water consumption and processing discharge.

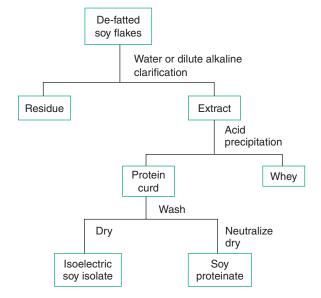
# **Chemical Compositions and Nutrient Contents**

#### **Proximate Compositions**

**Table 1** shows the proximate compositions of de-fatted soy flakes, three types of soy-protein concentrates, and soy-protein isolates. Despite the use of different manufacturing procedures, the overall



**Figure 1** Typical processes for the production of soy-protein concentrates.



**Figure 2** Typical process for the production of soy-protein isolates.

**Table 1** Proximate composition of soy-protein products (% dry basis)

	Protein (N × 6.25)	Fat (Pet. ether)	Crude fiber	Ash	Carbohydrates (by difference)
Defatted soy flour	54	1.0	3.5	6.0	38
Soy-protein concentrate					
Alcohol leach	66	0.3	3.5	5.6	24
Acid leach	67	0.3	3.4	4.8	24
Moist heat, water leach	70	1.2	4.4	3.7	21
Soy-protein isolate	92	0.5	0.3	4.5	2.5

compositions of the three soy concentrates are similar, with the protein content (on dry basis) ranging from 66% to 70%. The major nonprotein components are polysaccharides, including arabinan, arabinogalactin, cellulose, lignin, and pectin-like polysaccharides. Proteins in the alcohol-leached and moist-heattreated water-leached concentrates are denatured and insoluble, and the acid-leached proteins are more soluble, making the concentrates more suited in food applications. Protein contents of commercial soyprotein isolates are typically over 90% (dry basis) using a nitrogen-to-protein conversion factor of 6.25 (assuming a nitrogen content of 16%), a commonly used ratio in the food industry. This factor is considered too high since nitrogen contents of purified acid-precipitated soy proteins are higher than 16%.

#### **Mineral Contents**

Table 2 shows the mineral compositions of various soy-protein products. All soy-protein products contain minerals – such as calcium, iron, copper, phosphorus, and zinc - in nutritionally significant amounts. In whole soybeans, however, much of the mineral content is tied up in the form of phytates by forming complexes with phytic acid and fibers, hence lowering their bioavailability. In human studies, ingestion of soy concentrates did not result in any unfavorable trends in calcium, magnesium, zinc, or iron assimilation. Other studies show that zinc availability in humans is high when isolated soy proteins are present in diets containing adequate zinc contents. The availability of iron from soy isolates added to meat is lower, but can be compensated by increased iron content of the product.

#### **Essential Amino-Acid Contents**

Soybeans and other legumes are considered an excellent source of dietary proteins, and the amino-acid

Table 2 Mineral contents of soy-protein products (dry basis)

Element	Defatted soy flour	Soy-protein concentrate	Soy-protein isolates
Calcium	0.22%	0.22%	0.18%
Chlorine	0.13%	0.11%	0.13%
Chromium	0.9 ppm	< 1.5 ppm	< 1.0 ppm
Copper	23 ppm	16 ppm	12 ppm
Iodine	0.01 ppm	0.17 ppm	< 10 ppm
Iron	110 ppm	100 ppm	160 ppm
Magnesium	0.13%	0.25%	380 ppm
Manganese	28 ppm	30 ppm	17 ppm
Phosphorus	0.68%	0.70%	0.76%
Potassium	2.37%	2.1%	960 ppm
Sodium Sulfur	254 ppm 0.25%	50 ppm 0.42%	1.1%
Zinc	61 ppm	46 ppm	40 ppm

**Table 3** Essential amino-acid content of soy-protein products and suggested patterns (FAO/WHO) for amino-acid requirements (mg per g protein)

Essential		Age		FNB	Protein	Protein
amino acid	2–5	10–12	Adult	pattern	concen- trates	isolates
Histidine	19	19	16	17	25	28
Isoleucine	28	28	13	42	48	49
Leucine	66	44	19	70	79	82
Lysine	58	44	16	51	64	64
Methionine						
+ cysteine	25	22	17	26	28	26
Phenylalanine						
+ tyrosine	63	22	19	73	89	92
Threonine	34	28	9	35	45	38
Tryptophan	11	9	5	11	16	14
Valine	35	25	13	48	50	50

profile of soy proteins is recognized as the most complete of all vegetable protein sources. With the exception of sulfur-containing amino acids (methionine and cystine), the amino acid pattern of soy proteins resembles that of the high-quality animal proteins.

Table 3 shows the essential amino-acid compositions of soy-protein concentrates and isolates. When compared with the patterns for amino-acid requirements suggested by the 1980 Food and Nutrition Board (FNB) and the 1985 Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), the soy-protein products meet or exceed these guidelines. This demonstrates that isolated soy protein is a complete and adequate protein source for humans. Hence, soy concentrates and isolates can be blended or used interchangeably with other high-quality proteins.

# Nutritional Quality and Health Benefits of Soy-Protein Products

#### **Protein Digestibility**

Both animal tests and human clinical studies have shown that soy-protein products are comparable in digestibility to other high-quality proteins such as meat, milk, fish, and egg. In animal feeding experiments with rats and young pigs, the digestibility values of soy isolates and casein were similar. Studies with infants and young children demonstrated that the digestibility of different soy isolates were equal to or greater than that of milk proteins. Studies with young adult males showed identical digestibility values for isolated soy and meat proteins. The digestibility values of soy-protein concentrates and isolates range from 91% to 96%, comparable to that of milk.

#### **Protein Efficiency Ratio (PER)**

Small animal tests, particularly the protein efficiency ratio (PER) assay, have been used extensively to evaluate the nutritional quality of food proteins. The PER values of soy concentrates range from 2.0 to 2.2, and for soy isolates from 1.1 to 1.7, considerably lower than the PER value (2.50) corrected to casein. However, by supplemented soy-protein products with 1.5% methionine, the limiting essential amino acid in soy proteins, the PER values of soy concentrates and isolates can be increased to above 2.5 and 2.0, respectively.

#### **Antinutritional Factors**

Similar to many plant sources, soybeans contain a number of antinutritional factors that can influence its acceptability for food applications. These include protease inhibitors, hemagglutins, phytic acid, and lectins. Inhibitors of proteolytic enzymes – such as trypsin, chymotrypsin, carboxypeptidase, and elastase – lower the nutritional quality of soy protein. Trypsin inhibitors are the most important inhibitors in soybeans and two of them, the Kunitz and the Bowman-Birk inhibitors have been purified and studied in details. Both inhibitors cause enlargement of the pancreas in rats and chicks. Growth depression and pancreatic hypertrophy have been attributed to endogenous loss of essential amino acids in the enzymes secreted by the hyperactive pancreas in response to the inhibitory effect of the inhibitors. Although the significance of trypsin inhibitors to human health is not yet resolved, they should be eliminated from the diet.

Hemagglutinins cause clumping of red blood cells (hemagglutination) in *in vitro* tests. The major hemagglutin in soybean is a glucoprotein with a molecular

weight of 110 kDa, and has been shown to inhibit the growth of rats.

Both trypsin inhibitors and hemagglutins can be easily inactivated by heat. Since the production of de-fatted soybean flakes and flours involved heating, soy-protein concentrates and isolates contain only low levels of these inhibitors. Only 3% of original hemagglutinin is retained in unheated isolates, which is eliminated in normal heating steps. Similarly, normal processing conditions lead to  $\sim$ 70% reduction in trypsin inhibitor in isolated soy protein. Additional heat treatments result in further lowering in trypsin inhibitor activity to a level that does not cause health problems in both animals and humans.

#### **Health Benefits of Soy Proteins**

Recent studies have demonstrated the positive influence of soy proteins on human health. These include lowering of blood cholesterol, prevention of cancer, diabetes and obesity, and protection against kidney and bowel diseases.

High level of plasma cholesterol, particularly low-density lipoproteins (LDLs), increases the risk for car-diovascular diseases. Both animal feeding tests and human clinical trials have shown that consumption of diets rich in soy proteins lowers serum cholesterol and the ratio of LDLs to high-density lipoproteins (HDLs), a beneficial form of cholesterol. It has been suggested that the 7S subunit of soy protein can activate LDL receptors in human liver and lower plasma cholesterol by a mechanism different from that proposed for other diets and hypolipolemic drugs.

Epidemological studies have suggested that soy protein may be protective for cancer risks. However, the cancer preventive effects may be related to nonprotein components in soy-protein products such as dietary fibers and isoflavones. Many soy-protein products are rich sources of dietary fibers, which are known to decrease incidence of colon cancer. Isoflavones such as genestein are known as phytoestrogens, which can reduce circulating ovarian steroids and adrenal androgens and increase menstrual cycle length. Such effects may account for the decreased risk of breast cancer. Dietary fibers and isoflavones have other health beneficial effects, particularly when combined with soy proteins, e.g., lowering of serum cholesterol and modulation of glucose metabolism.

By providing a high-quality protein in concentrated form, soy concentrates and isolates can be used in specially designed low-calorie and high-nutrientdensity meals. These can be used for weight reduction in obese subjects. When compared to proteins from other food sources such as milk, seafoods, and nuts, soy proteins rarely incite allergenic responses in humans due to immune reactions. Soy-protein products can, therefore, be used in infant formula as an alternative protein source to replace milk proteins for infants allergic to milk proteins.

#### Functional Properties and Food Applications of Soy Concentrates and Isolates

Functional properties of food proteins are properties that affect their utilization in food systems. Soy proteins possess unique functional properties, which combined with relatively low cost, make them the most widely used protein ingredients by the food industry. Table 4 lists some examples of the food applications of soy protein products and the functional properties that are critical in these applications.

#### Solubility

Solubility plays an important role in many food applications for proteins, including beverages, food emulsions, and foams. Commercial soy-protein products exhibit a wide range of solubility highly dependent on pH and other components such as salts and sugars. Denatured proteins, found in soy concentrates prepared by alcohol leaching or moist heat treatment, have lower solubility than native proteins.

#### **Water Hydration and Binding**

Solubility, hydration, and water absorption or binding are different manifestations of protein—water interactions. Hydration or water absorption is a critical initial step in imparting the desired functionality to soy proteins. Water binding is the ability of a protein matrix to expand and absorb water without solubilization. Commercial protein products have a wide range of water-binding capacity. Highly soluble

protein powder absorbs little water, whereas insoluble dehydrated protein granules can rehydrate and bind up to 3–4 times their weight of water. Protein granules hydrate slowly and form lumps which need to be broken up by mechanical mixing.

#### **Emulsification and Foaming**

Emulsions and foams are two-phase dispersed systems, with a hydrophobic phase (oil droplets or air bubbles) surrounded by a continuous aqueous phase. By reducing the interfacial tension, soy proteins can act as a surface-active agent and reduce the energy required to create the large interfacial areas characteristic of foams and emulsions. Soy proteins have relatively high emulsification capacity and aid in the formation of oil-in-water emulsions. By forming a protective barrier at the oil—water interface, soy proteins can also stabilize the emulsion droplets, preventing their coalescence and breakdown. Soy-protein products are used extensively as emulsifiers in comminuted meats and in baked goods and soups.

Foaming properties of soy proteins are important in food systems such as whipped toppings, chiffon desserts, and angle cakes. Soy proteins do not form stable foams due to the presence of foam inhibitors, probably residual lipids, which can be removed by alcohol extraction. Various chemical modifications of such enzyme hydrolysis have been used to improve the foaming properties of soy-protein products.

#### **Fat Binding**

Soy-protein products are used in foods for two different purposes with regard to fat binding or absorption. In comminuted meat products, soy proteins promote fat binding and hence decrease cook loss and maintain dimensional stability in the cooked products. The mechanism of fat binding has been attributed partly to physical entrapment and is correlated with bulk density and particle size, with bulky samples

**Table 4** Functional requirements of soy protein in different food products

Food product	Required qualities	Functional properties			
Beverages	Flavor, emulsion stability, heat stability	Solubility, flavor binding, emulsion stability			
Meats, sausages	Flavor, aroma, water and fat binding, texture	Flavor binding, water holding, fat absorption, emulsification, gelation or texturization			
Whipped toppings, chiffon desserts, angel cakes	Foam density, foam stability	Foaming capacity, foam stability, solubility			
Bakery products	Flavor, moisture retention, loaf volume, texture	Flavor binding, water holding, film formation, gelation			
Aburages	Water and fat binding	Film formation, cohesion			
Instant tofu	Texture, flavor	Gelation, bland flavor			
Soups, gravies	Thickening	Viscosity, gelation			

absorbing more oil. In emulsified meat products such as frankfurters or luncheon meat, fat binding by soy proteins may also involve emulsion formation and stabilization.

In bakery foods such as pancakes and doughnuts, addition of soy-protein products can prevent excessive oil absorption during frying. This may be attributed to the denaturation of soy proteins to form a fat-resistant barrier at the product surface.

#### **Gelation and Viscoelastic Properties**

Soy protein in solution can form a three-dimensional network or gel matrix upon treatments such as heat, alkali, or calcium ion addition. The gelation of soy protein contributes to the structure and texture of some food products, including comminuted meats and bakery products. The gel matrix provides a medium to retain moisture and fats, and binds to flavors and other components. It also contributes to unique mouthfeel such as chewiness to the products. Gelation of soy proteins in formulated foods is influenced by a number of factors including protein concentration, pH, and other components including salts, sugars, and lipids.

Soy protein in solution also contributes to viscosity, a measure of resistance to flow. Soy-protein slurries at moderately high concentrations (10–12%) have relatively low apparent viscosity. Treatments that induce the formation of protein aggregates or coagulum can increase viscosity and contribute to the viscoelastic properties (e.g., thickening) of some food products such as soups and sauces.

#### **Film Formation**

The film-forming ability of soy protein is desirable in food products such as aburage, frankfurters, and bologna. When a dough of soy flour—water is autoclaved, a film is formed on the surface. This film acts as a barrier to water and solvents and can be broken by grinding or slicing when the dough is washed. When shredded meat is mixed with egg white and soy concentrate or isolate, the protein forms a coat and facilitates drying of the meat fibers. The protein film slows down flavor loss in the dried meat particles, assists dehydration, and provides desirable texture in the rehydrated product.

#### **Texturization**

Soy-protein products can be texturized to form preset structure with unique textural properties which are not changed upon further processing. The texturized soy proteins are generally insoluble but can be hydrated and swollen. Texturized soy proteins are used as ingredients to make simulated meat products including seafood, poultry, and ground meat products.

There are several types of texturized soy-protein products prepared by different processes. Texturized soy concentrates are prepared by thermoplastic extrusion or steam texturization. Soy concentrates are mixed with water, colors, flavors, and other ingredients and fed to a cooker-extruder, heated under pressure, and then extruded. The dried products are in granular form and the composition is similar to the source material.

Structured soy concentrates are prepared by passing high-concentration (30-75%) protein slurry with low fat and carbohydrate contents through an extruder into different sizes and shapes. Structured soy concentrates possess a fibrous rather than spongy structure, and can be flavored to resemble meat or poultry products.

Texturized soy isolates are manufactured by thermal extrusion as described above, or by extruding an alkaline-denatured protein dope slurry through spinnerets into an acid—salt bath that coagulates the protein to form fibers, which are washed and stretched to increase strength. The fibers can be combined with binders to form fiber bundles and fat, flavors, color, stabilizers, and other ingredients can be added. Pressure is then applied to form the simulated meat products. Complete meat analogue products, including ham, turkey, bacon, and sliced beef, have been prepared from texturized soy isolates, and are marketed as vegetarian-type foods.

#### **Specialty Soy-Protein Foods and Ingredients**

Partially hydrolyzed soy-protein products are prepared by either acid/alkaline treatments or by proteolysis with enzymes such as pepsin, trypsin, and papain. The products have reduced molecular weight range of 3–5 kDa. The hydrolyzed soy proteins have functional properties superior to that of the native proteins, particularly improved acid solubility and enhanced foaming and emulsifying properties. They are used as whipping agents in confections, toppings and dessert mixes, and in acidic beverages. Completely hydrolyzed soy proteins prepared by acid or enzyme hydrolysis can be used as flavoring agents.

Soy-protein isolates are used in soy-based infant formulas. These are suitable for infants suffering from allergy against milk proteins or intolerance to lactose. Special formulas using soy-protein products are also developed and manufactured for older infants and for geriatric, hospital and postoperative feeding. Soy-protein products have also been used in infant cereals and baby foods to increase the protein content, particularly in rice- and wheat-based products.

#### **Future Outlooks**

Soy-protein concentrates and isolates are valuable food ingredients in many fabricated food systems by providing the functional performance and sensory quality desired by the manufacturers and consumers. They also provide a nutritionally balanced, relatively low cost and high-quality protein, which can be used either alone or in combination with other protein sources. New technologies to remove undesirable soy colors and flavors have been reported, allowing soy-protein-based products with improved organoleptic quality to be developed, even at high levels of usage. Various physical and chemical processes, such as extrusion and partial proteolysis, have modified the functionality of soy proteins and greatly expanded their utilization in new food products.

With increasing consumer awareness on the relationship between diet and health, and the nutritional advantages of soy-protein-based products, such as low calorie, high fiber, and low in saturated fats, the demand on soy-protein products will be increasing. However, it should be noted that expanded utilization of soy-protein products and the successful establishment of soy-protein industry would also depend on securing viable markets for other major components, and in the case of soybeans, it is mainly oil. Although soybeans have to compete with many oilseeds in the vegetable oil market, soy protein is facing much less competition from other plant sources including oilseeds and starchy crops, mainly due to its established market and well-developed technology.

See also: Nutrition: Soy-Based Foods. Soybean: Grading and Marketing; Soymilk, Tofu, and Okara; Soy Concentrates and Isolates; Soy-Based Fermented Foods.

#### **Further Reading**

Circle SJ and Smith AK (1978) Processing soy flours, protein concentrates, and protein isolates. In: Smith AK (ed.) *Soybeans: Chemistry and Technology*, vol. 1, pp. 294–338. Westport, CT: AVI Publishing.

Friedman M and Brandon DL (2001) Nutritional and health benefits of soy proteins. *Journal of Agricultural and Food Chemistry* 49: 1069–1086.

Fukushima D (2000) Soybean processing. In: Nakai S and Modler HW (eds.) Food Proteins: Processing Applications, pp. 309–342. New York: Wiley-VCH.

Hettiarachchy N and Kalapathy U (1997) Soybean protein products. In: Liu KS (ed.) Soybeans: Chemistry, Technology, and Utilization, pp. 379–411. New York: Chapman and Hall.

Lucas EW and Rhee KC (1995) Soybean protein processing and utilization. In: Erickson DR (ed.) *Practical Handbook of Soybean Processing and Utilization*, pp. 117–160. Champaign, IL: American Oil Chemists' Society Press.

Pearson AM (1983) Soy proteins. In: Hudson BJF (ed.) Developments in Food Proteins, vol. 2, pp. 67–108. London: Applied Science Publishers.

Soy Protein Council (1987) Soy Protein Products, Characteristics, Nutritional Aspects and Utilization. Washington, DC: Soy Protein Council.

Wolf WJ and Cowan JC (1971) Soybeans as a Food Source. Cleveland, OH: CRC Press.

### Soy-Based Fermented Foods

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Soybean-based fermentations were developed in China and East Asia, and most depend on filamentous fungi that break down the proteins and carbohydrates, and yeasts and bacteria that produce flavor compounds. A small amount of added starch from starch-based grains promotes better microbial growth and flavor development. Products include those made with whole soybeans, with soymilk extracted from soybeans, and products that result in soybean pastes and condiments. The microbiological and biochemical processes involved in their production are now relatively well known and therefore pure microbial cultures are used in modern industrialized processes. In addition, with escalating soymilk production there is an increasing amount of "okara," a fibrous waste product that poses a waste disposal problem. Fermentations using traditional microorganisms can turn okara into useful human foods, and those using novel microorganisms have yielded novel bioactive compounds. For a long time soybeans have had a reputation for being beneficial to health and recent studies covering fermented soybean and fermented okara are discussed.

#### Introduction

Unlike other grains soybeans do not contain large quantities of starch, but they do contain large amounts of good-quality protein and oil. However, the protein is not easily digested and so it must be made nutritionally available by the first step of soaking and cooking the beans. This step makes the protein more digestible and destroys the antinutritional characteristics of the soybeans. The oil plays little or no part in soybean fermentations.

In most food fermentations easily available monoand di-saccharides, either as the sugars or as breakdown products from starch, are a prerequisite for the fermentation to proceed. In the case of soybean the amount of carbohydrate is ~30% in the dry matter and about a third of it is soluble (see Nutrition: Soy-Based Foods). The soluble component consists of sucrose ( $\sim$ 5%), raffinose ( $\sim$ 1%), and stachyose  $(\sim 4\%)$ ; the insoluble carbohydrate  $(\sim 2\%)$  of the dry matter) is made up of starch (<1%), cellulose  $(\sim 4\%)$ , hemicellulose  $(\sim 1\%)$ , and pectin  $(\sim 6\%)$ . In view of this composition soybean fermentations are sometimes augmented with small or large quantities of starch from other grains, because it promotes microbial growth. By far, the most important microorganisms for soybean fermentation are the filamentous fungi rather than bacteria or yeasts. The fungi produce a range of powerful enzymes capable of hydrolyzing protein, starch, and complex carbohydrates, and, despite low starch levels, acid-producing and proteolytic bacteria, as well as yeasts, often play a significant role.

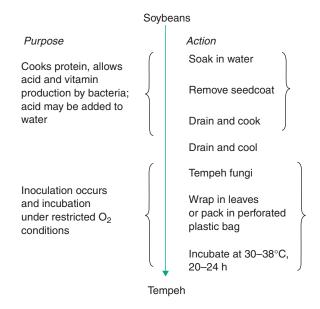
#### **Fermented Whole Bean Products**

The gross composition of some fermented soybean products is shown in Table 1.

#### **Tempeh**

"Tempeh" is a fermented soybean product and meat substitute that originated in Indonesia. It is probably the first "fast food" in that it can be deep-fried in 3—4 min or cooked in 10 min. The production of tempeh

is outlined in Figure 1 (see Soybean: Soymilk, Tofu, and Okara). In the first step the soybeans are soaked in water or acidified water at room temperature. During this stage a partial germination of the soybeans may occur depending on the amount of O<sub>2</sub> available to the seed, and acid is produced by bacteria growing in the soak water. Depending on the temperature during soaking, bacteria reach  $10^8 - 10^{10}$  colony-forming units per ml after 24-36 h. The pH drops from  $\sim$ 6.5 to  $\sim$ 4.5 due to the growth of the acid-producing bacterial species - e.g., Lactobacillus casei, Streptococcus faecium, Staphylococcus epidermidis, and Klebsiella pneumoniae – that are present naturally on the soybeans. The acid helps to prevent the growth of undesirable microorganisms, but any partial seed germination can affect the protein properties of the soybean and the subsequent fungal growth



**Figure 1** A flow diagram of the production of tempeh, an Indonesian fermented soybean product. The fungal mycelia knit the soybeans into a cake.

 Table 1
 The gross composition, g per kg, of some fermented soybean products

Fermented soy product	Moisture	Protein	Fat	Soluble carbohydrate	Fiber	Ash
Sufu (red)	555	146	57	58	6	171
Sufu (white)	565	144	112	48	7	124
Natto	585	165	100	101	23	26
Soybean miso	475	168	69	136	23	130
Chiang <sup>a</sup> (chunky)	486	116	52	272	21	74
Tempeh	640	183	40	110	17	10

<sup>&</sup>lt;sup>a</sup>The equivalent to Japanese miso.

phase. The bacteria that grow in the steeping water produce vitamin  $B_{12}$ , a significant nutrient in tempeh. The most desirable bacterial species for this stage is K. *pneumoniae*, but other pure bacterial starter cultures can perform the same function.

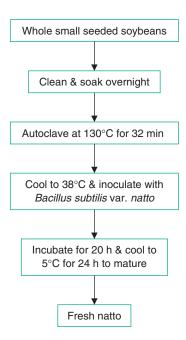
The soaked beans are de-hulled and carefully cooked to avoid overcooking or undercooking of the beans. The soybeans are then drained, cooled below 35°C, and dusted with wheat flour to provide a good source of fermentable carbohydrate, and inoculated. The desirable fungal species for successful tempeh production, whether arising from environmental inoculation or from pure starter inoculation, are Rhizopus oligosporus (e.g., NRRL-2710), R. stolonifer, R. arrhizus, R. oryzae, R. formosaensis, and R. achlamydosporus. The spores of R. oligosporus are produced commercially in Indonesia for industrialscale tempeh production. During the fungal growth phase the O<sub>2</sub> level must be controlled at a reduced level, otherwise the fungus will grow too quickly and form black spore masses that degrade the quality of the tempeh. The traditional way to control  $O_2$  is to wrap the inoculated beans in banana leaves, but a modern innovation is the use of microperforated polyethylene plastic. The fungus grows and mycelia knit the beans into a firm cake to give the characteristic meaty texture.

The enzymes from the fungi transform the soybeans making them more nutritious by hydrolyzing the protein and complex carbohydrates and increasing the levels of the vitamins – riboflavin, niacin, pantothenic acid, and vitamin  $B_6$ . Tempeh must be consumed fairly quickly. Defects include: (1) black patches due to fungal sporulation, (2) slime due to excessive bacterial growth because of too little  $O_2$  or a temperature of  $42^{\circ}C$ , and (3) a yellow color due to growth of toxic fungi. The yellow color indicates that the tempeh is highly toxic and it should not be eaten.

#### Natto

This is produced in Japan, Korea ("chung kook jang"), and Thailand ("thua nao"), but not in China. Traditionally the beans are soaked, boiled, and cooled and wrapped in rice straw and left for 1–2 days at a warm temperature to allow bacteria to grow on the beans and produce a sticky slime. The straw imparts a straw aroma to the fermented beans as well as being the source of the bacteria for the fermentation, and it absorbs some of the unpleasant aroma of ammonia released during the bacterial growth.

In modern manufacture, bacterial cultures of an obligate aerobe *Bacillus subtilis* var. *natto* are used, and the incubation temperature is kept at 38°C for



**Figure 2** A flow diagram showing how natto is produced. (Adapted from Liu K-S (1997) *Soybeans: Chemistry, Technology, and Utilization*. New York: Chapman and Hall.)

20 h followed by cooling (Figure 2). During growth the bacteria produce a viscous, sticky slime that pulls out into long strings when the soybeans are pulled apart. The viscous substance increases to  $\sim$ 2% of the "natto" after 48 h and its crude protein content is  $\sim 80\%$  of which  $\sim 20\%$  is the D-isomer of  $\gamma$ -polyglutamic acid and the remainder is mostly free amino acids. The bacterial fermentation results in a high concentration of vitamin K<sub>2</sub> (menaquinone-7), 100 times more than found in cheese. The fermentation also reduces the "beany" flavor and aroma of soybeans and increases the level of alkyl pyrazines that are responsible for the characteristic odor of natto. The amount of volatile compounds rises from  $\sim$ 35 µg per kg wet weight in cooked soybeans to 2-3.5 mg per kg wet weight in 72 h. Predominant volatile compounds in Japanese natto are 3-hydroxybutanone (acetoin), 2,5-dimethylpyrazine, trimethylpyrazine, and tetramethylpyrazine.

#### Salted Black Beans or Soy Nuggets

This product is made in China ("douchi"), Japan ("hamanatto"), the Philippines ("tao-si"), and India ("tao-tio"). Whole soybeans are soaked and cooked as usual. Under natural conditions, fungi/bacteria grow that depend on the ambient temperature, but under controlled conditions an inoculum of *Aspergillus oryzae* is used. The Chinese incubate for 3–20 days and then wash the beans to remove fungal spores, mycelium, mold odor, and bitter taste, and

then mix them with brine (or soy sauce) and spices which can vary widely (e.g., Indians add sugar), and ferment in a jar for several months. They are then dried. The Japanese use a soybean: wheat flour ratio of 2:1 and incubate for 50 h. They do not wash the beans, but sun-dry them immediately and then place them in brine under pressure for weeks to months. They are then redried and soy-sauce-pickled ginger may be added at the final stage.

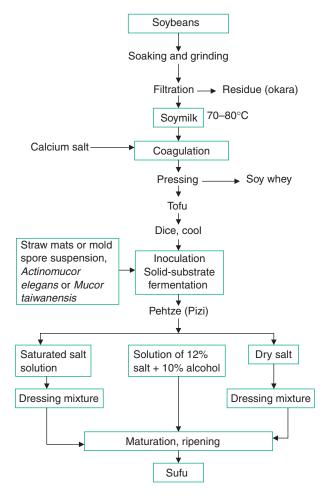
#### **Soymilk-Based Products**

#### **Fermented Tofu**

The general name for these products is "sufu" which means "molded milk" in Chinese, and in hieroglyphics it is known as "furu" which is its preferred Chinese name. Soymilk is extracted and coagulated to make a curd ("tofu") that is then cut into rectangular pieces on which mold is allowed to grow. Mold enzymes hydrolyze the protein, making it more digestible, and the soy oil, producing strong flavors. Tofu was invented by Liu An in  $\sim 179-122$  BC and the first production record dates from the Wei Dynasty in AD 220-265, but the origins of "sufu" are not known. Sufu production in China, domestically and in factories, is  $\sim 300\,000\,\text{t}$  per annum.

The sufu making process is as follows (Figure 3). Soybeans of suitable quality are processed in the usual way to produce soymilk (see Nutrition: Soy-Based Foods and Soybean: Soymilk, Tofu, and Okara). The coagulation step involves addition to soymilk at 70-80°C (in a suitable container) of calcium sulfate or magnesium sulfate at a rate equal to  $\sim 2.5 - 3.5\%$  of the dry weight of the soybeans used to produce the milk which is  $\sim 20\%$  more than used to produce regular tofu. After briefly stirring the mixture vigorously, it is left quiescent for the coagulum to form in 10-15 min. The curd is then pressed mechanically to remove excess water (soy whey) and cut into rectangular blocks  $32 \times 32 \times 16$  mm. Moisture content and pH vary between 70-79% and 6-7, respectively.

Mold stage The next stage, the "pehtze" (pizi) or mold stage, is akin to the "koji" stage in soy sauce and "miso" production. Traditionally, the spores came from straw mats and contaminated the surface, but in modern production methods, spores of a selected pure fungal culture, first isolated and identified in 1920, are applied to the surface of the cubes followed by incubation. Using pure mold cultures, production time is reduced from 5 to 15 days to ~48 h. Preferred mold cultures include *Actinomucor elegans* and *A. taiwanensis* used in Beijing and



**Figure 3** A flow diagram of the production of sufu, a kind of fermented tofu block. It depends on the growth of fungi on the surface of the tofu blocks to help in the production of flavors. Salting and dressing mixtures favor the development of flavors and introduce flavors to the final product.

Taiwan, respectively, but *Mucor sufu* and *M. wutung-kiao* also have desirable characteristics. The mold for the production of the best-quality pehtze must have:

- 1. a high proteolytic and lipolytic activity;
- 2. white appearance, or at most a slight yellow color that is attractive to consumers;
- the ability to produce a mycelial mat over the tofu surface with a texture that is dense and tenacious and able to retain the tofu block shape during subsequent processes; and
- 4. the ability to prevent unwanted bacterial growth, and the inability to produce off-odor, astringent taste, and mycotoxins.

Ideally temperature and relative humidity should be controlled for pehtze production, because *Actinomucor* and *Mucor* species only grow well in the  $20-30^{\circ}$ C range. The fungal starter culture, as a spore suspension containing  $\sim 10^{5}$  colony forming units per ml, is

prepared either by solid-state or by liquid culture in roux bottles. The solid-state culturing medium is bran and water (1:1.3 or thereabouts) and the liquid culture medium is soy whey with added maltose (2-3%) and peptone (1.5-2.0%).

The tofu blocks are sprayed with the inoculum and placed on wooden or bamboo strips in plastic trays and transferred to an incubation room at  $\sim 25^{\circ}$ C with relative humidity of 88-97%. Within 8-12 h a thin layer of mycelia is obvious, and by 36–40 h it is thick. The temperature is then reduced by aeration of the room and the pehtze observed regularly until a slight yellowish-white color appears. The total incubation time is  $\sim$ 48 h, but glutaminase activity that results in glutamic acid production and increased monosodium glutamate (MSG) levels is greatest within 3 days. In the final stage of pehtze production, the mold is flattened by hand.

Salting stage Salting does five things: it imparts a salty taste to the sufu, it prevents the growth of undesirable organisms, it stops the growth of the mold, it releases the proteolytic enzymes bound to the mold mycelia so they can penetrate into the tofu to transform it, and it removes some water from the tofu blocks. Salting can be by dry salting or brine salting. Dry salting takes longer and does not result in a consistent product. The layers of blocks are spread with dry salt, and then stored for 6-12days during which the salt content of the blocks rises to  $\sim$ 16%. The blocks are then removed, washed with water, and transferred to another container for further ripening. Brining is done with a saturated salt solution, or an alcoholic brine solution in which the blocks are immersed for 4-5 days, resulting in final moisture levels of 50-65% with  $\sim 12\%$  salt. The alcoholic brine combines the salting and aging or ripening steps. The ethanol has two effects: first, during ripening lipolytic enzymes release free fatty acids that combine with ethanol to produce aromatic esters, and second, it seems to interfere with protein degradation when compared with salted sufu so that the sufu does not degrade as much.

The final ripening stage of dry salted pehtze is conducted in various dressing mixtures that result in a range of different sufu products. The salted pehtze is placed in the dressing mixture in jars or bottles ranging in size from 0.25 to 101. For red sufu the dressing preparation commonly used is a mixture of 2% "angkak," 3-5% of a paste made with flour or soybean, 8–12% of alcohol, and 5–10% of spices. Further flavor variation is obtained by adding flavors such as rose essence for Rose Sufu or hot pepper to make a Hot Sufu. Traditional ripening periods extend up to 6 months, but modern methods require only 2-3 months. Reducing the size of the tofu cubes, lowering the salt content from  $\sim 14\%$  to  $\sim 10\%$ , lowering the alcohol content from  $\sim 10\%$  to  $\sim 6\%$ , keeping the ripening temperature higher and constant, and reducing the storage jar size can further reduce the ripening period. The higher salt concentrations are thought to reduce rates of proteolysis and lipolysis, but reduced salt content shortens the shelf life.

Types of sufu Types vary widely throughout China, but four types are distinguished based on the fermentation methods used, and four types based on the color and flavor of the product. The four fermentation types are given as follows:

- 1. mold-fermented sufu: use of pure mold culture, brine salting, and aging in a dressing mixture;
- 2. naturally fermented sufu: use of environmental fungi in straw mats and the subsequent process is the same as for (1);
- 3. bacteria-fermented sufu: the tofu is prepared and presalted and a pure bacterial culture is used followed by further salting and aging; and
- 4. *enzymatically ripened sufu*: use of mold-based koji (see soy sauce) at the dressing stage.

From the dressing stage four color and flavor types of sufu are recognized; they are given as follows.

- 1. Red sufu. Sufu blocks are red to purple outside and yellow to orange inside due to the angkak ("anka," red kojic rice or red "qu") prepared with the fungus Monascus purpureus that also imparts a specific flavor to the sufu.
- 2. White sufu. Sufu blocks are a light yellow color throughout and manufacture is similar to red sufu but without angkak and with a slightly reduced salt content.
- 3. Grey sufu. It has a strong pungent odor basically of unknown origin. It is probably derived from the use of a dressing mixture that includes soy whey from the soymilk manufacturing step, as well as salt and spices.
- 4. Other sufu. Sufu blocks are flavored with a variety of additives including vegetables, rice, bacon, and higher concentrations of alcohol.

Sufu may be further classified by size and shape.

#### Soymilk Yogurt

This is a modern product intended to imitate yogurt but the beany flavor due to natural n-hexanal and pentanal, and stachyose and raffinose, that cause human flatulence, detract from the product. Trials with the usual yogurt cultures did not produce

good results unless lactose or sucrose were added. However, *Bifidobacterium bifidum* can metabolize the sugars and the bean flavor substances to produce a satisfactory product thought to be beneficial to human health.

#### **Fermented Soybean Pastes**

#### **Soy Sauce**

Soy sauce is an ancient Chinese product whose precursor was mentioned ~3100 years ago in the Chinese literature. It was introduced to Japan in AD 552 and has also spread to other East Asian countries (Table 2). Soy sauce was derived from a Chinese food called "chiang yu" that was basically a mash made by growing yellow aspergilli on millet then mixing the molded grain and salt in an alcoholic liquor with added pieces of animal, poultry, or fish flesh and storing it for 100 days. (A similar product using soybeans is still made today in China.) A liquid extract from this type of product was first mentioned in the Han dynasty (AD 25–220) and

the first mention of the use of soybeans in this kind of mash dates from AD 535. However, it is the Japanese who have established the scientific basis for modern soy sauce production and they call it "shoyu." In AD 2000  $\sim$ 1800 manufacturers in Japan produced 1.046 million kiloliter (Mkl) of soy sauce of which 51% was produced by six manufacturers.

The best-quality soy sauces are naturally brewed but in practice some commercial soy sauces may contain added vegetable protein extract prepared by chemical or enzymatic hydrolysis. Briefly, production involves an initial solid-state mold growth phase followed by steeping in brine solution. Soy sauce is the liquid extract from the aged mixture. The varieties of soy sauces on the market owe their differences to the countries of origin where different processing conditions are used, and to the addition of wheat, a source of starch that promotes fungal growth and the production of mold enzymes. The soybean: wheat ratios used vary from 1:0 to 1:3. In Japan there are five main types of shoyu - "koikuchi," "usukuchi," "tamari," "saishikomi," and "shiro" - based on the soybean: wheat ratios and other factors (Table 3).

**Table 2** The general composition<sup>a</sup> of some soy sauces from various parts of the world

Product	Bé	NaCl	TN	RS (IS)	Alcohol	Color
Koikuchi shoyu, Japan	23.6	170	17	50.7	25	++
Soy sauce, Taiwan	25.6	156	20.5	59.5	8.6	++
Soy sauce, Korea	21.9	173	15	21	3.9	++
Soy sauce, Hong Kong	28.5	262	15.4	42.2	0	+++
Soy sauce, The Philippines	23.3	247	7.6	10.6	0.1	++
Soy sauce, Singapore	30.1	241	19.7	48.1	0	+++
Soy sauce, Malaysia	23.9	183	11.7	85	0.3	+++
Kecap asin, Indonesia		72	1.9	144.5	0.2	++
Kecap manis, Indonesia		59	1.9	111 (581)	0.9	+++
Soy sauce, USA	22.8	16.5	1.65	37	20.7	++

 $<sup>{}^</sup>a$ Bé=specific gravity, Degrees Baumé; NaCl=sodium chloride (g l<sup>-1</sup>); TN=Total nitrogen (g l<sup>-1</sup>); RS (lS)=reducing sugar (invert sugar) (g l<sup>-1</sup>); alcohol=ethanol (ml l<sup>-1</sup>).

Adapted from Yokotsuka T and Sasaki M (1998) Fermented protein foods in the Orient: *shoyu* and *miso* in Japan. In: Wood BJB (ed.) Microbiology of Fermented Foods, vol. 2, 2nd edn., pp. 351–415. London: Elsevier Applied Science.

Table 3 Typical percentage composition of the five types of soy sauce recognized in Japan

Soy sauce (Shoyu)	NaCl (w/v)	Total nitrogen (w/v)	Formol nitrogen (w/v)	Reducing sugar (w/v)	Alcohol (v/v)	Color	Soybean: wheat ratio	Comment
Koikuchi	16.9	1.57	0.94	3	2.3	Deep brown	1:1	Most popular in Japan, 83% of market
Usukuchi	18.9	1.19	0.8	4.2	2.1	Light brown	More wheat	
Tamari	19	2.55	1.05	5.3	0.1	Dark brown	10:1	Main Chinese type
Saishikomi	18.6	2.39	1.11	7.5	Trace	Dark brown	1:1	Raw shoyu, not fresh brine, is used
Shiro	19	0.5	0.24	20.2	Trace	Yellow/tan	Very high wheat	

Specific gravity is from 22° to 29° Baumé; pH is from 4.6 to 4.8.

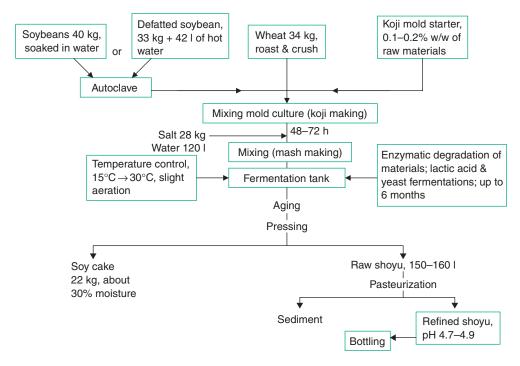
Adapted from Fukushima D (1989) Industrialization of fermented soy sauce production centering around Japan. In: Steinkraus KH (ed.) *Industrialization of Indigeneous Fermented Foods*, pp. 1–88. New York: Marcel Dekker.

Koikuchi is the most popular in Japan, and the main Chinese type of soy sauce is similar to tamari but soybean: wheat ratios of 2:1 and 1:1 are also common in China. Indonesians have two basic kinds of soy sauce — "kecap asin" and "kecap manis" — and they add sugar to the latter. Koikuchi has an alcohol content of 0.3% because it stabilizes the quality and adds flavor to shoyu, but ethanol is not a significant constituent in soy sauces from other parts of Asia. However, in taste tests, Koreans also favor  $\sim 3\%$  ethanol in their "kanjang."

Raw materials and their preparation The first stage in koikuchi soy sauce production is the choice of raw materials (soybeans and wheat) and their preparation (Figure 4) (see Nutrition: Soy-Based Foods and Soybean: Soy Concentrates and Isolates). The traditional choice is whole beans and whole wheat grains and the beans are cooked and the wheat roasted and cracked. However, the oil content of the beans is insignificant in soy sauce, so in modern production systems defatted beans are used, as well as wheat flour. Wheat bran is sometimes preferred, because it is a source of ferulic acid, a precursor for 4-ethylguaiacol, a desirable flavor component. Also the pentose in wheat bran enhances darker coloration

and color stability, but reduces the alcohol content. Best-quality soy sauces contain high soluble nitrogen levels, so one of the objectives of production is to maximize the extraction of the bean protein, which is achieved by using heating at far higher temperatures and for much shorter times than in the traditional boiling methods. Following preparation of the raw materials and cooling them, they are mixed and inoculated with fungi to make the koji. The most desirable fungi are strains of either *A. oryzae* or *A. sojae*.

Koji production The "tane" koji, or starter culture for the koji, is prepared by growing the molds through to the sporulation stage on steamed polished rice or wheat bran. Then ~0.1–1.0% w/w is mixed with the cooked soybean/wheat mixture. In traditional koji production, the inoculated soybean/wheat mixture is placed in a shallow layer (~3–5 cm) in trays in a koji room and incubated for 2–3 days for mold growth. The fungi require oxygen to grow and produce heat that must be dissipated (Figure 5), but in modern production systems special koji rooms or machines are used and conditioned air is mechanically driven through the inoculated layer of soybean/wheat mixture. The fungi produce amylase in the temperature range 35–40°C, but excessive



**Figure 4** A flow diagram of the production of koikuchi shoyu, the most popular type of soy sauce in Japan. The product is a salty extract of the soybean: wheat mixture modified by mold growth through the mixture, followed by yeast and bacterial growth in the brine that results in distinctive soy sauce flavors. (Adapted from Yokotsuka T and Sasaki M (1998) Fermented protein foods in the Orient: *shoyu* and *miso* in Japan. In: Wood BJB (ed.) *Microbiology of Fermented Foods*, vol. 2, 2nd edn., pp. 351–415. London: Elsevier Applied Science.)

amylase production results in sticky koji so the koji must be cooled by mixing at around 18 and 27 h of incubation. Good ventilation is important; however, overcooling can be detrimental. The fungal growth slows at  $\sim\!36\,\text{h}$ , and during the last stage the important proteases begin accumulating below 35°C. The process is stopped before fungal spore formation by gathering up and placing the prepared koji in a brine solution for the long steeping stage.

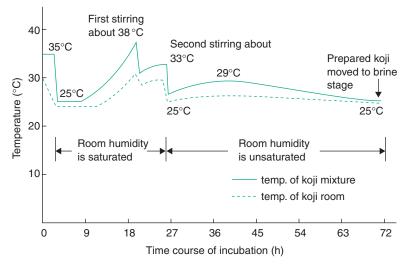
The production processes and names of the processing stages differ from country to country (**Table 4**). In Korea, during traditional manufacture instead of koji, a "meju" cake is made. Soybeans are steeped for 24 h, de-hulled, steamed for 2 h, and cooled to 50°C, and then crushed to a size of 10–15 mesh and natural environmental mold allowed to grow. The molded soybean cakes are dried for 2 days in air and then hung up with rice straw for a further 20–30 days to ferment in a koji room. The cakes are then broken into ~12 pieces before immersion in brine. In Indonesia, the koji is sun-dried before steeping.

Moromi stage In the steeping stage the brine solution is made up with water low in iron and copper, adjusted to pH 6.5-7.0 and cooled to  $0^{\circ}$ C. A complex enzymatic and fermentation process begins that is controlled by managing the temperature, and the addition of air and pure cultures. The high sodium chloride concentration kills the fungi and other incidental salt-sensitive bacteria and yeasts in the koji. The fungal enzymes continue to break down the starch and

proteins as well as cellulose, hemicellulose, and pectin in the soybeans (Figure 6). The objective during this stage is to maximize flavor production, release of proteins by hydrolysis of cell structural components by macerating enzymes (hemicellulase, pectinase, etc.), and protein hydrolysis. About 300 compounds contribute to flavor which depends on the sauce pH range being 4-5, but the important ones are glutamic acid (MSG) and ethanol, the characteristic soy sauce compound 4-hydroxy-2(or 5)-ethyl-5-(or 2)-methyl-3(2H)-furanone (HEMF), 4-ethylguaiacol, 4-ethylphenol. Glutamic acid is produced early by the fungal enzymes and is maximized by retarding the growth of the lactic acid bacterium *Tetragenococ*cus halophilus by keeping the temperature of the moromi low at  $\sim 15^{\circ}$ C for the first few weeks. The ethanol production depends on the growth of a yeast, Zygosaccharomyces rouxii, but the yeast will only grow after the pH of the mixture has been reduced to

**Table 4** The names by which the various stages of the soy sauce production system are known in some East Asian countries

Country	Solid-state fermentation stage	Steeping stage	Final product
Japan Korea Indonesia China The Philippines Thailand	koji meju bungkil chou	moromi baceman	shoyu kanjang kecap chiang-yu toyo see-ieu



**Figure 5** The production of koji is an aerobic process that depends on the growth of fungi. This illustrates what happens in a traditional process. The soybean: wheat mixture is inoculated and spread in a layer ∼5 cm deep. The tray is then put in a controlled atmosphere room and as the fungus grows it produces heat. This heat must be dissipated to reduce temperature so the layer is mixed twice, as indicated. The koji is harvested before the mold starts to sporulate when enzyme production is optimum. (Adapted from Fukushima D (1989) Industrialization of fermented soy sauce production centering around Japan. In: Steinkraus KH (ed.) *Industrialization of Indigenous Fermented Foods*, pp. 1−88. New York: Marcel Dekker.)

<pH 5.0 by bacterial growth. To maximize ethanol</p> production the moromi temperature is raised to 30°C below which ethanol production is almost nonexistent. Later other yeasts, Candida versatilis and C. etchellsii, grow and produce the other characteristic compounds, 4-ethylguaiacol and 4-ethylphenol, from ferulic and p-coumaric acids. All the yeasts are obligate aerobes; so to encourage their growth the moromi is stirred occasionally by pumping air to incorporate oxygen. The moromi maturation time in Japan is from 6 to 8 months. At the end of 2-3months, most of the fermentable carbohydrate has been converted to lactate (1% w/v), ethanol (2-3% v/v), and simple sugars (e.g., glucose and xylose).

The length of the moromi stage in other countries varies. In Indonesia it may be from 1 day up to 5 months, in Korea the traditional incubation period is 2 months. In China one method used involves a defatted soybean meal: wheat bran mixture at a 6:4 ratio that is incubated for 24 h for koji production followed by 3 weeks at a moromi temperature of 40-45°C.

Finally, the solid and liquid components are separated, either by drawing off the liquid or drawing off and pressing the residue. In some parts of China, the raw soy sauce is drawn off and further brine is added to the residual beans for a short period to produce different grades of soy sauce. The raw sauce is placed

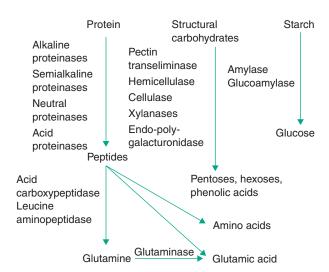


Figure 6 The fungal enzymes in the koji act on the three main components in the soybean: wheat mixture: the proteins, the structural carbohydrates, and starch. The amino acids and glutamic acid are important flavor and nutritive components, the pentoses, hexoses, and phenolic acids are important precursors for flavor compounds, the breakdown of the structural components eases the filtration of mash at the end of aging, and the glucose is an important precursor for acids, ethanol, and other flavoring compounds.

in a vat exposed to sunlight under a transparent cover for up to 6 weeks. The raw liquid is then heated to develop further flavor compounds in the final product. The heating promotes Strecker degradation reactions and Maillard reactions involving pentoses and amino acids that produce flavors.

A biotechnological approach, to reduce the soy sauce production time by employing bioreactors based on immobilized enzymes and microbial cells using ceramics, chitopearl, and alginate gels as the base, has shown some promise. Production times as short as 2 weeks have been achieved. The koji mixture is produced as usual and the enzymatic hydrolysis stage in the brine is controlled. The hydrolyzed extract is then pumped over the immobilized cells for the fermentation stage.

#### Miso

Miso is made with either added rice or added barley (Figure 7). The carbohydrates are cooked for  $\sim$ 40 min and the soybeans are soaked and cooked at 115°C for

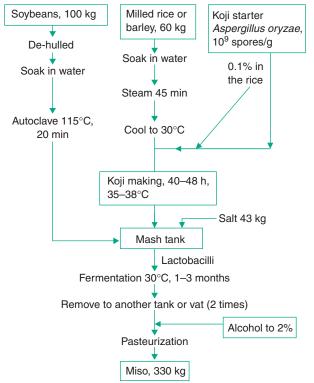


Figure 7 A flowchart for the production of miso. In this case the koji is prepared with the starch source alone and a slightly higher incubation temperature is used to promote amylase production over protease enzyme production. (Adapted from Yokotsuka T and Sasaki M (1998) Fermented protein foods in the Orient: shoyu and miso in Japan. In: Wood BJB (ed.) Microbiology of Fermented Foods, vol. 2, 2nd edn., pp. 351-415. London: Elsevier Applied Science.)

20 min. The koji mold, *A. oryzae*, is grown only on the rice or barley. It is inoculated and incubated at  $35-38^{\circ}$ C for 40-48 h to promote amylase instead of protease production. Salt is added immediately to prevent further mold growth and the salted rice or barley koji is mixed with the cooked soybeans. Lactic acid bacteria and yeast may be added. The mixture is packed in a tank and covered and incubated at  $\sim 30^{\circ}$ C. During aging, it is moved from one tank to another to mix the contents and to introduce some oxygen to promote yeast growth. The aged miso is then blended, mashed, and pasteurized, and  $\sim 2\%$  alcohol is added to prevent yeast growth.

#### Okara

The dry matter content of okara, the waste product from soymilk production, contains ~26% protein, 12% oil, and 52% complex carbohydrates such as hemicellulose, pectin, cellulose, etc. It is a waste product of growing significance, and the high insoluble dietary fiber content is a negative nutritional characteristic (*see* Soybean: Soymilk, Tofu, and Okara).

#### **Fermentation of Okara**

A traditional Chinese product is "meitauza" – produced with *A. elegans*. Acid protease enzyme from the mold releases ammonia and increases non-protein nitrogen eightfold, while pH rises from ~5.5 to 7.5. A natto-like product has been produced with selected bacterial cultures.

Fermentations are usually tempeh-like, so okara cakes are inoculated with fungi and incubated. Using the tempeh fungus, R. oligosporus, and the koji fungus, A. oryzae, the nutritional quality of okara is improved because, on a dry matter basis, there are increases in protein digestibility (80-84%), free amino acids (0.02-0.41%), acid-soluble nitrogen compounds (0.15-0.84%), free sugars (12–18%), and inorganic phosphorus, and decreases in fiber content (56.6–49.5%). Phosphorus increase is due to hydrolysis of phytic acid that binds iron, making the iron unavailable in the diet. Added rice bran improves okara tempeh. The mold *Neurospora* intermedia, used to make Indonesian "oncom," a fermented groundnut presscake with an orange color, when grown on okara presscake, increases the protein content (22-27%), and decreases insoluble fiber, and fat content 15-9%. The texture of fried okara oncom resembles chicken.

Citric acid Citric acid is produced from okara with added 0.1% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and using solid-state fermentation with two mold cultures: *Aspergillus terreus* 

NCIM 653 and A. niger NRRL 330. A. terreus saccharifies the okara and noncellulolytic A. niger produces the citric acid. Maximum citric acid yields of 5.1 g citric acid per 100 g of dry solids, are obtained when A. niger and A. terreus are grown together.

#### **Okara Fermentations for Nonfood Purposes**

Usually solid-state fermentations are used. B. subtilis NB22, incubated at 25°C, produces iturin A, a cyclic heptapeptide of  $\alpha$ -amino acids connected by long  $\beta$ -amino acids, that is effective against serious plant pathogenic fungi. Up to 11 g per kg dry-weight okara of iturin A has been produced. B. subtilis RB14 growing on okara produces surfactin, a lipopeptide consisting of iso-C<sub>15</sub>-hydroxy carboxylic acid and a seven-member ring structure of amino acids, that inhibits fibrin clotting and lyses erythrocytes, sphaeroplasts, and protoplasts, and is a powerful biosurfactant able to lower water surface tension from 72 to 27 mN m<sup>-1</sup>. Surfactin production as high as 2.0 g per kg wet weight was achieved during growth at 37°C. Fibrinolytic enzyme production by some B. subtilis strains was 2.5-fold higher when grown on okara compared with whole soybeans.

For *Penicillium simplicissimum* AK-40 okara was the best medium for the production of some novel compounds with insecticidal properties called okaramine A (C<sub>32</sub>H<sub>32</sub>N<sub>4</sub>O<sub>3</sub>) and B (C<sub>33</sub>H<sub>34</sub>N<sub>4</sub>O<sub>5</sub>), and another strain, ATCC 90288, produced the insecticidal agent, okaramine D.

# Health Benefits of Fermented Soybean Products

Fermented soybeans are thought to have some beneficial effects on health. The benefits arise from the fiber components in the products, and from metabolically transformed components.

#### Tempeh

Tempeh made with R. oligosporus has high free radical scavenging activity, one-third of which comes from isoflavones released by fungal  $\beta$ -glucosidase from the glycosides, and two-thirds from the peptides. One of the antioxidants is 3-hydroxyanthranilic acid (HAA), when it is at high levels in cell culture, inhibits production of the dominant membrane lipid, but at low concentration it accelerates membrane lipid formation. Also HAA inhibits cell growth and induces apoptosis in a human hepatoma-derived cell line (HuH-7), suggesting it may have anticancer properties.

To study the antioxidative, anti-inflammatory, and antithrombotic properties of tempeh, male Wistar

rats were fed an aqueous tempeh extract for 7 days and then their femoral arteries were observed using a γ-camera. When free radicals were induced in the rats, the animals fed the tempeh extract had lower interleukin-1 α-plasma levels, and reduced plasma thromboxane В2 plasma levels, suggesting a protective effect against atherosclerosis. Okarabased tempeh, when fed to rats, increased levels of cholesterol and bile acid in the faeces and significantly lowered them in plasma while liver cholesterol levels were lower compared with controls.

#### Natto

The B. subtilis var. natto used to manufacture natto produces enzymes that break down enterotoxins produced by Staphylococcus aureus. Natto has positive effects on osteoporosis. Ovariectomy-induced bone loss in rats was used as the model and when fed natto containing added isoflavone (446-924 mg per kg isoflavone, including genestin, genistein, daidzin, and daidzein) and zinc along with added calcium, serious bone loss in the rats over a 3 month trial period was prevented. A crude antioxidant preparation called NTX from nattofermented okara exerted an anti-inflammatory effect on the gastric mucosa better than α-tocopherol due to scavenging of the superoxide anion, and in the diet it repressed oedema when induced in rat foot pads by subcutaneous injection of croton oil. NTX also lowered levels of serum thiobarbituric acid-reactive substances, total cholesterol, low-density lipoprotein cholesterol, and triacylglycerol, as well as depressing atherosclerosis in rabbits. Natto intake also increases serum levels of vitamin K<sub>2</sub> (menaquinone-7) in humans.

#### Miso

A powerful antioxidant isolated from A. oryzae fermented soybean is 6-hydroxydaidzein in addition to 8-hydroxydaidzein and 8-hydroxygenistein. However, it was only produced when the soybeans were used to make the koji, not when rice or barley were used. It helps prevent oxidative deterioration of miso. Protein and fiber components in okara koji made with A. oryzae affect lipid metabolism in rats. Lower cholesterol levels in plasma and liver and lower triglyceride levels were found in rats fed a diet containing 50% okara protein enriched with A. oryzae compared with a casein-based control diet. The antioxidants in A. oryzae fermented okara are  $\gamma$ - and  $\delta$ -tocopherol, the isoflavones genistin, daidzein, genistein, and HAA.

The antimammary cancer effects of miso were tested in combination with tamoxifen in rats. The tamoxifen was implanted subcutaneously and the miso fed at 10% of the diet. Mammary cancer was induced and while both the miso group and the tamoxifen group showed reduced cancer rates, the two together showed a synergistic effect. The incidence of tumors in the control group, the miso fed group, the tamoxifen implanted group, and the miso+ tamoxifen group were 91%, 77%, 68%, and 10%, respectively.

Iron absorption in children can be improved by feeding fermented soybean. Microbial hydrolysis of the phytate reduces its iron-binding capacity. When fed to 437 children in China for 6 months, the incidence of iron deficiency dropped from 21.7% to 1.25% and the iron absorption rate was 21.8% in the children given fermented soy and only 14.2% on the control diet.

See also: Fermentation: Foods and Nonalcoholic Beverages. Nutrition: Soy-Based Foods. Soybean: Soy Concentrates and Isolates; Soymilk, Tofu, and Okara.

#### **Further Reading**

- Bei Z-H, Rombouts FM, and Nout MJR (2001) A Chinese fermented soybean food. International Journal of Food Microbiology 65: 1-10.
- Fukushima D (1989) Industrialization of fermented soy sauce production centering around Japan. In: Steinkraus KH (ed.) Industrialization of Indigenous Fermented Foods, pp. 1–88. New York: Marcel Dekker.
- Liu K-S (1997) Soybeans: Chemistry, Technology, and Utilization. New York: Chapman and Hall.
- Nunomura N and Sasaki M (1993) The shelf life of soy sauce. In: Charalambous G (ed.) Shelf Life Studies of Foods and Beverages: Chemical, Biological, Physical and Nutritional Aspects, pp. 391-408. Amsterdam: Elsevier.
- O'Toole DK (1997) The role of microorganisms in soy sauce production. Advances in Applied Microbiology 44: 87-152.
- O'Toole DK (1999) The characteristics and use of okara, the soybean residue from soy milk production (A Review). Journal of Agricultural and Food Chemistry 47: 363-371.
- Sasaki M and Nunomura N (1993) Soy sauce. In: Macrae R, Robinson RK, and Sadler MJ (eds.) Encyclopedia of Food Science, Food Technology and Nutrition, vol. 3, pp. 1786-1795. London: Academic Press.
- Wang HL and Fang SF (1986) History of Chinese fermented foods. In: Hesseltine CW and Wang HL (eds.) Indigenous Fermented Food of Non-Western Origin, Mycological Memoir No. 11, pp. 23–36. Berlin: J. Cramer.

Yokotsuka T and Sasaki M (1998) Fermented protein foods in the Orient: *shoyu* and *miso* in Japan. In: Wood BJB (ed.) *Microbiology of Fermented Foods*, vol. 2, 2nd edn., pp. 351–415. London: Elsevier Applied Science.

#### **Relevant Websites**

http://www.tempeh.info — A general site describing all aspects of tempeh including health aspects and food recipes using tempeh. Producers of tempeh in the USA, Canada, and Belgium are listed and suitable cultures for home production are available.

http://www.soyfoods.com — The US Soyfoods Directory with a free soyfoods guide available for downloading, as well as food recipes, mail order and new soy research. Descriptions of all kinds of soyfoods are given.

http://en.wikipedia.org — This is a site with a free, multilingual encyclopedia. Type in the name of a soy product or soy food to search for information.

http://www.japanweb.co.uk — A Japanese food and restaurant guide that contains a glossary of Japanese foods including soy foods. Information on the use of soy sauce in various recipes is also given.

http://www.foodreference.com — A site dealing in general with all aspects of food. It includes daily food and beverage news, a culinary quiz and a "Today in food history" section. There is a wealth of information.

## Soymilk, Tofu, and Okara

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Soybeans have been grown and consumed in China for over 4000 years, and about 2000 years ago the technology for extracting soymilk was developed. Following this, three main products are now produced using soymilk, tofu, yuba, and okara, the main waste product. With the increasing popularity of soymilk and soymilk-based products, okara is becoming an increasing disposal problem. Many approaches to using okara are being researched, including fermentation methods for food products or industrial products, and its use as a food additive. The production of tofu involves the use of the traditional calcium or magnesium salts, or the modern use of glucono-delta-lactone from which acid is produced

to coagulate soymilk. Growth conditions, soybean variety, and storage conditions influence soymilk characteristics that vary considerably and affect tofu production and yield, as well as characteristics. Yuba is the thin film of protein that forms on hot soymilk when heated openly. Soybeans contain antinutritional factors that must be destroyed by heating and the soymilk develops a "beany" taste that many Westerners find distasteful. This flavor is due to the action of an enzyme that oxidizes the long-chain unsaturated fatty acids.

#### Introduction

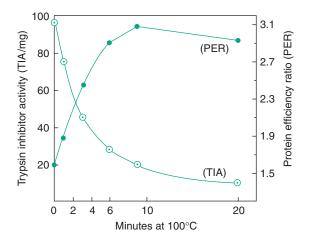
Soybeans, first cultivated in the Yellow River Valley in China ~5000 years ago and on record from ~ sixteenth to eleventh century BC, contain about 34% protein, 20% oil, and about 30% carbohydrate that is comprised of a soluble and an insoluble fraction. The soluble carbohydrate fraction, about 10% of the bean, is a mixture of sucrose (5%), raffinose (1%), and stachyose (4%), and the insoluble fraction consists of pectin (30% of insoluble carbohydrate), hemicellulose (50%), and cellulose (20%), and there is less than 1% starch. Soy hemicellulose consists mainly of arabinogalactan. About 12% of the protein is water soluble. In the beginning soybeans were cooked and eaten but then in the West Han Dynasty,  $\sim$ 2000 years ago, Liu An, who dates from  $\sim$ 164 BC, is said to have invented to fu that required the extraction of soymilk from the beans (see Milling and Baking, History). A major problem with soybeans is that they contain a trypsin inhibitor, an antinutritional agent that prevents the intestinal digestive enzymes from breaking down proteins, and that must be destroyed by heating for a few minutes (Figure 1) to obtain maximum nutritional value (see Nutrition: Beriberi, A Deficiency Related To Grains).

Because soybeans have a high protein content, they, and products derived from them, are seen as animal protein substitutes. In their raw state the concentration of nutrients is high, by comparison with beef, eggs, and milk (Table 1), but when prepared for consumption soybean products have significantly lower nutrient concentrations (Table 2). Apart from the fermented products obtained from soybeans (see Nutrition: Guidelines for Grain-Based Foods and Soybean: Soy-Based Fermented Foods), there are four main products and by-products: soymilk, tofu, yuba, and okara.

#### **Sovmilk Production**

The traditional soymilk production method involves soaking the beans to rehydrate them, rinsing,

resuspension in water and grinding of the beans, followed by filtration to separate the soymilk and fibrous residue. The Japanese developed a slight variation in the traditional Chinese method (Figure 2) that improved soymilk yield and improved filtration characteristics but at the cost of a tendency to burn the slurry



**Figure 1** The effect of heating time on the protein efficiency ratio (PER) and its relationship to the trypsin inhibitor activity (TIA) in soybean. As trypsin activity drops, the digestion of the protein improves as shown by increased PER.

and the need to use mechanical means to filter the hot mixture, as well as increased energy costs. Usually the ratio of water to beans is in the range from 8:1 to 10:1. About 70% of bean solids, 80% of bean protein, and 89% of the oil end up in the soymilk. The residue left is known by a variety of names: okara, draff, tofukasu, soy pulp, tofu residue, tofu cake, dou zha (Chinese), bejee (Korean), and tempeh gembus (Indonesian).

During soymilk production distinctive flavors develop that are variously described as "beany," "painty," "rancid," and "bitter." People from East Asia are used to the flavor but those in other places are generally not used to it. The flavor is due to the action of a native soybean enzyme, lipoxygenase (see Enzyme Activities). The enzyme reacts with the unsaturated fatty acids in the oil (linoleic acid, etc.) in the presence of water, and oxygen, by a process called autoxidation resulting in hydroperoxides (Figure 3) that are then broken down to aldehydes and ketones with strong odors and flavors, e.g., hexanal that has a flavor threshold of less than 1 mg kg<sup>-1</sup>. Normally, in the whole undamaged bean, the oil and the enzyme do not come into contact but, when the beans are ground, contact with the necessary elements of the reaction occurs so that the reaction can proceed. As heat

Table 1 The gross composition, and vitamin and mineral content of whole soybeans compared with other protein sources from animals in the uncooked state

Food (kJ/100 g)	Gross composition (g per 100 g)				Vitamins and minerals (mg per 100 g)					
	Carbohydrate	Protein	Lipid	Retinol	Thiamine	Riboflavin	Niacin	Ca	Р	Fe
Soybean (1653)	30 <sup>a</sup>	36.1	17.7	2	660	220	2.2	226	546	8.8
Beef (464)		21.0	3.0	5	130	170	5.5	12	224	3.2
Eggs (631)		12.3	11.3	530	100	300	0.1	73	224	3.1
Cow milk (264)	5	3.1	3.5	38	40	653	0.2	114	102	0.1

<sup>&</sup>lt;sup>a</sup> Nutritionally most of this carbohydrate is not available to humans. Vitamin contents may change when the foods are cooked.

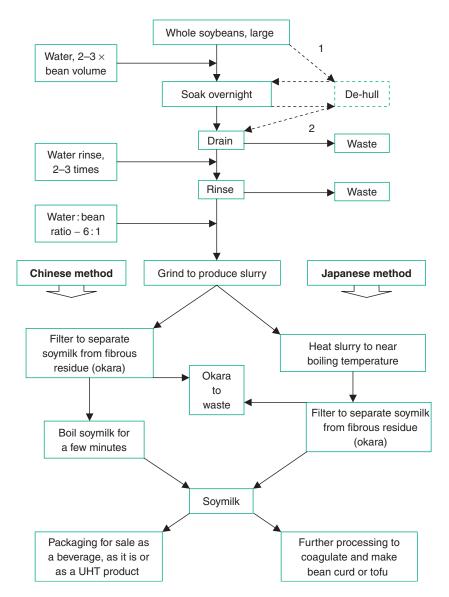
Table 2 The gross composition, and mineral and vitamin contents, of some soybean-based products

Food		Gross	compos	sition (g per 100 g	)		Mine	rals and	vitamin	s (mg per 100	) g)
	Water	Protein <sup>a</sup>	Fat	Carbohydrate	Crude fiber	Ca	Fe	Zn	Р	Thiamine	Riboflavin
Soymilk <sup>b</sup>	93.3	2.8	1.9	1.8	1.1	4	0.51	0.23		0.16	0.07
Tonyu <sup>c</sup>	89.4	4.5				22		0.52	78		
Okara	81.6	3.2	1.7	12.5	4.1	80	1.30			0.02	0.02
Okara <sup>c</sup>	78	5.1				50		0.6	86		
Tofu	84.6	8.1	4.8	1.9	0.1	105	5.36	0.80		0.08	0.05
Tofu <sup>c</sup>	86.3	7.0				78		0.75	105		
$Yuba^c$	7.1	46.2				321		4.08	707		
Tempeh	55.0	19.0	7.7	17.0	3.0	93	2.26	1.81		0.13	0.11

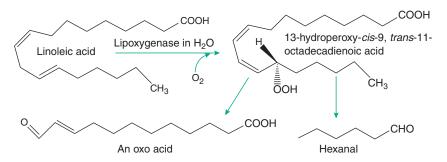
 $<sup>^{</sup>a}_{,}$  Kjeldahl N  $\times$  5.71.

<sup>&</sup>lt;sup>b</sup>Concentration of components in soymilk depends on the soybean: water ratio used to make the slurry.

<sup>&</sup>lt;sup>c</sup>Values from a Japanese paper, tonyu is the Japanese term for soymilk.



**Figure 2** A schematic diagram of the production of soymilk by the Chinese and the Japanese methods. Soybeans may be de-hulled before they are put in to soak or the hulls may be removed at the end of the soaking stage, or the hulls may not be removed at all. Okara is a major by-product from the process.



**Figure 3** The lipoxygenase enzyme is responsible for the "beany" flavor commonly noted in soymilk. The reaction catalyzed by the enzyme involves water, the unsaturated oil, and oxygen and the end-product hexanal is one of the compounds responsible for the beany flavor.

destroys the enzyme, possible processes to prevent bean flavor development include heating the dry beans, grinding the beans in hot water, and blanching the beans, but these processes render some soy protein insoluble, may introduce cooked flavor, and affect soy protein function. However, lipoxygenase is of value for the organoleptic qualities of tofu and soymilk, because tofu made with lipoxygenase-deficient soybeans lacks "mouthfulness" or richness, and soymilk made with similar soybeans results in soymilk with a darker vellow color.

Modern continuous production systems try to balance these factors. Recognized processes include:

- Cornell method. Soybeans are de-hulled and ground in a hot grinder with hot water at 80-100°C. The slurry is then boiled in a steam-jacketed kettle and constantly stirred for 10 min, followed by centrifugation or filtering to remove okara.
- Illinois method. Soybeans are de-hulled and either presoaked and placed in boiling water for 10 min, or placed dry in hot water for 20 min, then drained and ground in sufficient cold water to make a slurry with 12% solids that is then heated to 93.3°C and homogenized. Added sodium bicarbonate (0.25-0.5%) in the early stage may be used to hasten enzyme inactivation and has to be neutralized after homogenization. The resulting product, while bland in flavor, unfortunately has a chalky feel in the mouth. Soybean hulls are the only solid waste product.
- Rapid hydration hydrothermal cooking method. Soybeans are ground into flour, mixed with hot water, okara removed by centrifugation and soymilk pumped to a steam injection head where live steam is injected to increase temperature instantly (UHT conditions, 154°C for 30 s), and the soymilk is held in a holding tube to deactivate trypsin inhibitors. Okara waste results (e.g., the Tetra Alwin<sup>TM</sup> Soy system).
- Full fat soy flake method. The process begins with a product called MicroSoy Flakes made by the MyCal group of Iowa, USA, from de-hulled soybeans.
- Commercial integrated production systems. Some of these systems are designed to be continuous and incorporate steps to minimize "beany" flavor development, such as procedures to minimize incorporation of oxygen that reacts with lipoxygenase.

Large soymilk manufacturers may include a deodorization step at the conclusion of soymilk extraction. The hot soymilk is injected into a chamber under strong vacuum in which volatile off-flavors, such as sulfur flavors, short chain fatty acids and vinyl or sterol compounds, and air in the soymilk,

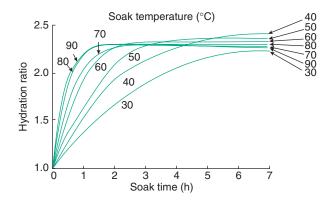


Figure 4 The time to maximum water uptake (hydration) by soybeans depends on the temperature of the soak water.

are vaporized and stripped out. The soymilk is also rapidly cooled as it expands into chamber.

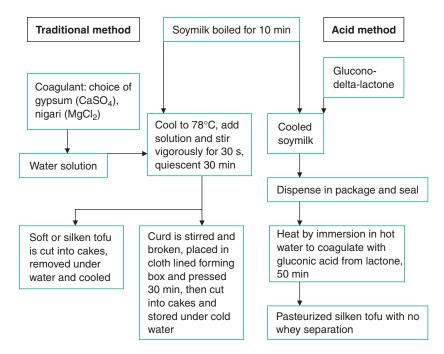
Whether or not the soybeans should be de-hulled before processing is debatable, because the process can damage the cotyledon thus releasing the enzyme but some benefits include elimination of a "green or raw" flavor, and reduced soaking time. Soaking time to full soybean hydration is fastest at 80-90°C and much slower at room temperature (Figure 4). During maceration to produce the slurry, the seedcoat does not behave the same way as the cotyledon cells.

#### Tofu

Tofu resembles a soft white cheese and can be defined as "a water extracted and salt- or acid-coagulated soy protein gel with water, soy lipids, and other constituents trapped on its network" that was developed in China and spread through East Asia (see Nutrition: Soy-Based Foods). It was introduced into Western cuisine less than 100 years ago. The manufacture of tofu with consistent quality and yield is difficult as many things can affect the outcome.

In the production of tofu, various coagulants are used to solidify the soymilk. They include magnesium chloride, calcium sulfate, and glucono-delta-lactone. The general process is outlined in Figure 5. Factors affecting the quality and yield of tofu include:

- Size, shape, and color of hilum and cotyledon of the soybean. Light colors are favored and although a large size, 200 mg per seed or larger, is a traditional Japanese requirement, there seems to be no scientific basis for it.
- Total protein and the 11S-7S protein ratio (see **Soybean:** Soy Concentrates and Isolates). Japanese manufacturers prefer soybeans containing 13% moisture with a 38% or greater protein content. The 7S fraction is  $\beta$ -conglycinin and the 11S fraction is glycinin and the concentrations and ratio



**Figure 5** A schematic diagram of the production of tofu. Traditionally calcium and magnesium salts have been used as coagulants but a more recent innovation is the use of an acid coagulant, gluconon-delta lactone that slowly hydrolyzes to gluconic acid to cause the coagulation of the proteins. The final product is manipulated in various ways to form the final shape and form of the tofu being produced.

**Table 3** Soybean cultivars vary in their protein content and the ratio between the protein fractions 11S and 7S and they have an impact on the tofu yield and tofu firmness

Cultivar	Soybe	an (g per 100 g sc	ybeans)	11S/7S ratio in protein	Tofu firmness (N)
	Moisture	Protein	Tofu yield		
Vintron-81	9.67	49.6	293	3.33	10.02
S-20F8	9.40	49.1	269	3.57	9.91
HP-204	9.06	48.5	320	3.10	8.53
IA-2034	9.66	47.9	322	1.85	8.19
Steyer	9.71	47.9	314	2.40	7.97
IA-2020	9.19	45.9	343	2.24	7.84
S-2020	9.04	42.9	290	2.05	6.93

of these vary with seed cultivar (**Table 3**). The 11S fraction controls hardness, brittleness, and gumminess of resulting tofu, and the 7S fraction requires more calcium or magnesium for coagulation. Hence, the ratio of these two proteins in a soymilk influences the process and product characteristics. The 11S fraction, the  $\alpha'$  polypeptide of 7S, and the basic polypeptide of 11S seem to each affect tofu yield but they are not related to tofu firmness. However, there is a relationship between tofu firmness and the 11S fraction, the 7S fraction, and their ratio. The effect of the 11S:7S ratio may be modified by the processing conditions.

• When calcium sulfate is used for coagulation, the concentration of the salt for optimum tofu quality declines from ~0.8% (w/w) to ~0.4% as the

concentration of protein in the soybeans rises from 31% to 38%. The recommended concentration is 0.02–0.04 mol, but in practice experienced tofu makers know if the amount is correct by whey color and curd texture. If concentration is just right, the whey is transparent with amber or pale yellow color and sweet taste, and if too much coagulating agent is added the whey is slightly bitter and the curd has a coarse texture.

As grinding temperature rises from 2°C to 50°C, the firmness of resulting tofu declines. This is due to the activity of the lipoxygenase enzyme whose reaction by-products, the peroxides, oxidize the – SH groups on the soybean protein being released during the grinding and so reduce protein cross-linking.

- The percentage of total solids in soymilk is related to the water: soybean ratio. The water value used is the total amount of water absorbed by the dry beans plus the final addition of water for grinding plus any rinse waters from the residual okara added back to the slurry. Maximum protein yields are obtained at a ratio of 10:1, water: soybean, but at a ratio of 9:1 pressed tofu has the lowest fresh yield and cohesiveness, and highest brittleness and hardness.
- The coagulation temperature depends on type and concentration of coagulant, how the coagulant is added in, and tofu type to be produced. Generally, as the temperature used is increased (50–90°C) tofu yield decreases, but at the lower temperatures tofu is soft and at higher temperatures tofu is hard and uneven. Consequently, the optimum temperature range is 70–80°C.
- If the soymilk is stirred with added coagulant, the resulting tofu has a hard curd and a low fresh yield is obtained. High-speed stirring favors hard curd production and low-speed stirring for a short time favors softer curds.
- Although soybean variety affects tofu qualities and yield, the growing season for the production of the soybeans has a major impact on tofu texture, due to the effect of environmental growth conditions on the 11S:7S ratio in the protein.
- When soybeans are stored under adverse conditions of high temperature and relative humidity, the tofu yield decreases and tofu hardness increases (Table 4).

During the production of tofu, additives are sometimes included to change the tofu characteristics or to improve textural characteristics. Added blood in Japan, algal cells (*Chlorella* sp.) in Korea, and chitosan have also been used to improve tofu texture. Commercial transglutaminase or glucose at a low level, when added at  $\sim$ 5 mg l<sup>-1</sup> to soymilk to make

**Table 4** The affect of storage of soybeans under adverse conditions, at 85% relative humidity and 30°C, on phytate concentration in the soybeans and the yield of tofu and the brittleness and hardness of the tofu measured in grams

Months of storage	(g per	Tofu yield 100 g of beans)	Brittleness (g)	Hardness (g)
0	1.33	512	1020	2090
1	1.21	505	1033	2182
2	1.18	481	923	2465
3	1.16	421	873	2704
4	1.10	389	920	3080
5	1.06	362	945	3278
6	1.05	233	>5000	>5000

glucono delta lactone tofu, suppresses retort-induced water release during sterilization at 115°C for 20 min.

Tofu is classified into soft (silken), firm, and extra firm varieties. Subsequent processing results in a range of products. Silken tofu contains 88–90% water and 6% protein and is made with soymilk containing 10–12% solids. The curds, when broken and pressed, result in firm and extra firm tofus. The firmness makes it easier to handle the small curd blocks so they keep their shape during slicing and cooking by deep frying or pan frying.

Products, depending on subsequent handling, include plain tofu, frozen tofu (a 1000–1500 year old Chinese tradition), dried-frozen tofu, deep-fried tofu, grilled tofu, and fermented tofu (see Soybean: Soy-Based Fermented Foods). Frozen tofu has an interesting characteristic. On freezing the tofu solids congeal into a firm network, and on thawing the water is released from the tofu block leaving behind a product with a sponge-like texture that is tough and chewy but it absorbs flavors easily. The thawed tofu, when dried (dried-frozen tofu), keeps well (up to 12 months), is easily handled and stored, and is a highly concentrated source of protein and energy.

#### Soymilk- and Tofu-Based Foods

Although the name "soymilk" is commonly used, there is some resistance to the use of "milk" in the name, because milk has been more commonly associated with the bovine-derived product. Consequently, it is sometimes named "soy beverage." Many modern products have been referred as second-generation soy foods because they are modern inventions as opposed to the traditional products above, but a clear-cut definition of these products is difficult. Broadly, they can be placed into three categories: dairy analogs, meat analogs, and tofu-based foods.

The soymilk-based dairy analogs are foods basically the same as dairy products, but soymilk and soy constituents are used instead of milk and milk constituents. Consequently, the soy-based analogs include frozen soy deserts such as soy ice cream and frozen soy yogurt. Generally, dairy ice cream is made from a mixture of ingredients that include water, high fructose corn sweetener (or sucrose), fruits, vegetable gums, salt, and colorant but in soy ice cream the dairy constituents are replaced with soy oil, tofu, isolated soy protein, and soy lecithin. Frozen soy yogurt is basically made from a mix similar to that found in soy ice cream except that it is cultured with the two yogurt lactic acid bacteria (*see* Soybean: Soy-Based Fermented Foods) and frozen and whipped as

for normal ice cream. The product resembles a regular soft serve frozen product.

Attempts to make fermented dairy analogs such as cheese and yogurt from pure soymilk have generally failed for a variety of reasons. They include that the soy protein molecules are larger than milk proteins and have different functional groups and characteristics; the fat to protein ratio in tofu is lower than in cheese, 0.55:1 versus 1.1:1, that results in a hard product; sugars in soymilk do not support growth of dairy bacterial cultures; and acid production by the usual dairy microorganisms in soymilk is variable. Consequently, processes used for dairy products manufacture cannot be easily adapted to soymilk. Products similar to cheese can be made but it requires addition of dairy constituents and enzymes to obtain products similar to dairy cheeses.

The idea of making meat analogs from soybean dates from the 1950s. There are two basic processes for manufacturing meat burger-like products, namely fiber spinning and thermoplastic extrusion. Fiber spinning is used to make fibrous meat-like products that are used in the production of "meat" pies, casseroles, seasoned vegetables, and sandwich fillings. Flavors such as bacon flavor can be added to the product. Thermoplastic extrusion is used to make textured vegetable protein (TVP) that can be formed into continuous slabs for eventual cutting into desired shapes such as burger shapes. Various flavors and spices can be added to make the product flavorful. These meat analogs can be marketed as low fat and cholesterol free as well as being vegetarian.

Tofu is very versatile and can be used to make very many Western style foods. Tofu has been used to make cheesecake, dips, pies, cream cheese, mayonnaise, Italian meatballs, and almost any Western style food.

#### Okara

About 1.1 kg of fresh okara is produced from every kg of soybeans processed for soymilk. Okara contains  $\sim 30\%$  of the whole soybean solids, 20% of the bean protein, and 11% of the oil. Huge quantities of okara are produced, e.g., in Japan  $\sim 700\,000$  ton (t) of okara were produced from the tofu production industry in 1986, and its disposal is a significant problem. The okara is used as an animal food, is dumped in landfills, is used as a fertilizer, and, in Japan, most is burnt. It has been used as a base for a low cost artificial food for silkworms for the first to the third larval instar stages of growth. Research is ongoing to devise uses for okara including the development of other processed human foods. When dry okara is mixed with rice powder in the ratio of 25:75 to prepare

a sauce-like product using a soy sauce manufacturing technique (*see* Soybean: Soy-Based Fermented Foods), the resulting product has an excellent flavor with a light and bright color, but total amino acids and glutamic acid are lower than found with a 1:1 mixture of defatted soybean meal and wheat. Okara is not suitable for ethanol production.

#### **Preservation of Okara**

Due to its high water activity and microbial contamination, okara can putrefy quickly so if it is not used immediately it must be preserved. It is desirable that during preservation the okara should retain its white color and ability to easily absorb water. For short-term storage a lactic acid fermentation will suffice, but drying is the preferred solution.

Fresh okara tends to be lumpy so there are homogeneity problems during drying. Successful drying systems depend on the initial moisture content of the okara. Dehydration methods include addition of water-absorbing synthetic polymers in various forms to the okara; the use of a patented process involving a pneumatic conveying drier and a pulverizer; or a pneumatic drying system with hot air  $(208-254^{\circ}\text{C})$  at a low velocity  $(192 \text{ m min}^{-1})$  in a large drying tower. Other drying systems include a vibro-fluidized bed under vacuum, and drying in pellet form (3 mm × 10 mm) in hot air. Sterilized wet okara can be produced continuously by finely dividing it and feeding it into a scraper-type heat exchanger at not less than 120°C followed by cooling, filling, and sealing aseptically in a suitable container.

#### **Okara Composition**

The main components of okara are ruptured cotyledon cells and the seedcoat. The proximate composition of the wet okara depends on the water extraction efficiency from the slurry phase and soybean cultivar from which it is extracted, amongst other factors. Okara has a pH of  $\sim$ 6.7, and on a dry-weight basis its proximate composition is 27% protein, 10% crude fat/oil, and 14% soluble fiber, 42% insoluble fiber, and 3% ash. The proximate insoluble fiber content is 12% hemicellulose, 5.5% cellulose, 11.5% lignin, and 0.2% phytic acid. Other nutritionally important compounds include vitamins: thiamine, riboflavin and nicotinic acid, as well as calcium, phosphate, zinc, iron, copper, and magnesium (Table 5). The phytic acid (inositol hexa-phosphate), when consumed in large quantities in the diet, reduces calcium balance and metal ion availability, and the zinc has low bioavailability. Soluble carbohydrates include sucrose as well as raffinose and stachyose that cause meteorism (flatulence) (Table 6).

The proximate composition, mineral analysis, and vitamin analysis on a dry matter basis of okara prepared from three cultivars of soybeans Table 5

Soybean cultivar			Proximate composition (g per 100 g)	osition (g	1 per 100 g)	_						Min	erals ar	ıd vita	mins (,	тд ре	Minerals and vitamins (mg per 100 g)		
	Protein <sup>a</sup>	liO	Protein <sup>a</sup> Oil Carbohydrate Phytic acid	Phytic acid	c Insoluble Soluble Total Ash fiber fiber fiber	Soluble fiber	Total fiber	Ash	Ca	Mg	Fe	Na	×	Cn	Zn I	An F	, Thiamii	Ash Ca Mg Fe Na K Cu Zn Mn P Thiamin Riboflavin Nicotinic acid	Nicotinic acid
Edgar	28.4	9.6	5.3	0.5	42.0	14.6	9.99	3200	260	163	6.2	16.2	163 6.2 16.2 1046 1.1 3.8	1.1	3.8	2.5 3	396 0.59	0.04	1.01
Hutton	25.4	10.9	3.8	1.2	43.6	14.5	58.1	3700	428	158	7.2	19.1	158 7.2 19.1 1094 1.1	<del>-</del> -	3.5 3	3.1 444	44 0.49	0.03	0.82
Prima	26.2	9.3	9.3 4.6	6.0	40.2	12.6	52.8	3000	286	165	8.2	18.4	165 8.2 18.4 1233 1.2 6.4	1.2	6.4 2	3.3	2.3 407 0.48	0.03	1.04
<sup>a</sup> Kjeldahl N × 5.71.																			

#### Fractionation of Okara

Okara, as a waste product, is a cheap resource, so to add value to it various components can be isolated in fractionating steps to produce high-value products such as proteins and carbohydrates that can be used as food additives.

Protein The protein in okara is of better quality than that from other soy products, e.g., the protein efficiency ratio of okara is 2.71 compared with 2.11 for soymilk (see Nutrition: Guidelines for Grain-Based Foods), but the ratio of essential amino acids to total amino acids is similar to tofu and soymilk. Protein extracted from heat-treated and nonheat-treated okara differs, the latter contains the same basic 7S globulin found in soybeans, unlike the former. Okara protein isolate is similar to commercial soy isolate in having comparable emulsifying, water and fat binding, and foaming properties.

Pepsin digests of proteins lower hypertension and blood pressure because they contain peptides that inhibit the angiotensin I-converting enzyme (ACE) that converts angiotensin I to angiotensin II, a substance that constricts arteries leading to hypertension and raised blood pressure. The peptides have IC<sub>50</sub> (concentration at which 50% inhibition is found) values between 14 and 53  $\mu$ mol.

Okara fiber component Cellulose concentration is higher in the seedcoat than in the cotyledon but hemicellulose is lower in the seedcoat and higher in the cotyledon, and although galactan hemicellulose is present in the two seed components, araban hemicellulose is present in cotyledon and pentosan hemicellulose in seedcoat. Arabinogalactan hemicellulose is the main constituent in the alkaline extract (24% KOH) from okara.

Demethylation of okara hemicellulose results in a low-viscosity water-soluble carbohydrate that can be used to stabilize soluble proteins under acid conditions, e.g., in acidified milk beverages.

The hemicellulose content of okara can be fractionated into hot-water-soluble, normal-soluble, and alkali-soluble hemicellulose at ratios of 5:19:4 after soaking at  $30^{\circ}$ C overnight. Hot water extraction from okara has been done by hydrolyzing in an autoclave at pH 4.5 in two volumes of water with or without a chelator (hexametaphosphate or ethylene diamine tetra-acetic acid (EDTA)). The extracted polysaccharides, molecular weight of  $\geq 10^{5}$ , have emulsification properties influenced by the amount of bound protein. The use of sodium hexametaphosphate results in protein-free pectic polysaccharides with characteristics similar to commercial pectic polysaccharides products.

Soybean cultivar	Monosaccharides (unspecified)		Oligosaccharides		Starch
		Stachyose	Raffinose	Sucrose	
Edgar	0.7	1.4	0.3	2.3	0.59
Hutton	0.6	0.9	0.3	1.3	0.68
Prima	0.7	0.9	0.4	1.8	0.79

Table 6 Carbohydrate (not fiber) contents on a dry matter basis, of okara from three cultivars of soybeans (g per 100 g)

The monosaccharides units in the polysaccharides in the insoluble fiber components, when enzymatically digested, include galacturonic acid, glucose, xylose, arabinose, and galactose.

#### **Human Consumption**

The dietary fiber content of okara is greater than 50%, so its energy content is only half that of wheat flour. Okara alone has some antinutritional qualities; however, fermented okara may have definite dietary advantages. It can act as a suitable replacement for digestible food in a food prepared to reduce calorie intake, when consumed, it can reduce cholesterol levels in the blood stream, and as a food that contains antioxidant activity, similar to vitamin E, it can reduce the level of free radicals in the body.

Nonfermented products Haarman and Reimer Corp. in the USA developed an okara-based snack bar, and the Japanese National Food Research Institute patented a process for converting okara to a textured soybean product. Biscuits fortified with 60% okara to give protein and dietary fiber contents of 8.72% and 5.98%, respectively, are acceptable to consumers. The Japanese patented a process to produce okara coated with shortening for use as a wheat substitute for making cakes acceptable to consumers. A nougat candy based on peanut, glucose, hydrogenated oil, sugar, and natural essences with added okara has been developed in Argentina. The candy contains 18.3% okara and 27.4% peanut. Acceptable corn tortillas can be made with 10% added okara, and the tortillas have increased lysine and tryptophan levels, amino acids that are nutritionally limited in corn.

Fermented products A traditional Chinese product is meitauza that is produced with okara using the fungus *Actinomucor elegans* in a solid-state fermentation, and another is pickled okara. However, much recent work has to do with using some traditional fungi and bacteria to produce nutritious and flavorsome okara-based products.

Okara-based natto has been produced using selected strains of *Bacillus natto*. Indonesian ontjom (oncom), an orange colored product, has been prepared from okara using the fungus *Neurospora* 

intermedia. The resulting product has no soybean flavor, smooth mouth feel, and, when fried, the texture of okara ontjom resembles chicken (see Soybean: Soy-Based Fermented Foods).

The tempeh fungus, *Rhizopus oligosporus*, and the soy sauce fungus, *Aspergillus oryzae*, improve protein digestibility, and availability of nutrients in the fermented okara along with its antioxidant activities.

In rats fed with a diet containing 50% okara protein enriched with okara koji fermented with A. oryzae, plasma- and liver-cholesterol levels and triglycerides (compared with a casein-based control diet) are lower. The protein and fiber components in okara koji made with A. oryzae and R. oligosporus affect lipid metabolism in rats. Plasma levels of cholesterol and bile acid are significantly lower in okara tempeh fed animals apparently due to cholesterol and bile acid being more strongly bound to okara fiber than cellulose fiber and so they are excreted in the faeces. Dietary fiber, particularly that from the soybean, may be effective in modifying colonic conditions in a positive way (see Nutrition: Guidelines for Grain-Based Foods). Rats fed with fermented okara absorb more iron than those fed with unfermented okara, possibly due to reduced levels of phytic acid.

Fermented okara, compared with unfermented okara, contains more of an antioxidant, called NTX, that has an anti-inflammatory effect on gastric injury in the gastric mucosa. In in vitro tests NTX scavenges the superoxide anion, a process that also occurs in vivo. When foot pad edema is induced in rats by subcutaneous injection of croton oil, NTX fed rats exhibit repressed edema but rats fed with a control diet or vitamin E show no repression of edema. The antioxidants in A. oryzae fermented okara seem to be  $\gamma$ -and  $\delta$ -tocopherol, the isoflavones genistin, daidzein, genistein, and 3-hydroxyanthranilic acid. In vitamin-E-deficient rats fed with okara supplemented with the oxidized oil, the body weight gain was lower and plasma peroxidase activities lower than in those fed with fermented okara suggesting that the antioxidants in the fermented okara scavenged lipid peroxides in vivo.

#### **Fermentation for Nonfood Products**

Some unusual and possibly useful chemicals, new and previously described, including iturin A and surfactin,

#### **Other Products**

The Japanese have patented a process in which okara is used to make a reinforced ceramic via the pozzolanic reaction. Fly ash (50-70%), clays (10-30%), and okara (10-20%) along with  $Ca(OH)_2$  (10-30%) are mixed and heated in an inert gas to 1300-1500°C. The carbonized okara reacts with  $SiO_2$  to form silicon carbide that strengthens the ceramic product.

#### Yuba

This product is made in Japan from soymilk. The soymilk is placed in a wide, open pan and brought to and held at a temperature close to boiling, the ideal temperature being  $82\pm2^{\circ}$ C. Over time a slowly thickening film forms that can eventually be removed with two sticks. The film is placed on a wire mesh shelf to dry out. From 10 to 20 sheets may be produced from one batch of soymilk. It has a very high protein content and is highly digestible and nutritious (Table 2). It is highly perishable and is best eaten fresh but it is also sold in dried or semi-dried forms.

The yield and characteristics of yuba are affected by 11S and 7S proteins in the soymilk. Yuba made with soymilk containing 11S proteins is smooth, opaque, and strong, but that made with 7S soymilk is translucent, full of creases, and weaker by comparison (*see* Soybean: Soy Concentrates and Isolates). The pH of the soymilk is important. The normal pH of soymilk is ~6.7 but if the pH is below 6.2 the film will not form, and if the pH is 9 the yield and protein incorporation are at a maximum. Generally, a pH of 7–8 is recommended.

#### **Detection of GM Soybean**

As genetically modified soybeans are of concern, detection of genetically modified soybeans, e.g.,

Roundup Ready beans, in a batch is of interest. Using a Polymerase Chain Reaction process the *Agrobacterium* derived enzyme, 5-enol-pyruvyl-shikimate-3-phosphate synase gene used in the modification process, can be detected at the level of one modified soybean in 5000 unmodified seeds.

See also: Milling and Baking, History. Nutrition: Beriberi, A Deficiency Related to Grains; Guidelines for Grain-Based Foods; Soy-Based Foods. Soybean: Soy Concentrates and Isolates; Soy-Based Fermented Foods.

#### **Further Reading**

- Bourne MC, Clemente MG, and Banzon J (1976) Survey of the suitability of thirty cultivars of soybeans for soymilk manufacture. *Journal of Food Science* 41: 1204–1208.
- Hou HJ and Chang KC (2003) Yield and textural properties of tofu as affected by the changes of phytate content during soybean storage. *Journal of Food Science* 68: 1185–1191.
- Ikeda S and Murakami T (1995) Zinc chemical form in some traditional soy foods. *Journal of Food Science* 60: 1151–1156.
- Kwok KC and Niranjan K (1995) Review: effect of thermal processing on soymilk. *International Journal of Food Science and Technology* 30: 263–295.
- Liu K-S (1997) Soybeans: Chemistry, Technology, and Utilization. New York: Chapman and Hall.
- Ma CY, Liu WS, Kwok KC, and Kwok F (1996) Isolation and characterization of proteins from soymilk residue (okara). *Food Research International* 29: 799–805.
- Matsuo M (1997) *In vivo* antioxidant activity of okara koji, a fermented okara, by *Aspergillus oryzae*. *Bioscience*, *Biotechnology and Biochemistry* 61: 1968–1972.
- Messina MJ, Persky V, Setchell KDR, and Barnes S (1994) Soy intake and cancer risk: a review of the *in vitro* and *in vivo* data. *Nutrition and Cancer* 21: 113–131.
- Mujoo R, Trinh DT, and Ng PKW (2003) Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. *Food Chemistry* 82: 265–273.
- Ohno A, Ano T, and Shoda M (1995) Production of a lipopeptide antibiotic, surfactin, by recombinant *Bacillus subtilis* in solid state fermentation. *Biotechnology and Bioengineering* 47: 209–214.
- O'Toole DK (1999) The characteristics and use of *okara*, the soybean residue from soymilk production: a review. *Agricultural and Food Chemistry* 47: 363–371.
- Van der Riet WB, Wight AW, Cilliers JJL, and Datel JM (1989) Food chemical investigation of tofu and its byproduct okara. Food Chemistry 34: 193–202.
- Wang HL and Cavins JF (1989) Yield and amino acid composition of fractions obtained during tofu production. *Cereal Chemistry* 66: 359–361.

Yamaguchi F, Ota Y, and Hatanaka C (1996) Extraction and purification of pectic polysaccharides from soybean okara and enzymatic analysis of their structures. *Carbohydrate Polymers* 30: 265–273.

#### **Relevant Websites**

http://www.dietobio.com — A combined French and English site concerned with vegetarian foods. Choose language and go to "Articles" for information on soy foods.

http://www.ellenskitchen.com — A site dealing with recipes and special diets for those with metabolic dietary problems, as well as vegetarian diets.

http://hcf-nutrition.org — The site of the HCF Nutrition Foundation with a focus on the nutritional aspects of foods, in particular soyfoods, and how they are all related to disease.

http://www.fao.org — The site of the FAO of the United Nations. Type "soybean" in the search window to obtain information. Go to "World Agricultural Information Centre" and under "Engineering, Technology and Research." Go to the

"Post Harvest Operations" to obtain comprehensive information about postharvest treatments of soybeans. The site is multilingual.

http://www.wishh.org — The site of the World Initiative for Soy in Human Health, an initiative from the United States of America. The site includes news items.

http://www.soya.be — Information on soyfoods including recipes as well as a wide range of foods. News items are also included.

http://www.aces.uiuc.edu - A University of Illinois website dealing with soybeans.

http://www.foodsubs.com — The Cook's Thesaurus and Encyclopedia covering thousands of recipes and kitchen tools.

http://www.soyfoods.com — The US Soyfoods Directory with a free soyfoods guide available for downloading, as well as food recipes, mail order and new soy research. Descriptions of all kinds of soyfoods are given. http://www.soybean.on.ca — From the Ontario soybean growers in Canada and includes information on the nutritive value of soybeans as well as recipes and descriptions of soyfoods.

### **STARCH**

Contents

Uses of Native Starch Analysis of Quality Chemistry Modification Synthesis

#### **Uses of Native Starch**

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Published by Elsevier Ltd.

#### Introduction

Foods high in starch have always been an important part of the human diet. Traditionally, these foods high

in starch have found their way into either human or animal food. Due to the abundance of these starchy natural materials, it is not surprising that applications other than its caloric value have been found for starch. Early in human history, starch was used to coat papyrus and the Romans documented the production of paste from white flour and vinegar. The variation found in the different sources of starch gives it great versatility in the types of uses. Starch can contribute to adhesion, viscosity, film forming, binding, and moisture retention. Starch is an attractive industrial material because of its abundant supply, low cost, renewability, and ease of modification. Commercially available starches are mostly isolated from either cereals (corn and wheat), and from tubers and roots (potato and cassava). Of the total world production of starch, 83% comes from corn (Figure 1). In the US only  $\sim 2\%$  of starch comes from sources other than corn. In the 2001 crop year, it is estimated that total cornstarch use, not counting cornstarch used for fuel ethanol and sweeteners, will be 250 million bushels. Using an average of 14.5 kg of cornstarch per bushel, estimated total use of cornstarch is  $\sim$ 3.6 billion kg in 2001. Smaller amounts of rice, sago, arrowroot, and pea starch are also available in commercial quantities. Of the wet-milled starch produced in the US,  $\sim$ 54% goes to sweeteners, 27% goes to manufacture of fuel alcohol, and 19% is used as starch. The largest user of starch is the paper industry, which accounts for 61% of the cornstarch uses. Food uses

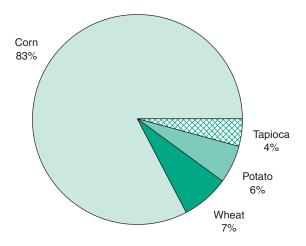


Figure 1 World production of starch.

Table 1 Structure and composition of native starches

account for another 15%; other nonfood uses, after paper, account for the final 14% of manufactured cornstarch.

#### **Composition and Properties**

Starch is deposited in the plastid of higher plants in the form of granules. The size and shape of these granules varies with botanical source (Table 1). The density of these granules is  $\sim 1.5 \,\mathrm{g \, cm^{-1}}$ . The granules are comprised of two polymers of D-glucose that are amylose and amylopectin. Amylose is a lightly branched polymer, with molecular weights varying from  $10^5$  to  $10^6$ , depending on the botanical source. Amylopectin is a highly branched polymer and its molecular weight ranges from  $10^7$  to  $10^8$ . The ratio of amylose to amylopectin varies with the source. Most starches contain between 17-27% amylose and 72-82% amylopectin. Some cultivars have been found that contain starch with no amylose and others have been found that contain up to 70% amylose. Native starch granules are partially crystalline with the amount of crystallinity depending on its botanical source. Amylopectin is the polymer that provides the partial crystallinity of starch, whereas amylose is completely amorphous. The native crystalline structure of amylopectin consists of parallel stranded double helices. Native starch granules also contain small amounts of proteins, lipids, and minerals. Generally, the amounts of these minor constituents of starch make up less than 2% of the granule for cereal starches and less than 1% for tuber and root starches.

Gelatinization is the process of disrupting the molecular structure within the starch granule as it is heated in the presence of water. During the gelatinization process, the viscosity is increased by the granules absorbing water and swelling. Pasting is the

Property	Dent corn	Waxy corn	High amylose corn	Wheat	Potato	Таріоса
Granule size (μm)	5-30	5-30	5-30	1-45	5-100	4–35
Average granule size (μm)	15	15	10	25	40	25
Granule shape	Round, polygonal	Round, polygonal	Round, polygonal, irregular	Round, lenticular	Oval, spherical	Oval, truncated, kettledrum
Amylose (%)	27	0	50-70	27	25	27
Amylose DP <sup>a</sup>	800	NA	700	800	3000	3000
Amylopectin (%)	72	99	30-50	72	74	82
Crystallinity (%)	27	28	_	20	24	24
Gelatinization temp. (EC)	62-74	63-72	95-130	52-64	56-69	52-64

<sup>&</sup>lt;sup>a</sup>DP: average degree of polymerization.

Adapted from (1) Shogren RL (1998) Starch: properties and materials applications. In Kaplan DL (ed.) *Biopolymers from Renewable Resources*, pp. 30–46. Springer; (2) Whistler RL and BeMiller JN (1997) Starch. In: *Carbohydrate Chemistry for Food Chemist*, pp. 117–151. St. Paul, MN: Eagan Press; (3) Rapaille A and Van Hemelrijck J (1992) Modified starches. In: Imeson A (ed.) *Thickening and Gelling Agents for Foods*, pp. 171–201. London: Blackie Academic and Professional; (4) Stevens ES (2002) *Green Plastics*. Princeton, NJ: Princeton University Press; and (5) Thomas DJ and Atwell WA (1999) *Starches Practical Guides for the Food Industry*. St. Paul, MN: Eagan Press.

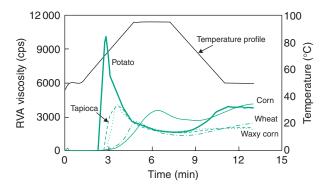
consequence of further heating after gelatinization. Additional heating causes the granules to become distorted, soluble starch is released into the solution, and eventually total disruption of the granules occurs. Many applications of starch rely on the rheological and textural properties of the starch paste. The properties of the paste depend on many factors including botanical source, amylose content, amylose/ amylopectin ratio, molecular weight, moisture content, shear rate, degree of granular disruption, additives, temperature, time, and chemical modification. Figure 2 illustrates the starch paste viscosity of different starches. Tuber and root starches swell more rapidly and have higher paste viscosity and solubilities than cereal starches. As the paste cools, amylose separates and retrogrades (crystallizes) quickly, whereas amylopectin requires a longer time to retrograde. Viscosity of the paste increases on cooling, and starches containing greater amounts of amylose show a larger increase in viscosity due to amylose retrogradation. Starch paste containing amylose will generally gel on cooling while waxy corn pastes, which contain no amylose, will remain fluid on cooling.

#### **Nonfood Uses of Starches**

Starch is used in many different industrial products (Table 2) and its function ranges from being an adhesive, binder, coating, flocculent, filler, fermentation ingredient, to being a viscosity modifier.

#### **Starch Used in Paper Production**

Approximately 1.7 billion kg of cornstarch went into the manufacture of paper for the 1996–97 year. The largest starch source for paper manufacture in the US is corn, but other starch sources can be and have been used. Most of the starch used in paper manufacturing needs to be modified. Starches can either be modified at the paper mill or purchased premodified. Currently,



**Figure 2** Pasting profiles of unmodified native starch. The pasting viscosity and temperature time profile were determined using a Rapid Visco Analyzer.

 $\sim$ 70% of the starch used in paper manufacturing is chemically modified, but in the past large amounts of unmodified starch were used in paper production. Starch use in paper manufacture is generally separated into three application areas: wet-end internal sizing, surface sizing, and coating.

Wet-end sizing can be divided among acid, alkaline, and neutral sizes. Alkaline conditions yield stronger paper, which allow mills to use more filler and less fiber, and thereby saving money. Increases in the use of alkaline sizing have led to an increase in the use of starch. Before the switch to alkaline sizing, manufacturers of uncoated paper used ~4.5 kg of starch per ton of paper. Mills using alkaline technology average between 4.5 and 8 kg of starch per ton of paper. The overall market for starch in wet-end sizing in North America was ∼317 million kg in 2000. Waxy cornstarch holds half the market for paper starches used in the alkaline process. Starch is used in the wet-end process to improve strength and product appearance. Starches used for sizing may be unmodified or specially modified, uncooked, cooked before addition, or pregelatinized and dried. Cationic starches, which are starch derivates, are the preferred wet-end starch additives. By being positively charged, they are

Table 2 Industrial products using native starch

Domestic products Briquets Diapers Typewriter ribbons Trash bags Twine, cord, string	Cosmetics Coating (food and drug) Dispersing agent Industrial alcohol Organic solvents Surgical dressing
Misc. industrial Explosives Filters Fireworks Drilling fluids Plastics Rubber	Paper and paper-related products Abrasive paper and cloth Bookbinding Labels Paper Straws Paste, adhesives
Tires Fermentation Fuel alcohol Beer Building materials	Adhesives Adhesives Binders Glues Gums Pastes
Cardboard Ceramics Coating (wood, metal) Wallboard Linoleum Fiberboard Ceiling tile Wallpaper Cork products	Textile Cord polishing Dyes Oil cloth Printing Sizing Window shades
Chemicals Acetic acid Lactic acid	

attracted to the negatively charged cellulose and the negatively charged fillers. This increases fiber to fiber and fiber to filler bonding, thereby increasing the strength and filler retention. These starches provide strength and drainage, as well as improved filler retention, in contrast to using native cornstarch.

The largest volume of starch used in paper production is for the purpose of surface sizing. Surface sizing improves the paper's finish, gives a better writing and printing surface, and minimizes lifting. Sizing is accomplished by running the paper through a sizing solution and then through sizing rolls. These rolls press the sizing into the paper and remove the excess from the surface of the paper. To make the sizing solution, paper mills usually purchase unmodified and unconverted starch and convert the starch at the mill. Preconverted starches such as acid modified, oxidized, acetylated, and hydroxyethylated are also widely used. Cooked unmodified starch is too high in viscosity for most sizing operations. Consequently, the viscosity of the starch paste is usually reduced. This is normally done at the mill by enzyme conversion, thermal conversion, or thermochemical conversion.

Paper coating refers to the addition of pigment, adhesives, and other materials to the surface of paper. The procedure is commonly called color coating. Some older literature refers to the procedure as pigment coating. The coating is done to provide whiteness, brightness, gloss and opacity, as well as an adequate surface for printing. The adhesive must act as a binder for the pigments, but also must act as a carrier for the pigments. Coating starches need to have lower viscosities than other starches used in the paper industry, because there needs to be a significant amount of starch present to bind the pigments and still be applied as a film. Due to the higher-starch solids, there is a greater potential for retrogradation to occur. Therefore, hydroxyethyl starch is an excellent coating binder. However, higher cost usually limits its use in conventional coatings. Most starches used in coating applications have been enzymatically converted to achieve higher starch levels in the coatings. Continuenzyme-converted starches have viscosities and a more stable paste than those of batch-converted starch of the same solid content. Thermochemical conversion of starch with oxidants is capable of producing high solids coating binder at low cost. Compared with other binders such as latex, starch has a lower adhesive strength, but is usually less expensive. Some coatings contain a mixture of starch and more expensive binders to obtain a compromise between adhesive strength and cost.

The other large consumers of starch in the paper industry are the corrugated board manufacturers. Nearly all the corrugated board produced uses starch

as the adhesive component. Corrugated board is a combination of liners (flat) and fluted paper. The material to be fluted is first softened by heat and moisture and then passed through a corrugated roll to form the flutes. Adhesive is applied to the tips of the flutes and to the face of the liner. The two are brought together under heat and pressure to form a single-facer web. A second liner is added to the single-facer web under similar conditions to form a flute double backer that is used in the familiar shipping containers. Additional single facers can be applied to double backer to build double and triple wallboard. Various weights and thicknesses of liners are used to produce corrugated board of different strengths for various applications.

The material used to join two layers of corrugated board is usually a two-phase starch adhesive. The liquid or carrier phase contains cooked (15-20%) of total starch) starch, sodium hydroxide, and water. The solid phase contains raw starch, borax, and water. The total solids of the adhesive are  $\sim 20\%$ . The sodium hydroxide is added to increase wetting of the cellulosic fibers and reduce gelatinization temperatures of the starch. The borax is used to cross-link the carrier starch and increase waterholding capacity. When the adhesive is applied to the heated flute tips, the raw starch is gelatinized in place. Then the fluted tips are brought into contact with the liner and passed through a roll to bond the material permanently.

Large amounts of starch or starch-based adhesives are used in the manufacture of bags and sacks. The three types of bag adhesives used are side seam, bottom paste, and cross-paste. The formulations of the adhesives for the three types of adhesive operation are quite different. During production of a single-layer bag, the paper is formed into a tube with the tube held together by side-seam adhesive. This adhesive needs to be slow drying, nonpenetrating, and made up of low solids having a high dry-bond strength. Side-seam adhesives usually contain white dextrin or acid-modified starch, so the viscosity will not be high. The adhesive needs to have low viscosity, because it is pumped to the applicator. Bottom-paste adhesive is used to close the bottom of the bag. This adhesive needs to have greater tack than the side-seam adhesive so as to keep the bag from reopening at the bottom seam. The bottom paste can be made from either white dextrin or starch and has a much greater viscosity than do side-seam adhesives. The cross-paste is very similar to the side-seam adhesive and is only used in the production of multiwall bags. Water resistance of bag adhesives can be improved by the addition of urea-formaldehyde or poly (vinyl alcohol).

#### **Starch-Based Adhesives**

Adhesives are used in a variety of applications, with more than 1000 different types of natural and synthetic adhesives used to manufacture many materials. Natural adhesives have  $\sim 32\%$  (or 1.8 billion kg) of the adhesive market. Natural adhesives are derived from corn and wheat starches, lignin, vegetable oils, rubber, and animal-based proteins. Starch-based adhesives are the largest segment of the natural adhesive market. Approximately 60% (1.2 billion kg) of the natural adhesives produced and consumed in the US were derived from corn and wheat starch. Environmental concerns have spurred the use of natural adhesives that have better biodegradability over their synthetic counterparts. The success of some starchbased adhesives can be directly related to solid-waste disposal problems and recycling operations. Starchbased adhesives are now preferred in some types of automated systems because of easy equipment cleaning and flow characteristics.

Starches must be converted before they can be used as adhesives. Methods of conversion include heating, oxidation, alkali, and acid treatment. Dextrins are also starch conversion products. They are produced by dry-roasting starch with an acid catalyst. Starches such as potato, tapioca, and sago are generally easier to convert to dextrins. However, due to the low cost of cornstarch, it is most often used to produce dextrins in the US. Dextrins can be divided into three categories based on their manufacture: white dextrins, yellow dextrins, and British gums. Dextrins are used as adhesive components in bag manufacture, lamination, carton seals, stamps, labels, envelope flaps, and tapes.

#### Other Uses of Starches

There are numerous other uses of starch that make up many niche markets. Starch is an excellent binder and is used in the manufacture of charcoal briquettes, ceramics, sand molds, gypsum board, crayons, and chalk. Starch used as a binder in foundry applications is expected to grow to over 61 million kg. By using starch in ceramics, few hazardous air pollutants are released during firing in contrast to the pollutants released by bitumen, pitch, or lignin sulfate.

Starch has a long history in the textile industry, but the volume used has declined as the industry has moved out of the US. About 80% of the starch consumed in textiles is used as warp sizing. Most of the starches used in warp sizing are modified. Various starches are coated onto yarn to increase strength and abrasion resistance during weaving, which are removed by washing (desizing) the finished fabric. Starches are also used for textile finishing. The finishes are intended to improve the appearance, "feel," and draping qualities of the material.

Starches are used in oil-field applications as viscosity modifiers and to prevent drilling-fluid losses. Drilling fluids are thixotropic solutions used to cool the drilling bit and to suspend solids. High viscosity is needed at low shear to suspend solids and low viscosity is needed at the high shear region around the drill bit where rapid fluid movement is needed to cool the bit. High viscosity is also needed to reduce drilling fluid loss. Starch use as drilling fluids is restricted because starch stability above 107°C is limited, it is highly susceptible to bacterial degradation, and starch solutions have reduced permeability when injected into cores.

Chemicals are produced from starch either by fermentation or biochemical production. Because starch yields pure dextrose upon hydrolysis, it is now being used in many fermentation processes. Ethanol is the chemical produced in greatest quantity from starch. Other chemicals produced from fermentation include acetic acid, citric acid, lysine, lactic acid, and gluconic acid. The fermentation of glucose to lactic acids, from a mass balance point of view, is better than fermentation of glucose to ethanol. The molar yield of lactic acid is 2 mol of lactic acid per mole of glucose where commercial strains are used in anaerobic fermentation. Fermentation of lactic acid does not produce carbon dioxide as ethanol fermentation does. Other alcohols besides ethanol can also be fermented from converted starch or glucose such as butanol and isopropanol. Butanol and acetone can be produced by one of the oldest industrial bacterial fermentation processes (Weizman process) dating back to 1916. The yield of solvent and the ratio of butanol and acetone vary depending on the bacterial species used. Other chemicals can also be fermented from glucose such as butyric acid, formic acid, and acetaldehyde.

Polyhydroxy compounds can be made from starch. Glucose is the most common polyhydroxy compound obtained from starch. Yields obtained from acid or enzymatic depolymerization are  $\sim 90-95\%$ . Glucose can be converted to a variety of cyclic and acrylic polyols, aldehydes, ketones, esters, and ethers. The most widely used polyol made from glucose is sorbitol. Its functional properties (e.g., sweeteners, humectants, and bulking agents) make it suitable for a variety of applications.

#### **New and Future Uses of Starch**

Since the 1980s, there has been a keen interest in replacing petroleum-derived, disposable plastic articles with biodegradable polymers. Plastics

intended for one-time use such as food packaging are difficult to recycle and constitute more than 7.7 billion kg a year market in the US alone. Because of starch's biodegradability, relative low cost, and ready availability, it has received considerable attention as an additive to impart biodegradability.

Starch can be used to make plastic-like materials in many ways. A loose-filled packaging material containing 95% starch was first introduced in 1990. Expanded packaging foams (peanuts) are made by extruding moist starch where water serves as the blowing agent. The original foam was prepared from a slightly hydroxpropylated high-amylose cornstarch. A small amount of poly (vinyl alcohol) was co-extruded with the starch in the presence of 16% moisture. The commercial loose-fill foams on the market generally contain more than 90% starch. Starch-based loose fill has now captured ~15-20% of the 4 million kg per year loose-fill market. Starchbased loose-fill foams have two main drawbacks: (1) its resilience is not as good as that of expanded polystyrene (ESP) and (2) the bulk density of the starch-based loose fill is greater than EPS loose fill. Greater bulk densities of starch-based loose fill make it more expensive per volume basis. It also adds more weight to the freight cost, as more of it is needed to cover the same volume. Starch-based loose fill does have the market advantage of being water dispersible and biodegradable. It also has the unexpected property of being antistatic. Applications for antistatic loose fill include its use in packaging of static-sensitive electronic components, foods, and drugs.

Thin-walled molded articles such as plates, cups, trays, and package cushioning (Figure 3) can be prepared by cooking starch in a mold. This technology is similar to waffle manufacture in the food industry.



**Figure 3** Starch-foamed articles manufactured by baking technology.

An article is prepared by feeding aqueous starch slurry into a mold, with subsequent heating, and evaporation of the water. Molded articles can be made that contain 100% starch, but plant fibers, minerals, and plasticizer are usually added to improve the physical properties. Some items are already on the market in Europe and the US. Potato is the starch source most often used in the manufacture of these foamed articles, due to its greater foaming ability and its lack of odor. Cereal starches can be used where it is feasible.

Gelatinized starch, referred to as destructured starch, is used to make starch-based plastic. The term "destructurized" describes starch extruded at 5-30% moisture under elevated pressures and temperatures that are above its glass transition temperature and melt temperature. Starch that is prepared in this manner is thermoplastic and will flow upon heating. The thermoplastic starch resins usually contain various synthetic polymers (poly(vinyl alcohol), poly(ethylene-co-acrylic acid), polycaprolactone, and poly(ethylene-co-vinyl alcohol)), plasticizers, and lubricants that improve the properties of the resin and/or the properties of the final product. Thermoplastic starch resins generally contain at least 60% starch. These types of starch-based resins can be injection-molded into cutlery and golf tees, as well as blown into leaf and lawn compost bags and mulch. They also can be blow-molded into bottles and other containers. Unfortunately, starch-based resins have not made much of an impact in the market. This is mostly due to their greater cost and their physical properties being inferior to comparable synthetic resins.

Granular starch has been used as a filler in some types of plastic since the 1970s. Generally, starch is added to allow for or increase the biodegradation and/ or lower the cost of the final plastic product. In these systems where starch is a filler in a composite, starch is diluting the resin, which is the continuous phase. This can lead to the reduction of some of the physical properties of the composite such as tensile strength, impact strength, and elongation. The reduction of tensile properties usually limits granular starch contents in composites to 40% or less. Much of the early work was done using starch in nonbiodegradable synthetic polymers such as polyethylene and polyvinyl chloride. In the early 1990s, new biodegradable polymers began to be produced such as poly( $\beta$ -hydroxybutyrate-co-hydroxyvalerate), poly(ε-caprolactone), and poly(lactic acid). These new polymers had excellent properties, but were more expensive than their nonbiodegradable counterparts. Because of starch's biodegradability and low cost, it was naturally thought of as a possible filler in these new polymers

to reduce the overall cost of the resins. These filled composites suffered from the same problem as the nonbiodegradable filled synthetic polymers that being a reduction of their tensile properties.

#### **Food Uses of Starches**

Currently,  $\sim 15-18\%$  of the starch produced in the US is used in food. Starch serves many functions in food such as thickeners, gelling aids, texturizers, emulsifiers, bulking agents, adhesives for coatings, water binding, and fat substitutes. Table 3 lists the food applications of starches in food. Using starch as a food ingredient requires that the functional properties of the starch match a particular application. Table 4 lists some of the functional properties of native starches.

Granular starch is commonly used as dusting agents for candy, starch molds for gumdrops, and as a carrier for baking powder. Smaller granular starch or starch particles ranging from 1 to 2  $\mu$ m have been used as fat substitutes, because they resemble fat micelles.

Most food uses of starch require that the granular structure be disrupted and the starch polymers released. Native starches, although widely used in the food industry, have limited resistance to the physical conditions used in processing. To improve the effectiveness of starch as a functional ingredient, native starches are chemically and physically modified. Starch can be physically modified in a number of ways such as using different drying techniques, redried starches, agglomerated starches, and coldwater-swelling starches. Combinations of different starches are also employed to achieve the functional effectiveness needed to meet processing needs. Native starches without chemical modifications are now finding greater acceptance in food products due to consumers' demands for natural products. Starch manufacturers have developed native waxy

starches that can withstand extremes in heat and shear used in food processing. These native starches can be used in hot retorts and hot filled foods. They also work well in dressings, soups, condiments, yogurt, and, puddings.

Table 3 Food products using native starch

Baking snack foods Frozen desserts Baking powder Frozen puddings **Biscuits** Ice cream or milk Bread and rolls Powder mixes Cakes Sherbets Cookies Meat products Crackers Bologna Doughnuts Fish, seafood Frosting Mincemeat Pies Sausage Pretzels Surimi Canning Misc. food **Berries** Baby food Fruits Cheese spread Fruit fillings Coffee whitener Soups Precooked frozen meals Tomato sauce Powder beverage mixes Vegetables **Pudding** Confectionery Mixes, prepared Chewing gum Cake Chocolates Coating and breading **Dusting powders** Cookies, brownie Marshmallows Dessert **Nougats** Frostina Condiments Gravy Catsup Instant breakfast foods Gravies Pancake Mayonnaise Quickbread Mustard Seasoning Salad dressing Soups Sauce mixes Fats and oil Margarine Pan coatings

Table 4 Properties of native starch pastes

Property	Corn	Waxy corn	Wheat	Potato	Tapioca
Paste viscosity	Medium	Medium high	Medium low	Very high	High
Paste stability shear	Medium high	Medium low	Medium high	Medium	Medium low
Paste stability acid	Low	Low	Medium low	Very low	Low
Paste stability freezing	Low	Medium	Low	Low	Low
Retrogradation rate	High	Very low	High	Medium low	Low
Water binding capacity <sup>a</sup>	15	22	13	24	20
Texture	Short	Long	Short	Long	Long
Clarity	Low	Medium high	Low	Very high	High

<sup>&</sup>lt;sup>a</sup> Parts of water per parts of dry native starch to give same hot viscosity.

Adapted from Whistler RL and BeMiller JN (1997) Starch. Carbohydrate Chemistry for Food Chemist, pp. 117–151. St. Paul, MN: Eagan Press; Rapaille A and Van Hemelrijck J (1992) Modified starches. In: Imeson A (ed.) Thickening and Gelling Agents for Foods, pp. 171–201. London: Blackie Academic and Professional; and Maurer HW (2001) Manufacture and composition of unmodified starch. In: Starch and Starch Products in Surface Sizing and Paper Coating, pp. 25–28. Atlanta, GA: TAPPI Press.

The primary function of a starch in the food industry is to control viscosity. It is used to thicken, add texture and to control viscosity in a variety of prepared foods ranging from canned foods and salad dressings to pie fillings and puddings. Starches used during retort are fill viscosity starches designed to give viscosity during the initial mixing and filling process of canning. A viscosifying starch is used to retain viscosity during retort and as such cannot break down at high temperatures and when shear is applied. For salad dressing, starch is needed for both texture and viscosity control. High-amylose starches are used to provide the set (or spoonable nature) and mouth feel, whereas a waxy starch would provide the viscosity control. Pourable salad dressing uses starch to modify the viscosity, shorten the texture, and add creaminess to the dressing. Starch is used in fillings to modify the viscosity, add stability, and to give smooth short texture. The starch should also not mask or alter the flavor of the filling. For fruit fillings, it is important that the starch gives a clear gel.

See also: **Noodles**: Starch Noodles. **Starch**: Analysis of Quality; Chemistry; Modification; Synthesis.

## **Further Reading**

Jarowenko W (1976) Starch based adhesives. In: Skeist I (ed.) *Handbook of Adhesives*, pp. 192–211. New York: Van Nostrand.

Kennedy HM (1989) Starch- and dextrin-based adhesives. In: Hemingway RW and Conner AH (eds.) *Adhesives* from Renewable Resources. Washington, DC: American Chemical Society.

Maurer HW (2001) Manufacture and composition of unmodified starch. *Starch and Starch Products in Surface Sizing and Paper Coating*, pp. 25–28. Atlanta, GA: TAPPI Press.

Maurer HW and Kearney RL (1998) Opportunities and challenges for starch in the paper industry. *Starch* 9: 396–402.

Nordquist CE (ed.) (2001) Corrugated Adhesives Preparation, 5th edn. Atlanta, GA: TAPPI Press.

Radley JA (ed.) (1976) *Industrial Uses of Starch and its Derivatives*. London: Applied Science Publishers.

Rapaille A and Van Hemelrijck J (1992) Modified starches. In: Imeson A (ed.) *Thickening and Gelling Agents for Foods*, pp. 171–201. London: Blackie Academic and Professional.

Shogren RL (1998) Starch: properties and materials applications. In: Kaplan DL (ed.) *Biopolymers from Renewable Resources*, pp. 30–46. New York: Springer.
 Stevens ES (2002) *Green Plastics*. Princeton, NJ: Princeton University Press.

Thomas DJ and Atwell WA (1999) Starches, Practical Guides for the Food Industry. St. Paul, MN: Eagan Press.

Whistler RL and BeMiller JN (1997) Starch. Carbohydrate Chemistry for Food Chemist, pp. 117–151. St. Paul, MN: Eagan Press.

Whistler RL, BeMiller JN, and Paschall EF (eds.) (1984) Starch Chemistry and Technology, 2nd edn. Orlando, FL: Academic Press.

#### **Relevant Websites**

http://www.corn.org — Corn Refiners. http://www.starch.dk — International Starch Institute. http://www.pulpandpaper-technology.com — Pulp and Paper Technology.

# **Analysis of Quality**

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#### Introduction

Starch is the main polysaccharide produced in photosynthetic tissues. Recently, there has been a growing demand for starch in both food and nonfood industries because of its wide array of applicability in diverse systems. Functionality is the key for the industrial utilization of starch. Functional properties can be defined as the characteristics that govern the behavior of a food component during processing, storage, and preparation. Factors such as botanical source, structure and physicochemical properties, and pretreatments of starch directly affect functional properties of starch. Analysis of starch-related functionality should provide a clear picture of the relevant property that will allow better use of starch in food and nonfood systems, for example, to maintain consistent quality and functionality of starch-based products in every batch, otherwise the product will not survive in the competitive market. Methods that are implemented for the analysis of starch functional properties should be simple and fast, technically feasible, nondestructive, and able to provide reproducible results with high accuracy. Although several methods have been developed recently to monitor some of the functional properties, individual methods are sometimes not capable of providing adequate data on all the dimensions of a functional property,

especially in the determination of retrogradation and gelatinization. Therefore, it is often worth applying at least two methods to get complementary data. This article aims to discuss some functional properties of starch from the perspective of the applicability of methods that are currently employed to determine those properties.

## **Properties, Functions, and Analysis of Starch Macromolecules**

#### **Amylose**

The two major compounds of starch are amylose and amylopectin. The two polymers are structurally different, the minor component, which is linear, consisting mainly of  $\alpha(1-4)$ -linked glucose. However, in some starches, amylose chains have shown a slight degree of branching. Incomplete conversion to maltose by  $\beta$ -amylase suggests the presence of branched points in the amylose polymer. The branched chains can also act similar to unbranched chains. Amylose isolated from tuber and root starches such as potato and cassava has a larger molecular weight than that isolated from cereal starches such as maize and wheat. Although amylose is a linear molecule, its conformation tends to change due to the large number of hydroxyl groups that can produce higher hydrogen bonding capability with strong internal forces. The conformation of amylose has been the subject of controversy and has been shown to vary from helical to an interrupted helix, to a random coil. In alkaline solutions (KOH) and dimethyl sulfoxide (DMSO), amylose probably has an expanded coil conformation, while in water and neutral aqueous potassium

chloride solutions, it is a random coil with short, loose helical segments. Although helical conformation is common in amylose, double helices form when different helices are packed together. The helical arrangement of amylose forms a hydrophobic core inside the amylose molecule, which permits complexing with guest molecules such as lipids and iodine. The amount and molecular characteristics of amylose have a significant influence on starch functionality. Higher amylose content decreases the granular swelling power, whereas increasing amylose concentration decreases the gel stickiness but increases the gel firmness. Reassociation of amylose chains on cooling of a starch paste shrink the starch gel resulting in water accumulation on an aging gel, which decreases the storage stability and usually the quality of an affected starch-based food product.

Methods used in amylose estimation Conventional quantitative determination of amylose is based on its ability to form a deep blue color complex with iodine. Amylose content of starch therefore can be quantified either by spectrophotometric means or by potentiometric titration. Table 1 shows amylose content of some common starches. However, interference from amylopectin and other intermediate materials in the blue color formation reaction biases the estimation of actual content of amylose. Amylopectin-iodine complex is not stable due to shorter unit chain length of amylopectin, but long chains of amylopectin, e.g., long B chain of waxy maize amylopectin can bind with iodine in a similar way to amylose. This would lead to an overestimation. Despite shortcomings such as this, and the time-consuming nature

**Table 1** Some physical and chemical properties of common starches

Starch	Granular size (μm)	Granular shape	Amylose (%)	Swelling power at 95°C	Solubility at 95°C	Taste
Barley	2-35	Round, elliptical, lenticular	22			Low
Maize						
Regular	5-25	Round, polygonal	26	24	25	Low
Waxy	5-25	Round, oval	~1	64	23	Low
High amylose		Round	Up to 80	6	12	Low
Potato	15-100	Egg-like, oyster indentations	22	1000	82	Slight
Rice	3–8	Polygonal clusters	17	19		Low
Rye	2-35	Elliptical, lenticular	23			Low
Sago	20-60	Egg-like, some truncate forms	27	97		Low
Sorghum	5-25	Round, polygonal	26	22	22	Low
Tapioca	5-35	Round, oval, truncated on side	17	71	48	Fruity
Wheat	2-35	Round, elliptical, lenticular	25	21	41	Low
Oats	2-10	Polygonal, compound	27			Low

Adapted from Collado LS and Corke H (2003) Starch properties and functionalities. In: Kaletunc G and Breslauer KJ (eds.) Characterization of Cereals and Flours, pp. 473-506. New York: Marcel Dekker.

of the assay, the amylose-iodine reaction is still the basis of the most widely used method to determine amylose content of starch because of its accuracy, reproducibility, and easy operation. However, the excessive time needed limits its use in quality control applications. Instead of conventional thermal dissociation of amylose in DMSO, low-temperature gelatinization in CaClB<sub>2B</sub> could be employed to reduce the time requirement. High performance size exclusion chromatography (HPSEC), that has shown a good correlation with blue value measurements of amylose content, can be employed to separate starch and de-branched starch. Ability to directly monitor the effect of de-branching on the molecular size distribution of starch and high-molecular weight linear amylose content from this technique provides the estimation of long-chain amylopectin chains that contribute to the apparent amylose content. Recently another technique that is able to eliminate the effect of long-chain amylopectin in the estimation of amylose content has been introduced. In this method, starch completely dissolved in DMSO is subjected to de-fatting with ethanol. The precipitate after defatting is dissolved in acetate/salt solution. A special reaction mixture (concanavalin-A) is then added to the solution to precipitate the amylopectin component and the resulting supernatant after centrifugation contains only the amylose component. However, a comparative study of size exclusion chromatography (SEC), differential-scanning calorimetry, iodinebinding capacity (IB), and concanavalin-A, where SEC was used as the reference method, has revealed that IB, differential-scanning calorimetry (DSC), and concanavalin-A resulted in an overestimation in the determination of amylose content of mutant maize starches (Table 2). As other alternative methods to

**Table 2** Amylose content in maize mutant starches as estimated with size exclusion chromatography (SEC), Concanavalin (Con. A), differential scanning calorimetry (DSC), and iodine binding capacity (IBC)

Starches		Amylos	e (%)	_
	SEC	Con. A	DSC	IBC
ae wx	nd	7	nd	14
du	27	45	55	45
su2	24	50	58	45
du su2	34	58	66	60
ae du	30	56	64	56
ae	54	63	60	63

nd: not detected.

Source: Planchot V, Gerard C, Bertoft E, and Colonna P (2001) An approach to structural analysis of granules using genetically modified starches. In: Barsby TL, Donald AM, and Frazier PJ (eds.) *Advances in Structure and Function*, pp. 104–128. UK: The Royal Society of Chemistry.

the conventional iodine—amylose complex formation, spectroscopic methods have been developed recently. These techniques provide advantages such as ease of sample preparation, speed, and applicability to online monitoring in the process control situation compared with other techniques. Near-infrared (IR)-reflectance analysis has been successfully introduced in determining the amylose content of waxy, normal, and high amylose maize, unground brown or milled rice, and whole grain maize samples. Raman spectroscopy on the other hand is becoming more popular as a quantitative analytical technique in the food industry and has shown potential applicability in the estimation of amylose content in maize starches.

#### **Amylopectin**

Amylopectin, the highly branched molecule, is usually the major component in the starch granule with  $\alpha(1-4)$ -linked glucose linear chains and  $\alpha(1-6)$ linked branch points. Crystalline domains of the starch granules are due to the clustered branches of amylopectin chains that are packed together, whereas the free amylose, amylose complexed with lipids, and branch points of the amylopectin are found in the amorphous region. Alternative arrangement of crystalline and amorphous region was proposed for the semicrystalline starch granule. However, there is no clear demarcation between amorphous and crystalline regions. Crystalline region is less susceptible to enzymatic hydrolysis, water penetration, and other chemical reactions than amorphous region. Amylopectin has a lesser tendency to gelation, retrogradation, and syneresis because of the branched structure. The amount of amylopectin varies among different starches. Waxy varieties contain almost 100% amylopectin. The extent of functional characteristics of starch (viscosity, gelatinization, solubility, texture, gel stability, retrogradation, shear resistance) are directly affected by the amylose/amylopectin ratio. Although amylopectin is the major component of the starch granule, there is no convenient method developed for direct estimation of amylopectin and studies on amylopectin are dependent on development of enzymatic and instrumental methods. Average structural properties of whole molecule and impact of internal structure of clusters on crystallization have been studied by means of enzymatic and SEC. It has been shown that the combined application of preparative and analytical size exclusion chromatography with multiple detection, precipitation techniques, and enzymatic de-branching provides the most detailed analytical insight on the microstructural properties of amylopectin.

## **Function Related to Morphological** Characteristics of Starch Granules

Granules are the basic physical structural unit of starch. The size, shape, and other morphological characters of starch granules are extremely diverse (Table 1). The shape of the starch granule can be spherical, disk, polygonal, or elongated. In general, cereal starches have smaller granules compared to tuber and root starches. Most tuber and root starch granules have a simple size distribution. In some starches, such as barley and wheat, two different granular size populations exist, i.e., a bimodal distribution. Granular size and morphology have recently received more attention, e.g., the size of granules is important in determining taste and mouth feel of some starch-based fat mimetics. Some specific industrial applications of starch are related to size and the size distribution of starch granules. For example, the small size of rice starch granules makes it highly suitable for laundry use. Most of the physicochemical properties of native starch are highly correlated with the size of the starch granules. Larger size granules tend to swell more than smaller granules, whereas smaller size granules are more susceptible to enzymatic hydrolysis. The outer surface of starch granules also plays a key role in many applications of starch. Electron microscopic images have revealed that tuber and root starches show no sign of any pores on the granular surface. However, pores are present on the surface of maize, sorghum, and millet starch granules. Pores on granule surfaces increase accessibility of α-amylase to the granule interior resulting in increased enzymatic hydrolysis. Microscopic analysis provides information on surface features and size of starch granules.

## **Characterization and Analysis of Starch Thermal Properties**

#### Gelatinization

Gelatinization is one of the most important processes affecting starch. It occurs when starch is heated in excess water, when irreversible granular swelling, native crystallites melting, loss of granular order, loss of birefringence, and starch solubilization take place. The extent of these changes depends on type of starch, starch concentration, temperature, presence of other solutes, and shear applied during the gelatinization process. As a result of the above changes during gelatinization, the starch paste develops viscosity, the basis of most technological usefulness of starch as an ingredient in food and nonfood applications. In the majority of food applications, starch functions

(e.g., imparts texture on consumption) in a gelatinized form. Several concepts have been proposed to explain the gelatinization process. Granular swelling acts primarily as a driver to destabilize the starch crystallites. Recently, a model was proposed to explain the phenomena involved in gelatinization and hydration of starch based on the side-chain liquid crystalline. In this model, lamellae in starch are considered in terms of three components - (1) backbone, (2) sidechain, and (3) double helices. The degree of mobility of these three components, coupled with the helixcoil transition, give starch its distinctive properties in gelatinization. Gelatinization is influenced by many factors such as, botanical source of starch, water content, and added solutes. Several methods have been proposed to study the effect of water content on thermal stability. Depending on the water content available during gelatinization, changes in endothermic transition have been detected in terms of number and the position of the differential scanning calorimetric endotherms. endotherm is detectable at higher levels of moisture content while two endothermic transitions are reported at the low-moisture-content level for some starches where low temperature and higher temperature endotherms are designated as G1 and M1 respectively (Figure 1). In addition to two endothermic transactions at lower moisture content, peaks are shifted to higher temperature, indicating need for higher thermal energy to melt the starch crystallites at low moisture content. If there is sufficient water available in gelatinization, then crystallites melt cooperatively, resulting in a single endotherm. Two endothermic transition peaks in insufficient water could be attributed to the differences in the stability of starch crystallites, which melt over different temperatures, less stable crystallites melt first and the

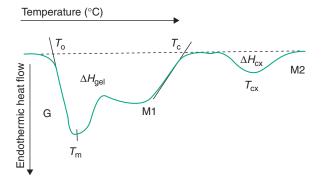


Figure 1 Schematic representation of DSC thermograms obtained for wheat starch heating. (Eliasson A-C (2003) Utilization of thermal properties for understanding baking and salting processes. In: Kaletunc G and Breslauer KJ (eds.) Characterization of Cereals and Flours, pp. 65-115. New York: Marcel Dekker.)

others are next causing the second endotherm. Redistribution of water between crystalline domain and gelatinized starch could also result in two endothermic transitions, in which the first endotherm could be attributed to melting of starch crystals in excess water. Much research has been conducted to investigate the influence of added solutes such as sugars, emulsifiers, and electrolytes (such as sodium chloride), which are widely applied in food industry on starch behavior. Sugars have shown to increase the gelatinization temperature but not to affect endothermic heat absorption. Quality of various food products, bread and cakes, thickening and gelling of sauce, pie filling, and extrusion of cereals are highly dependent on starch-water interaction. Increased gelatinization, when sugars are added to starch paste could be due to decrease of water availability for the gelatinization, binding water to sugar molecules. However, it was reported that sugar acts as an antiplasticizer rather than gelatinizer through the effect on water-binding capability. Increased sugar concentration decreases the plasticization effect requiring more heat energy to achieve the gelatinization. The extent of the influence of sugars on gelatinization, swelling, and viscosity differs depending on the type of sugars and salts used when compared on a molar basis. Monosaccharides are less effective than disaccharides except maltose; among disaccharides, sucrose is more effective.

Methods in the analysis of starch **gelatinization** There are several methods to analyze the gelatinization process, such as DSC, Kofler hotstage microscopy, light microscopy, electron microscopy, X-ray crystallography, enzymatic analysis, nuclear magnetic resonance (NMR), pulsed nuclear magnetic resonance, small angle X-ray scattering, and small angle neutron scattering, Brabender viscoamylography (BV), and Rapid Visco Analysis. DSC, the most widely used method, measures the dissociation parameters, TB<sub>oB</sub> (onset), TB<sub>pB</sub> (peak), TB<sub>cB</sub> (conclusion), and  $\Delta H$  (endothermic heat absorption), of starch crystallites in gelatinization (Figure 1).  $\Delta H$  is the area below the transition endotherm. Gelatinization parameters of starches from different botanical sources measured by DSC are presented in Table 3. In addition, DSC can be used to study the glass transition temperatures of various starchy food products. Gelatinization temperatures are influenced more by the granular architecture than the amyloseamylopectin ratio and depend on the degree of starch crystallite perfection, whereas endothermic heat absorption reflects the quality and amount of starch crystallites. Analysis of gelatinization parameters of starch using DSC was first reported in 1971.

**Table 3** Gelatinization parameters of some starches measured by differential scanning calorimetry

Starch	Starch- water ratio	To	$T_{p}$	T <sub>c</sub>	$T_{\rm o} - T_{\rm c}$	ΔΗ
Wheat	1:3	57.0	62.0	67.0	10.0	9.7
Maize normal	1:3	65.3	71.3	80.9	15.6	11.0
Maize waxy	1:3	62.9	72.8	84.3	21.4	13.6
Potato	1:3	59.6	66.3	76.0	16.4	16.3
Cassava	1:3	63.0	71.5	81.5	18.7	12.3
Sweet potato	1:3	60.0	69.0	82.5	22.5	7.1
Taro	1:3	76.8	83.0	95.2	18.4	14.5
True yam ( <i>Dioscorea</i> )	1:3	75.0	80.0	90.2	16.5	17.8

 $T_{\rm o}$  onset temperature (°C);  $T_{\rm p}$  peak temperature (°C);  $T_{\rm c}$  conclusion temperature (°C).  $\Delta H$  enthalpy (J g<sup>-1</sup>).

Sources: Gunaratne A and Hoover R (2002) Effect of heat-moisture treatment on the structure and physicochemical properties of tuber and root starches. *Carbohydrate Polymers* 49: 425–437; and Hoover R, Vasanthan T, Senanayake NJ, and Martin AM (1994) The effects of defatting and heat-moisture treatment on the retrogradation of starch gels from wheat, oat, potato, and lentil. *Carbohydrate Research* 261: 13–24.

**Table 4** DSC parameters of amylose—lipid complex of some cereal starches

Starch	Condition	$T_{\sf cx}$	$\Delta H_{cx}$	
Wheat	50% water	110.1	1.4	
Rye	50% water	107.8	8.0	
Barley	50% water	110.3	1.8	
High-amylose barley	50% water	110.8	2.8	

 $T_{\rm ex}$  (peak temperature);  $\Delta H_{\rm ex}$  (transition enthalpy). Source: Fredriksson H, Silverio J, Andersson R, Eliasson A-C, and Aman P (1998) The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. *Carbohydrate Polymers* 35: 119–134.

Gelatinization parameters measured by DSC can be affected by sample preparation, baseline application, pan selection, method of adding water, sample mass, heating rate, and moisture equilibration time. Thermal behavior of amylose-lipid complex as well as formation of amylose-lipid inclusion with various molecules such as fatty acids and emulsifiers can also be detected by DSC. Characterization of amylose-lipid complex in cereal starches and influence of amylose-lipid complex on starch properties are well documented. Some of the internal lipids in cereal starch is thought to be complexed with amylose to form the amylose-lipid complex, which is dissociated at higher temperature (M2 endotherm in Figure 1) than that of normal starch crystallites (Figure 1 and Table 4). Although DSC is widely used to study gelatinization, the small sample size used can limit its real applications. In a comparative study of maize starch gelatinization with DSC and NMR, it was reported that NMR could provide much better performance in the analysis of gelatinization for larger samples than DSC. Gelatinization temperature of

Table 5 Gelatinization temperature range of some starches as measured with different techniques: DSC, NMR, BV, and RVA

Starch	Starch-water ratio	Gelatinization temperature range			
		DSC	NMR	BV <sup>a</sup>	RVA <sup>b</sup>
Maize	1:2	58.1-79.6	59-67		
Waxy maize	1:2	56.4-81.3	60-67		
Potato	1:2	49.2-73.9	53-60	76-86	75-86
Cocoyam	1:2	74-87		78-95	85-95
Peruvian carrot	1:2	56-73		62-95	68-95

<sup>&</sup>lt;sup>a</sup>Starch concentration (4%) except Peruvian carrot (6%).

Sources: Perez EE, Breene WM, and Bahnassey YA (1998) Gelatinization profiles of cassava, sagu, and arrowroot native starches as measured with different thermal and mechanical methods. Starch/Starke 50: 14-16 and Gonera A and Cornillon P (2002) Gelatinization of starch/gum/sugar systems studied by using DSC, NMR, and CSLM. Starch/Starke 54: 508-516.

Peruvian carrot, potato, and maize starch measured by Rapid Visco Analyzer (RVA) and BV, and DSC have shown different values for different methods. This indicates the need for multiple analytical techniques for a meaningful understanding of gelatinization along with careful specification (and preferably standardization) of experimental conditions used. Table 5 shows the differences in gelatinization parameters measured by different techniques for the same starch.

#### **Pasting properties**

Starch heated in excess water undergoes various changes as a result of heat and moisture transfer. Gelatinization and pasting occur in the same system and have often been used to describe all the changes that occur. Gelatinization may be used to refer to early changes whereas pasting includes later changes. Starch gelatinization is defined as the collapse of the starch granule manifested in irreversible melting, loss of birefringence, and starch solubilization. The point of gelatinization, and the range over which it occurs is governed by starch concentration, method of observation, granular type, and heterogeneity. Pasting is defined as the phenomena following gelatinization in the dissolution of starch, involving granular swelling, exudation of molecular components from the granule, and eventually total disruption of the granules. The schematic representation of granular changes and viscosity development that occurs during pasting is shown in Figure 2.

Measurement of pasting properties The BV is the most widely established method for determining pasting properties, although it has some methodological and geometrical shortcomings. This apparatus measures the development of viscosity when a starchwater suspension is subjected to a programmed heating and cooling cycle under a shear force. The

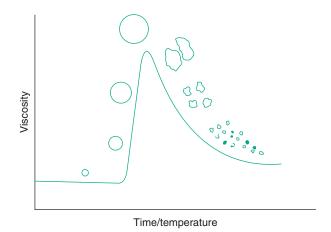


Figure 2 Schematic representation of granular changes and viscosity development during starch pasting (Daniel JR and Whistler RL (1985) Principal changes in starch during food processing. In: Richardson TR and Finley JW (eds.) Chemical Changes in Food During Processing, pp. 305-326. Westport, CT: Avi Publishing Co.).

temperature at which viscosity begins to increase is termed pasting temperature. With further heating, granular swelling increases the viscosity to reach a peak. Further heating at elevated temperature under shear force tends to disintegrate the swollen granules resulting in decrease of viscosity. Upon cooling the starch paste in the next stage, there is a tendency, mainly due to amylose chain reassociation, to increase viscosity. Therefore, a typical pasting profile exhibits three distinct viscosity developments, peak viscosity, hot paste viscosity, and cold paste viscosity (Figure 3). Pasting curves vary according to botanical source of the starch, starch concentration, and the programmed heating-cooling cycle chosen. Among the native starches, potato shows the highest peak viscosity, and generally, low peak viscosity and higher setbacks can be detected in normal cereal starches compared to tuber and root starches. Waxy cereal starches, on the other hand, behave

<sup>&</sup>lt;sup>b</sup>Starch concentration (8%).

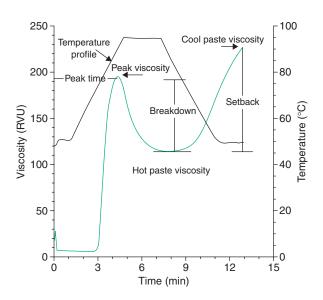
more like the tuber and root starches (Figure 4). According to the characteristics of pasting curves, starch has been categorized into four groups as, high swelling starch (potato, cassava, waxy cereals, ionic starch derivatives), moderate swelling starch (normal cereal starch), restricted swelling starch (cross-linked starch), and highly restricted starch (starches >55% amylose). The above four different categories of starch exhibit the following pasting properties respectively: high peak followed by rapid and major thinning during cooking; lower peak and much less thinning; no peak but maintain high viscosity during cooking; and no swelling to gave a viscous paste at normal concentration.

Because of some technical shortcomings with the BV, such as large sample size requirement and inability to program the temperature profiles, a more recent equipment, the RVA, has become popular for analyzing pasting properties. The RVA differs from the Brabender instrument due to rapid heating rate and stronger mixing action. However, controlled heating

rate to 1.5°C min<sup>-1</sup> in RVA provides similar results to those observed in the BV. Comparative study of pasting properties of cassava, "sago" (sagu), and arrowroot using Brabender and RVA has shown that both techniques provide similar pasting patterns (**Table 6**). Pasting curves of some of the starches determined by BV and RVA is presented in **Figures 4** and 5.

## **Swelling and Solubility**

Native starch granules are insoluble in water. Although small amounts of water can be absorbed at room temperature, granular swelling is limited in intact granules. During heating in excess water, after the onset of gelatinization, granules begin to swell rapidly, losing the polarization crosses. The extent of the swelling power and solubility depends on the magnitude of the starch chain interaction within the amorphous and crystalline domain, size of the starch granules, amylose content, bound lipids, starch



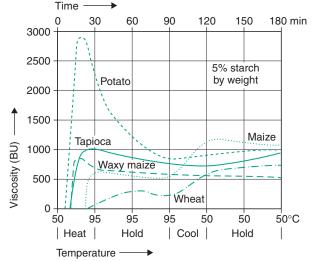


Figure 3 Characteristics of a typical pasting curve.

**Figure 4** Brabender Viscoamylogram of starches from different botanical sources.

Table 6 Pasting properties of cassava, sago, and arrowroot as determined by BV and RVA

Starch	Method	GT	PV	FV at 95°C	V at 50°C	BD	SB	Consistency
Cassava	$BV^a$	68-90	50	40	40	10	-10	0
	$RVA^b$	73-90	23	9	17	14	-6	8
Sago	BV	68-90	300	460	660	-160	360	200
Ü	RVA	73-95	60	22	37	38	-23	15
Arrowroot	BV	75-90	150	150	170	0	20	20
	RVA	79-92	30	14	18	16	-12	4

<sup>&</sup>lt;sup>a</sup>Starch concentration 4.3%.

<sup>&</sup>lt;sup>b</sup>Starch concentration 8%.

GT – gelatinization temperature range; PV – peak viscosity; FV – final viscosity; V – viscosity; BD – breakdown; SB – setback. Source: Perez EE, Breene WM, and Bahnassey YA (1998) Gelatinization profiles of cassava, sagu, and arrowroot native starches as measured with different thermal and mechanical methods. *Starch/Starke* 50: 14–16.

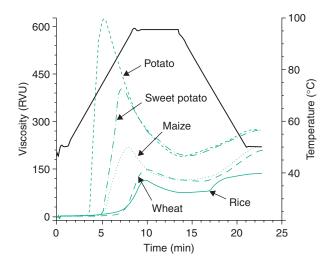


Figure 5 Pasting curves obtained for different starches from Rapid Visco Analyzer.

damage, temperature, and molecular characteristics and the amount of amylose-amylopectin ratio. Table 1 presents the swelling and solubility of some starches at 95°C. High amylose content lowers the swelling whereas larger granules show greater swelling. The swelling power and solubility provide information on the relative strength of bonding within the granules.

Methods used to analyze starch swelling Swelling volume, swelling power or swelling factor, all measure the extent of granular swelling at a given temperature, when starch is heated in excess water. However, swelling factor measures only the intergranular water content and hence reflects the true swelling of starch granules at a given temperature. Swelling power measures the weight of swollen starch sediment relative to the initial starch dry weight, and is expressed as the ratio of the wet sediment to the initial weight of the dry starch. Measuring the soluble carbohydrate that dissolved in the supernatant either by oven drying or colorimetric method enables solubility, the ratio between the amount of soluble carbohydrate to the initial weight of dry starch to be obtained. Swelling volume is a modified version in which programmed shaking of starch suspension is used instead of stirring, and measurement is of the volume expanded due to granular swelling. Swelling factor is the ratio of volume of swollen starch granule to the volume of the dry starch. Swelling factor measures only the intragranular water content. Calculation of the swelling factor is based on starch weight adjusted to 10% moisture assuming the density of 1.4 mg ml $^{-1}$ . The basic principle of this method is the measurement of the volume of water absorbed by starch granules heated in excess water, based on the observation that

blue dextran dye (molecular weight  $2 \times 10P^{6P}$ ) will dissolve in supernatant and interstitial water. The advantages of this method over the others are the ability to measure the true swelling and the high accuracy.

Retrogradation Reassociation of starch polymers via hydrogen bonding in gelatinized starch on cooling is generally termed retrogradation, and is time- and temperature-dependent. Starch gels tend to undergo structural changes during storage as they are metastable and nonequilibrium systems. Both amylose and amylopectin are involved in retrogradation, where rapid amylose aggregation causes a shortterm development of starch gel providing initial firmness. Branched amylopectin recrystallization, particularly outer branches of amylopectin molecule, is correlated with long-term development of starch gel. Several factors influence starch retrogradation, such as, starch concentration, storage temperature, initial heating temperature, chain length distribution of amylopectin, molecular size of amylose, lipids, and physical and chemical modification of starch.

Effect of retrogradation on quality of starch-based **food products** Retrogradation has desirable as well as undesirable effects on quality of starch-based food products. Mostly it is undesirable. Bread staling is one of the main undesirable effects of retrogradation. Although several factors contribute to bread staling, it has been found that retrogradation is the key physical change associated with bread staling. Retrogradation sometimes aids processing in some food products such as hardening of parboiled rice and to improve textural characteristics of certain types of noodles.

Determination of starch retrogradation Several methods have been developed to determine starch retrogradation, because of its great influence on industry-based food and nonfood products, such as DSC, X-ray analysis, rheological methods, and spectroscopic methods.

Differential scanning calorimetry Several studies on retrogradation behavior have been examined using DSC techniques. DSC is probably the best thermal analysis method to study the starch-aging process for different systems. However, dissociation parameters of stronger crystals that formed due to the association of amylose are usually difficult to detect using DSC, since those crystals dissociate at higher temperature (>120°C). Therefore, DSC usually measures the melting of recrystallized amylopectin, that occurs at the same temperature interval as gelatinization

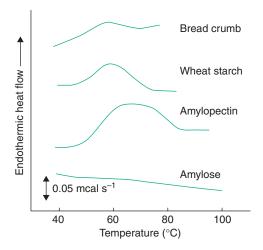


Figure 6 Differential scanning calorimetric curves for different retrograded food systems. (Eliasson A-C (2003) Utilization of thermal properties for understanding baking and salting processes. In: Kaletunc G and Breslauer KJ (eds.) Characterization of Cereals and Flours, pp. 65-115. New York: Marcel Dekker.)

Table 7 DSC characteristics of amylopectin retrogradation of wheat and potato starch gel (1:1) under different storage conditions

Starch	Storage condition <sup>a</sup>	$(T_{\rm o}-T_{\rm c})$	$\Delta H (J g^{-1} AMP)$
Wheat	6/6	40.4-63.3	8.1
	6/30	48.5 - 64.7	7.7
	6/306/30	49.3 - 65.4	9.4
	6/40	58.0-68.5	4.5
	6/40/6/40	58.2-69.0	5.9
Potato	6/6	35.2-78.2	13.3
	6/30	48.8 - 78.3	11.7
	6/30/6/30	49.7-78.1	13.1
	6/40	59.2-78.3	9.9
	6/40/6/40	60.7-79.5	10.6

<sup>&</sup>lt;sup>a</sup>Each number indicates the temperature for one day of storage; 6/40 means one day at 60°C, followed by one day at 40°C.

(Figure 6), when reheating of retrograded starch gel, in which heat absorption, the area under the endotherm  $(\Delta H)$ , and transition temperatures can be detected (Table 7). Crystals formed by the association of amylose chains would be possible to detect using DSC pans, that can withstand a higher temperature range (Figure 7). Characteristic smaller endosperms were usually reported for the retrograded gel compared with the gelatinization endotherm of their native counterpart. However, it was reported that retrograded starch crystals melt over a wide range of temperature indicating the more heterogeneous crystal perfection than those of native starch crystals.

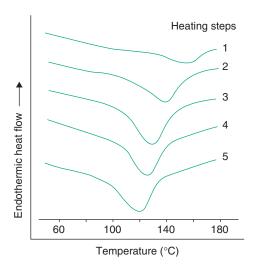


Figure 7 DSC curves obtained for amylose retrogradation. (Eliasson A-C (2003) Utilization of thermal properties for understanding baking and salting processes. In: Kaletunc G and Breslauer KJ (eds.) Characterization of Cereals and Flours. pp. 65-115. New York: Marcel Dekker.)

Table 8 X-ray pattern and crystallinity of different starches

Starch	X-ray pattern	Crystallinity
Rice	A	38
Oat	Α	33
Wheat	Α	36
Rye	Α	34
Amylomaize	В	15-22
Corn	Α	40
Waxy rice	Α	37
Potato	Α	30
Cassava	С	37
True yam (Dioscorea)	В	32
Taro	Α	31
Sweet potato	С	38

Sources: Zobel (1988b) and Gunaratne A and Hoover R (2002) Effect of heat-moisture treatment on the structure and physicochemical properties of tuber and root starches. Carbohydrate Polymers 49: 425-437.

Melting temperature indicates the perfection of recrystallized amylopectin, the quality and heterogeneity.

Although DSC has some advantages especially that it is not time consuming, has a wide range of applications, and measures directly, it has some weakness particularly its small sample size and inability to determine retrogradation in dilute starch paste. More often DSC coupled with X-ray analysis provides better performance in characterizing retrogradation.

X-ray analysis X-ray analysis can be applied to detect the presence and nature of crystallinity in native starch granules as well as crystals formed in aging starch gels. In native starch, the crystallinity is due to the amylopectin components and the crystal

 $T_0 - T_0$  - transition temperature range;  $\Delta H$  - enthalpy; AMP - amylopectin. Source: Silverio J, Fredriksson H, Andersson R, Eliasson A-C, and Aman P (2000) The effect of temperature cycling on the amylopectin retrogradation of starches with different amylopectin unit-chain length distribution. Carbohydrate Polymers 42: 175-184.

Table 9 Gel textural properties of maize starches differing in amylose content

Sample	Probe type	Hardness (g)	Adhesiveness	Springiness	Cohesiveness
Regular Waxy	5 (mm)	75	219	0.94	0.51
High amylose		19	18	0.61	0.031
Waxy	20 (mm)	28	18	0.94	0.81
High amylose		128	160	0.85	0.35

Starch paste after RVA analysis kept at 25°C for 24 h.

Source: Liu H, Ramsden L, and Corke H (1999) Physical properties and enzymatic digestibility of hydroxypropylated ae, wx, and normal maize starch. Carbohydrate Polymers 40: 175-182.

domains are constructed mainly of A chains and outer B chains of amylopectin. Native starches can be categorized into three groups according to their X-ray diffraction pattern. The A type crystallinity is found mainly in cereal starches. Most tuber and root starches exhibit typical B type X-ray pattern and C type is intermediate between A and B types, shown in legumes, cassava, and some varieties of sweet potato (Table 8). X-ray analysis of retrograded starch has shown that aging gels form B-type crystals irrespective of the native starch. Some research using wide range and small angle X-ray diffraction, shows that crystalline formation in starch paste occurred primarily due to amylopectin aggregation, whereas amylose provides a template effect and highly ordered amylose aggregation does not necessarily possess a crystalline nature but accelerates the amylopectin reassociation. Recently, more information on bread staling has been revealed by X-ray analysis. Aggregation of starch polymers that formed crystals in bread staling was shown from X-ray analysis; however, NMR and FTIR techniques are better at detecting minor differences of starch polymer aggregation than X-ray analysis.

Rheological methods Measurement of pasting parameters, analysis of textural properties of starch paste using texture analyzer, and small deformation dynamic techniques oscillatory rheometry can be applied to the behavior of starch. Pasting behavior of starch indicates the trend of starch retrogradation. In a typical pasting profile, the magnitude of setback reflects the tendency of mainly amylose polymer reassociation in a starch paste. Several investigations have been performed to study the properties of aging breads using viscometry and have observed a tendency of decreased peak viscosity when bread staling progresses. Recently there is a growing interest, especially in industry, in use of texture analyzers that can directly and rapidly measure the textural properties such as hardness, stickiness, cohesiveness, adhesiveness, fracturability of starch paste (Table 9). Long-term reliability and accuracy,

direct measurement, time saving, and technical feasibility of the texture analyzer increases its attractiveness among food technologists. The main issue which requires continuing work is that of instrumental to sensory correspondence, i.e., whether the texture analysis profile is adequately predictive of the sensory traits important to the consumer.

Many additional methods can be employed to detect retrogradation. No individual method is able to fully characterize all the events of the starch-aging process. DSC measures only the transition of heat energy in the melting of crystal aggregates, X-ray measures the presence and characteristics of retrograded starch crystals, and texture analysis provides textural parameters for the aged starch gel. Therefore, it is clear that a multiple analytical technique approach for the advance detection of retrogradation behavior is necessary. For example, a study of bread staling with multiple techniques, DSC, FTIR, and NIR, led to greater understanding of the process than would be possible with each individual technique used alone.

See also: Noodles: Starch Noodles. Starch: Uses of Native Starch; Chemistry; Modification; Synthesis.

## **Further Reading**

Collado LS and Corke H (2003) Starch properties and functionalities. In: Kaletunc G and Breslauer KJ (eds.) Characterization of Cereals and Flours, pp. 473-506. New York: Marcel Dekker.

Daniel JR and Whistler RL (1985) Principal changes in starch during food processing. In: Richardson TR and Finley JW (eds.) Chemical Changes in Food During 305–326. Westport, CT: AVI *Processing*, pp. Publishing Co.

Eliasson A-C (2003) Utilization of thermal properties for understanding baking and salting processes. In: Kaletunc G and Breslauer KJ (eds.) Characterization of Cereals and Flours, pp. 65–115. New York: Marcel Dekker.

Fredriksson H, Silverio J, Andersson R, Eliasson A-C, and Aman P (1998) The influence of amylose and amylopectin characteristics on gelatinization and

- retrogradation properties of different starches. Carbohydrate Polymers 35: 119-134.
- Gonera A and Cornillon P (2002) Gelatinization of starch/ gum/sugar systems studied by using DSC, NMR, and CSLM. Starch/Starke 54: 508-516.
- Gunaratne A, and Hoover R (2002) Effect of heat-moisture treatment on the structure and physicochemical properties of tuber and root starches. Carbohydrate Polymers 49: 425-437.
- Hoover R, Vasanthan T, Senanayake NJ, and Martin AM (1994) The effects of defatting and heat-moisture treatment on the retrogradation of starch gels from wheat, oat, potato, and lentil. Carbohydrate Research 261: 13-24.
- Jane J, Kasemsuwan T, Leas S, Zobel HF, and Robyt JF (1994) Anthology of starch granules morphology by scanning electron microscope. Starch/Starke 46: 121.
- Karim AA, Norziah MH, and Seow CC (2000) Methods for the study of starch retrogradation. Food Chemistry 71: 9-36.
- Liu H, Ramsden L, and Corke H (1999) Physical properties and enzymatic digestibility of hydroxypropylated ae, wx, and normal maize starch. Carbohydrate Polymers 40: 175-182.
- Perez EE, Breene WM, and Bahnassey YA (1998) Gelatinization profiles of cassava, sagu, and arrowroot native starches as measured with different thermal and mechanical methods. Starch/Starke 50: 14-16.
- Phillips DL, Xing J, Liu H, Pan DH, and Corke H (1999) Potential use of Raman spectroscopy for the determination of amylose content in maize starch. Cereal Chemistry 76: 821-823.
- Planchot V, Gerard C, Bertoft E, and Colonna P (2001) An approach to structural analysis of granules using genetically modified starches. In: Barsby TL, Donald AM, and Frazier PJ (eds.) Advances in Structure and Function, pp. 104–128. UK: The Royal Society of Chemistry.
- Praznik W, Huber A, Watzinger S, and Beck RHF (1994) Molecular characteristics of high amylose starches. Starch/Starke 46: 88-94.
- Silverio J, Fredriksson H, Andersson R, Eliasson A-C, and Aman P (2000) The effect of temperature cycling on the amylopectin retrogradation of starches with different amylopectin unit-chain length distribution. Carbohy*drate Polymers* 42: 175–184.
- Tester RF and Morrison WR (1990) Swelling and gelatinization of cereal starches 1. Effects of amylopectin, amylose and lipids. Cereal Chemistry 67: 551-557.
- Yu L and Christie G (2001) Measurement of starch thermal transitions using differential scanning calorimetry. Carbohydrate Polymers 46: 179-184.
- Zobel, HF (1988) Molecules to granules: a comprehensive starch review. Starch/Starke 40: 44-50.

#### **Relevant Website**

http://www.starch.dk - Website of the International Starch Institute, Denmark, with useful links to other starch-related sites.

## Chemistry

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#### Introduction

Starch is the major energy reserve of higher plants. This makes it a major energy source in the diet of humans and many other animals, including livestock animals grown for their meat and milk as well as pest animals which consume grains in storage. Starch comprises  $\sim 70\%$  of the dry matter of the endosperm of a mature cereal grain and ~16% of the fresh matter of a mature potato tuber.

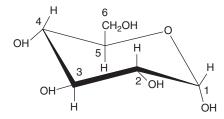
Starch may be loosely defined as a polymer of glucose which is distinguished from other glucans by a number of key features.

First, it is  $\alpha$ -linked, unlike cellulose and other  $\beta$ -linked glucans. The majority of these links are  $\alpha$ -1,4 (also written as  $\alpha$ -(1  $\rightarrow$  4)), leading to chains of varying lengths, and the chains are joined together by  $\alpha$ -1,6 linkages. These branching points occur in a highly ordered fashion, leading to double-helix formation and a type of crystallinity, minimizing water-holding capacity. This feature is in significant contrast to glycogen, an  $\alpha$ -(1 $\rightarrow$ 4), (1 $\rightarrow$ 6) mixed-link glucan found in animals and bacteria. Ordering in glycogen is very limited, so it holds much more water than an equivalent mass of starch. It is thus not as efficient an energy store where water availability is limited, such as in a dry, dormant seed.

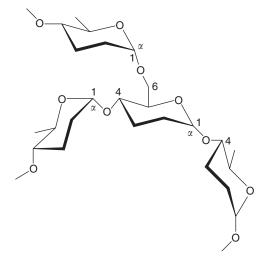
Starch is deposited in granules between 1 and 100 µm in diameter within membrane-bound organelles. In the actively photosynthetic parts of the plant, these organelles are the chloroplasts, and in storage organs, they are the modified chloroplasts called amyloplasts. Granule structure has a number of features which are consistent across species, while granule size, morphology, and composition vary widely among species. These aspects as well as starch composition affect the processing properties of the starch.

## The Monomer and Linkages

The building block of starch is D-glucose, in its hexagonal pyranose ring conformation (Figure 1). Carbon 1 of this ring is a highly reactive, aldehydic, "reducing" end. Most commonly, during polymer formation in a plant, it attaches to carbon 4 of another glucopyranose residue (Figure 2). When the monomers are  $\alpha$ -D-glucopyranose, the resulting polymer is a chain of starch, and when they are  $\beta$ -D-glucopyranose, it is a chain of cellulose.



**Figure 1** p-glucopyranose. As shown, this is the  $\alpha$ -conformation. When the hydroxyl group and hydrogen attached to carbon 1 are in the opposite orientation, it is the  $\beta$ -conformation.



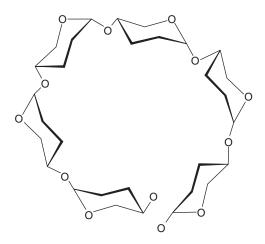
**Figure 2** Glucose polymer with  $\alpha$ -1,4 and  $\alpha$ -1,6 branching types.

An  $\alpha$ -1,4 linked glucan is at its most stable in a left-handed helix, with a complete turn taking six residues (Figure 3) and occupying 2.1 nm. Hydrogen bonding between adjacent glucose residues helps to stabilize the helix. The helix can allow a second parallel helix to fit into it, creating a double helix. The helical structure of starch is an important factor in its processing properties and in its enzymic degradation during digestion. Cellulose, in contrast, is highly crystalline, ribbon like rather than helical, and relatively few organisms have the appropriate enzymes to hydrolyze it.

The presence of  $\alpha$ -1,6 bonds (Figure 2) allows the development of much larger and less soluble polymers through the introduction of branching points connecting glucose chains of various lengths. Starch is an excellent example of the evolutionary conservation of a successful structure throughout the plant kingdom.

## **Starch Polymer Types**

Two subclasses of starch, "amylose" and "amylopectin," have been widely recognized, based on chain length, branching pattern, and overall mass. Both



**Figure 3** Helix of  $\alpha$ -1,4 glucose residues, seen from above.

types have a single reducing group per molecule and a variable number of nonreducing ends. Most plant starches comprise about one-fifth to one-third amylose and the remainder amylopectin (Table 1). Plant breeders and geneticists have considerably enhanced this range since the beginning of the twentieth century.

Amylose is usually described as being "essentially linear." A typical amylose molecule comprises two to eleven chains of 200-700 α-1,4 linked glucose residues. The chains are connected by  $\alpha$ -1,6 bonds, leading to an overall polymer size of 1000-5000 glucose residues and a molecular mass of  $(1.6-8) \times 10^{3}$ . Chain length and branch number are species specific. Within the starch granule, the amylose chain is present as a single helix. This is demonstrated by the ease with which it may be leached out of the granules by water at moderate temperatures and its full release at 60-80°C, whereas an amylose double helix should not be released until the temperature greatly exceeds 100°C. The single helix retains a hydrophobic channel or lumen along its axis, into which a fatty acid, for example, may interpolate. These amylose-lipid complexes have critical effects on the processing properties of the starch, as described below (starchbound lipids). Amylose concentration increases during the course of starch deposition in the storage organ and, in maize, is greater in proximal than in distal grains.

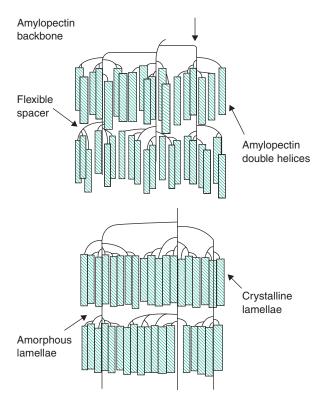
Amylopectin is a much more complex entity and is usually described as "highly branched." The overall molecular mass is of the order of  $10^6-10^8$ , making this polymer one of the largest known in nature. Chain length distribution is multimodal and there are three main types of chain. The A-type chain is the outermost in the branching pattern and contains 15-30  $\alpha$ -1,4 linked D-glucose residues. It is attached to a B-type chain by an  $\alpha$ -1,6 bond between the

Source	Amylose concentration (%)	Granule average diameter (µm)	Lipid concentration (%)	Protein concentration (%)
Maize (corn)	25	15	0.80	0.35
Wheat, barley, rye A-granules	27	25	0.90	0.40
Wheat, barley, rye B-granules	24	5	0.90	0.40
Rice	20	3		
Oat	27	5	1.10	
Amaranth	20	1.5	1.10	0.49
Quinoa	9	1.5	0.11	0.91
Canary-grass	17	2	0.02	0.20
Potato	20	30-40	0.10	0.10
Cassava	17	8	0.10	0.10
Pea, chickpea, common bean, faba bean, lentil, cowpea, pigeonpea	33	25	0.1	0.5

Data from a variety of sources.

reducing C1 of the A chain and a C6 of the B chain. The A-chain: B-chain ratio is commonly 1-1.5:1. The B-chain has one to several A-chains attached to it and is itself attached either to other B-chains or to the C-chain, which carries the reducing end of the polymer. The B-chain is classified as B1 to B4, with the numeral indicating how many crystalline lamellae, or zones of the starch granule, it passes through. A B1-chain passes through only one lamella and is similar in chain length to the A-chains. A B2-chain passes through two such lamellae and has a chain length of  $\sim$ 45–50 glucose residues. The B3- and B4-chains are correspondingly longer and rarer. The currently accepted model, supported by a range of analytical methods, is that the branching points are clustered within any given molecule and also coordinated in adjacent molecules, rather than random (Figure 4). The model shows double helices, often of an A-chain and a B1-chain, as the state of the outermost branches. The lumen of a double helix is smaller than that of a single helix and hence less likely to include guest molecules. In potato amylopectin, phosphate groups are attached to some of the hydroxyl groups of the glucose residues. This is associated with greater hydrophilicity and may contribute to the high swelling power of potato starch, which is 10 times that of many other starches.

Numerous biochemical tests have been devised to evaluate the relative proportions of amylose and amylopectin in a starch. One of the most common involves the use of iodine, which may be quantified in two ways. Amylose can bind 20% of its mass in iodide, whereas amylopectin binds less than 1%. This may be measured accurately, if slowly, by potentiometric titration. In a more approximate and considerably more rapid method, the light absorption of the solution may be measured. Chains of tri-iodide ions bound by the amylose helix absorb strongly at longer



**Figure 4** Representation of the amylopectin molecule as a liquid crystal. The shaded blocks represent double helices. In the dry state (top), they are disordered and in the hydrated state (bottom) they are aligned. (Reproduced with permission from Donald AM, Perry PA, and Waigh TA (2001) The impact of internal granule structure on processing and properties. In: Barsby TL, Donald AM, and Frazier PJ (eds.) *Starch: Advances in Structure and Function*, pp. 45–52. Cambridge, UK: Royal Society of Chemistry.)

wavelengths of light, 600–640 nm, causing the starch—iodine solution to appear blue. Each turn of the helix holds about two iodine atoms. Shorter chains infrequently included in amylopectin helices absorb at a lower wavelength, 530–550 nm, and

confer a tan, reddish, or purplish color to the solution. Any lipid molecules in the solution compete with the iodide ions for the amylose helices and thereby reduce the apparent amylose concentration. Various authors have shown that simultaneous measurement of absorbance at two to six wavelengths across the visible spectrum greatly increases the accuracy of this method over the traditional measure at a single wavelength in the 600-640 nm range. High-pressure size exclusion chromatography is a modern method for amylose determination with a reasonable balance of throughput and accuracy.

"Intermediate material" was described in maize starch for many years, having some of the features of amylose and others of amylopectin, e.g., amylopectin-type branching but with much longer chains. This area is discussed further below.

Phytoglycogen is found in certain plant mutants such as sweet corn. It is due to a mutation in the gene for starch debranching enzyme or isoamylase. Its structure is very comparable to that of animal glycogen, and its lack of higher-order structure, such as double helix formation, allows it to remain largely water soluble. This aspect limits the amount of storage polysaccharide that can accumulate in a grain, so when it dries down at maturity, it is shrunken and wrinkled.

## **Levels of Organization**

The next order of structure is an alignment of the short, stiff double helices of the amylopectin molecules. This takes place in two ways. First, they align parallel to each other, along the radius of the starch granule. This is what gives the polarization of light, leading to a characteristic "Maltese cross" appearance when starch granules are viewed between crossed polarizers in a microscope. Second, the double helices pack together in an energy-minimizing fashion (Figure 5). In some starches, generally those from tubers and stems, the packing is hexagonal with a lumen in the center, which leads to a consistent pattern, termed B-type, in X-ray diffraction. In other starches, mostly those from grains, the double helices pack much more closely, without a lumen, leading to a different, A-type, X-ray diffraction pattern. Starches from grain legumes and from most roots have an intermediate diffraction pattern, termed C-type, which implies that some of the hexagons have a lumen and others do not. A final X-ray diffraction pattern, V-type, is given by amylose which encloses a lipid chain. High-amylose maize gives a B-type diffraction pattern, suggesting that amylose does not pack as tightly as amylopectin. Some species of yam (Dioscorea) show A-type diffraction patterns

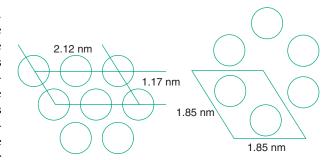


Figure 5 Packing of amylopectin double helices: left, monoclinic crystal giving A-type X-ray diffraction pattern; right, open crystal giving B-type X-ray diffraction pattern. (Modified from Parker R and Ring SG (2001) Aspects of the physical chemistry of starch. Journal of Cereal Science 34: 1-17.)

and some B-type. Starches with the B-type pattern generally retain or take up more water than those with an A-type pattern.

The crystalline double helices are thus aligned in a crystalline lamella. This alignment is possible because the branches in amylopectin are clustered, leading to an amorphous (noncrystalline) region (Figure 4). The thickness of this amorphous/ crystalline ring is commonly 9 nm. This is commensurate with a helix of  $\sim$ 18 residues (6.3 nm) plus a few more residues to join with the B-chain in the amorphous lamella. This, in turn, accounts for the 15-30unit length of the majority of amylopectin chains.

Alteration of packing can occur in the presence of gentle hydration, leading to the model that in the hydrated starch granule, the rod-like double helices on their flexible attachments function as a liquid crystal (Figure 4).

Stacks of ~16 amorphous/crystalline lamellae form semicrystalline layers of ~140 nm in thickness. These layers are separated by amorphous layers of comparable thickness. This level of order was visible in early electron micrographs. Experimental results published in 1962 suggested that these "growth rings" disappeared in plants grown in continuous light. Nevertheless, some controversy remains about the association of these rings with diurnal light cycles. Most starch granules show the alternating semicrystalline and amorphous layers across their entire cross-section. Certain double mutants in maize, however, show clearly biphasic growth. In ae du and ae wx lines, the core 30–60% (by volume) of the starch granule shows the typical concentric rings, but the remaining outer mass of the granule is shapeless and disorganized. It was proposed that there was a change in the relative expression of the starch synthetic enzymes and in substrate concentration.

## Starch Granule Morphology

Starches of wheat, rye, barley, and many of their wild relatives in the cereal tribe Triticeae have biphasic granule initiation, rather than biphasic growth. One major starch granule, termed A-type, originates in each amyloplast. About 15 days into grain filling, membrane tubules appear around the equator of the A-granule and several subsidiary, B-type, granules form. At grain maturity, the A-granules are approximately lenticular or lentil shaped, with a long diameter about twice the short diameter and with a detectable equatorial groove. Mean A-granule diameter can be as low as 15 µm in diploid wheats such as Triticum monococcum or as large as 30 µm in some tetraploid and hexaploid Aegilops species, but is 20-25 µm in cultivated rve, barley, durum wheat, and common wheat (Table 1). The B-granules are roughly spherical and average 5-6 μm in diameter in most of these species. There are other differences between A- and B-granules. B-granules lack the central cavity or hilum found in A-granules and also in the granules of other species. They also lack at least one granulebound protein and they can have amylose contents 2–4% lower than A-granules. With their greater surface area they adsorb more water per unit mass than A-granules. The factors determining A-granule size and B-granule concentration or initiation are the subjects of continuing research. Many scientists view a third population of small B-granules as a distinct C-granule type. This bimodal or trimodal granule size distribution is not found in any other group of plant species.

In maize, starch granules are spherical when isolated from "mealy" regions of the mature grain where there is space between the granules, but are polyhedral when isolated from "vitreous" regions where the granules have been in close physical contact since partway through grain filling. Faceted granule surfaces are thus attributable to restricted growing space rather than active differences in granule development. A-type granules of wheat and barley often show indentations where other granules have

impinged on them. Oat and rice have compound starch granules. Grain legume starch granules are generally ovoid or elliptical, and root or tuber starches are usually round to ovoid.

Zero-amylose (waxy) starches have a fairly normal gross morphology. Granules of high-amylose starches, in contrast, such as those of amylomaize or wrinkled pea, are shapeless, fissured, and nonbirefringent. These features demonstrate the critical role of amylopectin in determining granule crystallinity and the much less critical role of amylose.

## **Starch-Bound Lipids**

As mentioned above, long amylose chains and lipids can form complexes. The concentration of lipid in starch is very strongly correlated with the amylose concentration of the starch. The amylose-lipid complexes resist both their own leaching from the starch granule and also the entry of water into the granule. This latter aspect may be the biological importance of starch-bound lipids in the quiescent or germinating seed. Starch processing properties are also affected by the presence of bound lipid, which increases gel temperature and the temperature of maximum viscosity, decreases gel strength, and delays staling by interfering with retrogradation. Removal of lipids lowers gel temperature and increases peak viscosity.

Starch-bound lipids are generally either free fatty acids (commonly linoleic) or lysophospholipids. The ratio of these two classes differs among species (Table 2). In some species, di- and tri-glycerides are also found bound to starch. Lipid concentration of extracted starch varies up to tenfold depending on the extraction method.

#### Starch-Bound Proteins

Numerous types of protein are found in close association with starch granules, some on the surface and others bound within. The biological functions of some of these proteins remain to be determined. The surface

Table 2 Composition of selected starch-bound lipids

Species	Free fatty acids	Lysophospholipids	Triglycerides	Diglycerides
Wheat, barley, rye	10	90		
Rice, oat	30	70		
Millet, sorghum	45	55		
Maize	60	40		
Cow cockle (Saponaria vaccaria L.)	39	10	45	6

proteins tend to be smaller than the internal proteins. Altered sequences in the tryptophan-rich surface proteins, puroindoline "a" and puroindoline "b," have been strongly linked with difference in grain hardness, but their causative role in this difference is controversial.

Granule-bound starch synthase (GBSS), which has a relative molecular mass of  $\sim 60 \,\mathrm{kDa}$ , is responsible for synthesizing amylose and is thus absent from socalled "waxy" mutants (currently identified in a wide range of crops including maize, barley, wheat, rice, and pea). Confocal laser scanning microscopy of fluorescent-dyed proteins has shown that GBSS is localized in concentric spheres within the starch granules of maize, wheat, and potato. These spheres appear to be the amorphous layers of the growth rings. Other unidentified proteins were shown by this method to be concentrated in the hilum of the granule. A large, 140–152 kDa form of starch branching enzyme (SBE I) has recently been shown to be associated only with A-granules, not B-granules, of wheat.

The total protein concentration of extracted starch is higher in cereal grains than in roots or tubers (Table 1), although the exact figure depends a great deal on the extraction method.

## **Dissociation Chemistry**

Gelatinization has been defined as "the collapse (disruption) of molecular order within the starch granule, manifested in irreversible changes in properties such as granule swelling, native crystalline melting, loss of birefringence and starch solubilization." Pasting is what happens after gelatinization – "granule swelling, exudation of molecular components from the granule and, eventually, total disruption of the granules."

Starch granules in cold water can reversibly absorb 30% of their weight in water. When starch is heated in an excess of water, a sequence of events occurs as the kinetic energy of the polymer increases and the hydrogen bonds rupture. At the "pasting" or "gelatinization" temperature, between 60°C and 70°C, energy is absorbed in de-crystallizing the crystalline regions of the molecule. It also leads to an endotherm, as seen in the differential scanning calorimeter (DSC), due to the absorption of energy. Typical values of "heat of gelatinization" or "gelatinization enthalpy" are in the range of  $8-12 \,\mathrm{Jg}^{-1}$  but are as high as 32 in amylomaize. The loss of crystallinity leads to a rapid swelling of the granule combined with a considerable loss in structure, as seen in a rapid increase in viscosity in a pasting curve (Figure 6). Amylose leaches from the granule but amylopectin remains associated.

Further heating without stirring in an enclosed vessel, such as a DSC, causes dissociation of amylose from lipid. This process also absorbs energy and shows up as an endotherm between 102°C and 110°C.

If, however, the heating is in the presence of stirring in a nonpressurized container, such as a Brabender Viscoamylograph or a Rapid Visco Analyzer (RVA), the viscosity of the suspension continues to increase as the amylose molecules get more dispersed and the granules become more porous, gelatinized, and swollen. Further stirring at a high temperature results in shear thinning, where the molecular entropy is not enhanced but granule structure is gradually further destroyed until a new plateau is reached. This phase is known as "breakdown." As the paste is cooled and stirred, viscosity reaches a final value. The RVA takes  $\sim$ 15 min for the process, instead of about an hour for the Viscoamylograph. Recent models of the RVA have the very significant advantage that they give readings in SI units (cP), in contrast to the arbitrary units of older models and the Viscoamylograph.

In a set of rice starches of equivalent amylose content, most RVA parameters were not significantly affected but breakdown was correlated negatively with the proportion of long amylopectin chains and positively with the proportion of short. In a separate study it was noted that increased firmness of cooked rice was associated with increased content of long amylopectin chains. The short chains are types A and B1, the long B2 to B4. Perhaps the presence of more interconnections between crystalline lamellae, given by a higher content of B3 and B4 chains especially, helps to maintain structure of the gelatinized starch granule. Furthermore, the radius of rotation of the

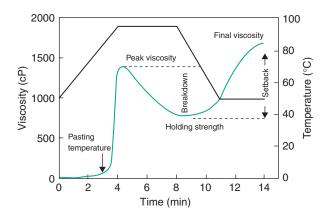


Figure 6 Starch pasting profile, as determined with an RVA (Newport Scientific Pty Ltd., Warriewood, NSW, Australia). Key measurements are named on the graph. Thin line shows temperature profile and thick shows viscosity profile.

chain increases with its degree of polymerization, and the viscosity increases as the cube of the radius, so small increases in chain length can lead to large increases in viscosity.

## **Reassociation Chemistry**

As stated above, the minimum energy state for an α-1,4 linked glucan is as a double helix with another such chain. As a starch solution cools, the chains reassociate in a process termed "retrogradation." Long amylose chains have a considerable radius of rotation and freedom of movement in a solution and may thus entangle with many other long chains at concentrations as low as 1-1.5%. Amylose retrogradation thus entraps a lot of water, involves many molecules, makes a clear hard gel, and is essentially complete when the cooked product has cooled to room temperature. The double helices are generally limited to a few dozen (perhaps 50) glucose residues, leaving great lengths of single helix which can entrap enough iodide ions to give a strong blue color. Amylopectin retrogradation, in contrast, is more likely to involve chains from the same molecule, which are already much shorter than their amylose counterparts. This process can take up to several days and makes a translucent, soft paste. Loss of water from the gel or paste during storage is termed "syneresis."

Some of the components of a retrograded starch are so well crystallized that they are resistant to enzyme attack and are known as "resistant" starch (Table 3). These can contribute to dietary fiber. Recrystallized autoclaved amylomaize starch is sold as resistant starch by at least two manufacturers. Similar material is used as an additive to white bread by an Australian bread manufacturer in order to increase insoluble dietary fiber content without adversely affecting the color or texture of the product.

#### **Mutant Starches**

Starch synthesis mutants have been well characterized in several species (see Starch: Synthesis). These have

Table 3 Classification of resistant starch

Туре	Description	Example
1	Physically inaccessible	Grain fragments
2	Native granules	Uncooked banana
3	Retrograded after heat/ moisture treatment	Cooled, cooked potato
4	Chemically or thermally modified	

been valuable not only as industrial and food components but also for elucidating the relative roles of amylose and amylopectin in starch functionality.

GBSS mutants do not synthesize amylose (or do so to only a limited extent) so starch is 100% amylopectin, which is known as "waxy" starch on account of its appearance. These wx mutants have long been known in barley, maize, and rice ("glutinous") and have more recently been developed in common wheat and pea. The gel temperature is usually lower and peak viscosity higher in waxy starches than in their normal counterparts. In many cases, final viscosity is higher as well. The isolation of further waxy starches, e.g., from other grain legumes and pseudocereals, may give us even more interesting properties.

High-amylose starches have been harder to develop, even though one has been known for a very long time and was an important part of Mendel's original elucidation of the principles of genetics. The distinction between "round" and "wrinkled" peas, called "rugosus," is now known to be due to a mutation in starch branching enzyme I. The wrinkled phenotype is associated with an amylose concentration of up to 80% in a reduced mass of starch. High-amylose barley (amo-1) is 45% amylose, whereas high-amylose maize starch can exceed 85% amylose. Amylose appears to be digested more slowly than amylopectin in the gut of monogastric animals. High-amylose starches have been associated with increased satiety (i.e., reduced desire for food), reduced glycaemic index, and reduced insulin release in comparison with normal-amylose starches. Highamylose foods will thus be an attractive option for people with diabetes and other metabolic problems related to insulin, glycaemia, or obesity.

Many mutations affect maize starch synthesis, including ae (amylose extender), du (dull), h (horny), sh1 and sh2 (shrunken), and su1 and su2 (sugary). The double mutant ae wx maize has no true amylose, owing to the wx mutation, but has longer amylopectin chains (average 52 instead of 30 glucose residues) than the wild type due to the *ae* mutation. Starches of other double or triple mutants of maize have desirable properties which emulate or exceed those of chemically modified starches. These include good freeze-thaw stability (wx sh1) and low viscosity when hot during processing but high viscosity when cool, which is desirable for canning (du h, du ae, du su2, ae su2). Starch of the ae du wx triple mutant has high paste viscosity, shear resistance, and acid resistance, and the paste has a creamy texture making it a suitable fat substitute. Chemical analysis of chain length and branching pattern has not yet been at sufficiently high resolution to provide mechanistic

explanations of these differences in processing properties.

#### Conclusions

Numerous techniques have been used to elucidate aspects of the physical and functional chemistry of starch. Although it is a homopolymer consisting entirely of glucose, its structure is extremely variable, showing differences in chain length, branching pattern, and the incorporation of other molecules such as proteins and lipids. These differences lead to a wide range of processing properties. Aspects of the nomenclature may be confusing to the novice, in particular the use of the letters A, B, and C to indicate such diverse traits as different types of amylopectin chain, granule size, or X-ray diffraction pattern. Many questions remain to be answered about the various levels of organization within the starch granule, including the nature of the initiation of the starch granule itself.

See also: Carbohydrate Metabolism. Maize: Genetics. Noodles: Starch Noodles. Rice: Genetics. Starch: Uses of Native Starch; Modification; Synthesis.

## **Further Reading**

Atwell WA, Hood LF, Lineback DR, Varriano-Marston E, and Zobel HF (1988) The terminology and methodology associated with basic starch phenomena. Cereal Foods World 33: 306-311.

Barsby TL, Donald AM, and Frazier PJ (eds.) (2001) Starch - Advances in Structure and Function. Cambridge, UK: Royal Society of Chemistry.

Donald AM, Perry PA, and Waigh TA (2001) The impact of internal granule structure on processing and properties. In: Barsby TL, Donald AM, and Frazier PJ (eds.) Starch: Advances in Structure and Function, pp. 45-52. Cambridge, UK: Royal Society of Chemistry.

Ellis RP, Cochrane MP, Dale MFB, et al. (1998) Starch production and industrial use. Journal of the Science of Food and Agriculture 77: 289-311.

Morrison WR (1995) Starch lipids, starch granule structure and properties. In: Schofield JD (ed.) Wheat Structure, Biochemistry and Functionality, pp. 261–270. Cambridge, UK: Royal Society of Chemistry.

Myers AM, Morell MK, James MG, and Ball SG (2000) Recent progress toward understanding the biosynthesis of the amylopectin crystal. Plant Physiology 122: 989-997.

Parker R and Ring SG (2001) Aspects of the physical chemistry of starch. *Journal of Cereal Science* 34: 1–17. Rahman S, Li Z, Batey I, et al. (2000) Genetic alteration of starch functionality in wheat. Journal of Cereal Science 31: 91-110.

Stone BA (1996) Cereal grain carbohydrates. In: Henry RJ and Kettlewell PS (eds.) Cereal Grain Quality. London, UK: Chapman and Hall.

Thomas DJ and Atwell WA (1999) Starches. St. Paul, MN: American Association of Cereal Chemists.

#### Relevant Websites

http://www.bagkf.de - Bundesforschungsanstalt für Ernährung und Lebensmittel (BFEL), Federal Research Centre for Nutrition and Food, Detmold, Germany.

http://www.starch.dk - International Starch Institute, Aarhus, Denmark.

http://www3.interscience.wiley.com – Starch/die Stärke (Journal home page), Wiley Interscience Publishing.

http://www.corn.org - Corn Refiners Association, Inc, Washington DC, USA.

http://www.strath.ac.uk - The University of Strathclyde, Glasgow, UK.

http://www.jic.bbsrc.ac.uk - The John Innes Centre, Norwich, UK.

http://www.vtt.fi - VTT Technical Research Centre of Finland, Espoo, Finland.

## Modification

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Commercial sources of starches include cereal grains such as corn/maize, wheat, and rice. (Roots and tubers, such as potato, cassava/tapioca, and arrowroot, and the sago palm are other sources.) Corn is by far the principal source of starch, with corn starch making up more than 80% of the world's commercial supply of starch. An additional 8% is obtained from wheat. Most starch used in food or other industrial applications is physically and/or chemically modified in at least one way, and often in multiple ways, before use. Modification is done to enhance the starch's desirable attributes and to minimize its undesirable characteristics. Starch that is to be used in papermaking (the largest industrial use of starch other than that used to produce ethanol of syrups), which utilizes approximately two-thirds of the remaining starch, undergoes modifications different than those to be used in

food applications. Nevertheless, modified food starches exemplify why and how starches are modified.

## **Background**

Food processors generally require starches with better behavioral characteristics than provided by native starches. Cereal starches produce particularly weak-bodied, cohesive, rubbery pastes, and undesirable gels when cooked. However, via modification, the functional properties of starches can be improved. Modification is done to introduce specific functionalities and to make resultant cooked products better able to withstand the conditions of heat, shear, and pH (acid) associated with processing conditions. The final products, modified food starches, are abundant, functional, and useful food ingredients, generally macroingredients.

Modifications can be chemical or physical. Chemical modifications are oxidation, cross-linking, stabilization, and depolymerization. Physical modifications make pregelatinized and cold-water-swelling products. Chemical modifications have the greatest effects on functionalities. Modifications can be single modifications, but modified starches are often prepared by combinations of two, three, and sometimes four processes. Chemical derivatives found in modified food starches (in the USA) are the following:

- 1. Stabilized starches
  - a. hydroxypropyl starches (starch ether);
  - b. starch acetates (starch ester); and
  - c. starch octenylsuccinates (monostarch ester); and
  - d. monostarch phosphate (ester).
- 2. Cross-linked starches
  - a. distarch phosphate and
  - b. distarch adipate.
- 3. Cross-linked and stabilized starches
  - a. hydroxypropylated distarch phosphate;
  - b. phosphorylated distarch phosphate;
  - c. acetylated distarch phosphate; and
  - d. acetylated distarch adipate.

Property improvements that can be obtained by chemical modifications include the following:

- 1. Hypochlorite-oxidized starches
  - a. whiter;
  - b. lower gelatinization and pasting temperature;
  - c. decreased maximum paste viscosity; and
  - d. softer, clearer gels.
- 2. Stabilized (hydroxypropylated or acetylated) starches
  - a. lower gelatinization and pasting temperatures;

- b. improved freeze—thaw stability of pastes and gels;
- c. decreased setback of pastes and gels (improved paste stability);
- d. easier redispersibility when pregelatinized; and
- e. greater clarity of pastes and gels.
- 3. Cross-linked (phosphorylated) starches
  - a. increased gelatinization and pasting temperatures;
  - b. increased shear resistance;
  - c. increased acid stability;
  - d. decreased setback of pastes and gels (improved paste stability); and
  - e. increased viscosity of pastes.
- 4. Cross-linked and stabilized starches;
  - a. lower gelatinization and pasting temperatures, but increased paste viscosity and
  - b. other attributes of stabilized and cross-linked products.
- 5. Thinned (depolymerized) starches
  - a. decreased viscosity of pastes;
  - b. lower gelatinization and pasting temperatures; and
  - c. increased solubility.

Any starch (corn, potato, tapioca/cassava, wheat, rice, etc.) can be modified, but modification is practiced significantly only on corn (both common corn and waxy maize) and potato starches and, to a much lesser extent, on tapioca and wheat starches. This article is written primarily from the point of view of corn and waxy maize starches.

# Methods of Production and Applications

Cross-linked and/or stabilized starch products are prepared by chemical derivatization of a starch, most often in an aqueous slurry in a batch process. In such a process, a slurry of 30–45% solids (starch) as obtained from the mill is introduced into a stirred reaction tank. Sodium chloride or sodium sulfate is added to inhibit granule swelling. The pH is adjusted with sodium hydroxide (up to values of  $\sim 11.5$ , depending on the reaction). Chemical reagents are added. Temperature is controlled. Reactions may be done at temperatures up to 50°C, but gelatinization must be avoided to allow recovery of the modified starch in granule form by filtration or centrifugation. Because the gelatinization temperature may be lowered by the modification, there may be, and often is, a limit to the degree of substitution that can be made in this manner. (The degree of substitution is the average number of hydroxyl groups per α-D-glucopyranosyl unit (the monomeric unit of starch) that have been derivatized, the maximum

being 3.) In some reactions, the pH needs to be controlled by the metered addition of dilute sodium hydroxide solutions. Following modification to the desired level, the starch is recovered by centrifugation or filtration, washed, and dried.

Chemical reactions currently both allowed and used to prepare modified food starches in the USA are as follows:

- esterification with acetic anhydride, succinic anhydride, the mixed anhydride of acetic and adipic acids, 1-octenylsuccinic anhydride, phosphoryl chloride, sodium trimetaphosphate, sodium tripolyphosphate, or monosodium orthophosphate;
- etherification with propylene oxide;
- acid modification with hydrochloric or sulfuric acids;
- bleaching with hydrogen peroxide, peracetic acid, potassium permanganate, or sodium hypochlorite;
- oxidation with sodium hypochlorite; and
- various combinations of these reactions.

Other reagents may be used in other countries.

Waxy maize starch modifications are especially popular in the US food industry because the inherent properties of waxy maize starch are preferred over modifications to common corn starch.

#### **Cross-Linking**

Cross-linking is the most important modification of a food starch. Cross-linking occurs when starch granules are reacted with difunctional reagents to connect hydroxyl groups on two different molecules within the granule. Cross-links reinforce the granule and reduce both the rate and the degree of granule swelling and subsequent disintegration, i.e., reduce sensitivity to processing conditions (high temperature; extended cooking times; low pH; high shear during mixing, milling, homogenization, and/or pumping). Cooked pastes of cross-linked starches are more viscous, heavier-bodied, shorter-textured, and less likely to break down with extended cooking times, greater acidity, or severe agitation than are pastes of the native starches from which they are prepared. Only a small amount of cross-linking is required to produce a noticeable effect; for example, one cross-link for every approximately 1200 α-D-glucopyranosyl units greatly reduces both the rate and the degree of granule swelling, greatly increases paste stability, and changes dramatically both the viscosity profile as the starch is cooked and the textural characteristics of its paste. Three times that much cross-linking, for example, produces a product in which granule swelling is restricted to the point that a peak viscosity is never reached in a slurry heated to 95°C and

held at that temperature with moderate stirring. As the number of cross-links increases, the granules become more and more tolerant to physical conditions and acidity, and swell and disintegrate (solubilize) upon cooking less and less. Energy requirements to reach maximum swelling and viscosity are also increased.

By far the most common cross-links are distarch phosphate esters. These distarch phosphates are prepared with either phosphoryl chloride or sodium trimetaphosphate. Phosphoryl chloride is very reactive and undoubtedly reacts near granule surfaces. To prepare cross-linked starches with phosphoryl chloride, the reagent is added to an aqueous starch suspension of pH 8-12. To cross-link a starch with sodium trimetaphosphate, it is slurried in a solution of the reagent at pH 5.0-8.5; the suspension is filtered, and the starch is dried. In this case, the cross-links are undoubtedly more evenly distributed throughout the granule.

A relatively small amount of cross-linked starch is made by reaction of corn starch with the mixed anhydride of adipic and acetic acids in aqueous alkaline suspension.

#### **Stabilization**

Derivatization of a starch with monofunctional reagents reduces the intermolecular associations which result in gelation of its paste and/or precipitation of the starch polymers (combined processes termed retrogradation or setback). Pastes of unmodified starches generally will gel, and the gels will usually be cohesive, rubbery, long-textured, and prone to syneresis. (Waxy maize starch pastes gel to a very limited extent at room temperature, but will become cloudy and chunky and exhibit syneresis when stored under freezing conditions.)

The most common derivatives employed for starch stabilization are the hydroxypropyl ether and acetate and monostarch phosphate esters. Acetylation is accomplished by treating a starch slurry with acetic anhydride at pH 7-11, the optimum pH depending on the reaction temperature. Acetylation of starch lowers the gelatinization temperature, an indication of a weakening of granules. Upon cooking, a higher peak viscosity is obtained due to greater granule swelling. Upon cooling of the resulting paste, the viscosity becomes lower than that obtained from the unmodified starch, an indication of improved stability, i.e., less retrogradation. Acetylated starches with an acetyl content of up to 2.5% (degree of substitution, DS, 0.09) can be used in food products (USA). (A DS of 0.09 indicates an average of nine acetyl goups per 100 α-D-glucopyranosyl units.)

Sodium phosphate monoesters are prepared by impregnating the starch with a solution of sodium tripolyphosphate. After adjustment of the pH to 5.0-8.5, the slurry is mixed, then filtered, and the filter cake is dried and heated. Sodium tripolyphosphate is used to make products of up to 0.002 DS (one phosphate group per 500  $\alpha$ -D-glucopyranosyl units), the maximum allowed in the USA. Monosodium orthophosphate in the pH range 5.0-6.5 is also used to produce monostarch phosphates in the same way.

Monostarch phosphates produce stable pastes that are clear and have a long, cohesive texture. Paste viscosity can be controlled by varying the concentrations of phosphate salt, time of reaction, temperature, and pH. Increasing substitution lowers the gelatinization temperature; products become cold-water-swelling at DS 0.07. Corn starch phosphates of DS 0.01–0.03 produce pastes with hot viscosity, clarity, stability, and texture more like those of potato starch. Starch phosphates are good emulsion stabilizers and produce pastes with improved freeze—thaw stability.

Hydroxypropyl ether derivatives of starches are prepared by reacting an alkaline slurry with propylene oxide. To the starch slurry is added sodium sulfate and sodium hydroxide. The reactor is charged with propylene oxide and sealed. Reaction is continued for ~24 h at ~49°C. The maximum allowable moles of substitution in the USA is 0.2 (7.0% of hydroxypropyl groups). (Moles of substitution, MS, is essentially the same as DS but is used in place of DS because each hydroxypropyl group contains a hydroxyl group that can itself be etherified, so that the maximum number of substituent groups per glucosyl unit can be more than 3.)

Low-MS hydroxylpropylstarches behave much like low-DS starch acetates and are used because of similar improvements in texture and appearance. The hydroxypropyl ether linkage is, however, much more stable than an ester linkage.

Starch succinate half-esters are prepared by reacting starch with succinic anhydride.

#### Starches with Hydrophobic Groups

Reaction of starch with 1-octenylsuccinic anhydride introduces hydrophobic substituent groups. Such derivatives can be used as emulsifiers and emulsion stabilizers in products based on oil-in-water emulsions, such as pourable dressings and flavored beverages. Flavor oil emulsions containing a thin-boiling starch or dextrin (see below) derivatized with 1-octenylsuccinate ester groups may be spray-dried. The flavor oil in the resulting powder is protected against oxidation, and the emulsion will reform when the

powder is stirred into an aqueous medium. Gum arabic is, however, usually the material of choice for this application. Higher-DS products are nonwetting and are used as release agents for dusting on dough sheets and as processing aids. The maximum DS level allowed in the USA is 0.02.

#### **Acid Modification**

Thin-boiling starches are prepared by treating a suspension of a native or derivatized starch with dilute mineral acid at a temperature below the gelatinization temperature. When a product that gives the desired paste viscosity is produced, the acid is neutralized, and the product is recovered by centrifugation or filtration, washed, and dried. Even though only a few glycosidic bonds are hydrolyzed, granules disintegrate more easily and after only a small degree of swelling. Acid-modified starches form gels with improved clarity and increased strength, even though their pastes are less viscous. Thin-boiling starches are used as film formers and adhesives in products such as pan-coated nuts and candies, whenever a strong gel is desired, e.g., in gum candies such as jelly beans, jujubes, orange slices, and spearmint leaves, and in processed cheese loaves. To prepare especially strong and fast-setting gels, a high-amylose corn starch is used. More extensive modification with acid produces dextrins.

#### **Oxidation**

Depolymerization, viscosity reduction, and decreased pasting temperature can also be achieved by oxidation with sodium hypochlorite (chlorine in an alkaline solution). Oxidation also reduces association of amylose molecules, i.e., results in some stabilization via introduction of small amounts of carboxylate and carbonyl groups. Oxidized starches produce intermediate-viscosity and soft gels and are used when these properties are needed. They are also used to improve adhesion of starch batters to fish and meat and in breadings. Mild treatment with sodium hypochlorite, hydrogen peroxide, or potassium permanganate simply bleaches the starch and reduces the count of viable microbes.

#### **Pregelatinization**

Pregelatinized starches are precooked starches that can be dispersed (dissolved) in water at temperatures below the gelatinization temperatures of the parent starches; thus, these "instant" starches need no cooking. To prepare a pregelatinized starch, a slurry is simultaneously cooked and dried on hot drums. Because pregelatinized starch products are powders prepared from dried pastes, generally no granules are

present, although granule fragments may be. Both chemically modified and unmodified starches can be used. The resulting products contain no intact starch granules. If chemically modified starches are used, the properties introduced by the modification(s) are found in the pregelatinized products; thus, paste properties, such as freeze-thaw stability, can be characteristics of pregelatinized starches. Several physical forms of pregelatinized starches are produced. For example, some will produce smooth solutions; others will produce pulpy or grainy dispersions and find use in fruit drinks and tomato products. Pregelatinized starches are often used in dry mixes, as are maltodextrins, because they disperse readily, even when mixed with other ingredients. Starches that are not pregelatinized are known as cook-up starches.

## **Cold-Water-Swelling Starches**

Starch products that are gelatinized starches, i.e., starches that have lost their crystallinity, but which retain their granular form, in contrast to standard pregelatinized starches, are called cold-water-swelling starches. There are several ways that such products can be prepared; one way is to heat a starch in an aqueous alcohol solution with sufficient water to allow gelatinization and sufficient alcohol that granule integrity is maintained. Cold-water-swelling products swell rapidly and thicken unheated aqueous systems. (A granular, cook-up starch requires heating a slurry to the pasting temperature before thickening occurs.)

#### **Multiple Modifications**

Modified food starches are tailor-made for specific applications. Most modified food starches are made by cross-linking, introduction of monosubstituent groups (stabilization), or a combination of these two approaches. Many products, in fact, have received two or more modifications. For example, a modified food starch may be a cross-linked and stabilized waxy maize starch; another may be a stabilized, acid-thinned, and pregelatinized common corn starch. Characteristics that can be controlled/improved by multiple modifications include, but are not limited to, one or more of the following:

- adhesion,
- clarity of solutions/pastes,
- emulsion stabilization,
- film formation,
- flavor release,
- hydration rate,
- moisture retention and control in product,

- mouth feel of product,
- oil migration control in product,
- paste texture/consistency,
- product form (liquid, semisolid, solid),
- sheen of product,
- shelf-stability of product,
- stability to acids,
- stability to heat,
- stability to shear,
- tackiness,
- temperature required to cook, and
- viscosity (hot paste and cold paste).

## **Digestion and Metabolism**

Various regulations concerning reagents that may be used and the maximum allowable modification of a starch for food use, alone or in combination with another modification, are in effect around the world. Generally, the level of substitution in a derivatized food starch is below DS 0.1 and in the range DS 0.002–0.2. Because of this low level of modification, the digestion, metabolism, and caloric values of modified food starches are reduced only to a minor, usually unmeasurable, extent as compared to native starches. Because only monosaccharides (D-glucose in this case) are absorbed, fragments containing esterified, etherified, or oxidized α-D-glucopyranosyl units should not be absorbed from the small intestine.

See also: Maize: Wet Milling. Starch: Uses of Native Starch; Analysis of Quality; Chemistry. Wheat: Wet Millina.

## **Further Reading**

Eliasson A-C (ed.) (1996) Carbohydrates in Food. New York: Marcel Dekker.

Light JM (1990) Modified food starches. Cereal Foods World 35: 1081–1092.

Thomas DJ and Atwell WA (1997) Starches. St. Paul, MN: Eagen Press.

Whistler RL, BeMiller JN, and Paschall EF (eds.) (1984) Starch: Chemistry and Technology, chapters 10, 17, 19. Orlando, FL: Academic Press.

Wurzburg OB (ed.) (1986) Modified Starches: Properties and Uses, chapters 2-4, 6, 7, 9, 12. Boca Raton, FL: CRC Press.

#### **Relevant Website**

http://www.foodstarch.com - This website provides information on the most innovative food ingredients in the global food industry.

# **Synthesis**

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### Introduction

Starch makes up by far the largest component of cereal grains and is also found in fruits, roots, tubers, and leaves. It is a very important source of energy in our diets. Most starch is easily digested; however, some starches termed resistant starch are digested only after they reach the large intestine and recent research shows that this type of starch has positive attributes for bowel health. Starch also performs two basic roles in the food industry, as a stabilizer providing viscosity, texture, mouth feel, and consistency to food products and as a processing aid to facilitate manufacturing. As a consequence of its low cost and diverse functionality, starch is used in a range of food and nonfood industries.

Starch is a polymer of glucose monomers linked by  $\alpha$ -1,4 and  $\alpha$ -1,6 linkages, so it is a glucan. Cellulose is also a glucan; however, the single type of glucose linkage between the glucose molecules ( $\beta$ -1,4) provides very different properties. In starch the frequency of  $\alpha$ -1,6 linkages defines the two major components of starch - amylose and amylopectin. Amylose and amylopectin differ in their degree of polymerization (DP) or number of glucose residues and branching frequency. Amylose is mainly linear with <1%  $\alpha$ -1,6 linkages and a DP of 500–5000, whereas amylopectin is highly branched with  $4-6\% \alpha-1.6$  linkages and a DP of  $50\,000-500\,000$ . The ratio of amylose to amylopectin and the details of the branching (such as the length of the branches and distance between branches) are important determinants of the suitability of starch for specific end uses. Generally, the amylose content of starch is  $\sim 25\%$ , but it can vary from 0% to 80% depending on alterations in the starch biosynthetic pathway.

In nature starch is found in granules. X-ray diffraction studies have revealed a semicrystalline structure for such granules. The X-ray diffraction pattern is determined by the proportion of the external chains that are aligned in crystalline arrays and by the packing in the array. Amylopectin molecules are radially arranged with their nonreducing ends pointing towards the surface. Starch is packaged into alternating crystalline and amorphous lamellae. The location of amylose chains within this structure is unclear and it has been suggested that it is present in the amorphous

cavity and/or interspersed in the amylopectin crystalline region.

Starch is synthesized in plants within organelles that are either photosynthetic (such as the chloroplast of leaf cells) or nonphotosynthetic, such as the amyloplast of cereal endosperm cells. The starch in chloroplasts is synthesized during the day and broken down at night and it is often termed transitory starch. In contrast, the starch synthesized in seeds is broken down only on germination and the starch is termed reserve starch. There are some differences in the detail of biosynthesis between the two types of starch. This article is mainly concerned with the starch deposition within the nonphotosynthetic amyloplast.

#### **Starch from Different Cereals**

Starch granules in the reserve tissues vary widely in size distribution and shape between species (see Table 1 and Figure 1). Maize and rice starches exhibit a unimodal granule size distribution ranging from 5 to 20 µm for maize and 2 to 5 µm for rice. Starches of wheat, barley, and rye are characterized by a bimodal distribution composed of an A granule population of 10-35 µm diameter which are lenticular in shape with a characteristic equatorial groove and a B granule population of generally spherical granules with a diameter less than 10 µm. In barley, a trimodal distribution of starch granules with "C" granules of diameter less than 5 µm is also reported. In wheat, the larger granules, which are initiated during the early stages of endosperm development, constitute 70-80% of the starch by weight and the more numerous smaller granules, which are started later, contain only 20-30% of the total starch in grain. There have been some reports of slight variation in amylose content between the A and B granules and between different regions of the granule.

**Table 1** Typical properties of starch from different sources

Source	Starch granule size range (µm)	Starch granule size distribution	Apparent amylose content	Gelatinization onset temperature <sup>a</sup> (°C)
Wheat	3-35	Bimodal	25	57
Barley	2-35	Trimodal	22	56
Rice	2-5	Normal	18	70
Maize	5-20	Normal	28	64

<sup>&</sup>lt;sup>a</sup> Gelatinization temperature measured by differential scanning calorimetry using starch mixed with water in a ratio of 2 parts of water: 1 part of starch. The heating rate was 10°C min⁻¹ over a temperature range of 25−150°C. Data compiled from Jane J, Chen YY, Lee LF, et al. (1999) Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry* 76: 629−637; and Rahman S, Li Z, Batey I, Cochrane MP, Appels R, and Morell M (2000) Genetic alteration of starch functionality in wheat. *Journal of Cereal Science* 31: 91−110.

The amylose/amylopectin ratio does not normally vary greatly between sources, with a ratio of 1:3 being common. However, mutations where amylose is completely missing are fairly common and such starches are known as waxy starches. In some food preparations such starches are preferred, e.g., in the preparation of many Asian sticky rice desserts. Waxy starches are also often preferred as thickeners because of the greater solubility of starches without amylose. High amylose starches are best known in maize among cereals. Here the amylose content is ~80% and the starch granules appear to be distorted. High amylose starches are in demand because such starches are useful starting points for the production of resistant starches in foods and can be used in packaging materials and adhesives.

An important property of starch granules is the gelatinization onset temperature, the temperature at which the granules begin to lose internal order and crystallinity. The gelatinization temperature of starches can vary considerably, from ~57°C for barley starches to over 75°C for rice. Waxy starches appear to have slightly lower gelatinization temperatures. The differences in gelatinization correlate with differences in the branch lengths of starches from different sources and in the packaging of branches in the granule. Another important property is the viscosity of the gelatinized starch.

A principal difference between starches from cereals and tubers is in the proportion of phosphate groups found in the starch. Potato has  $\sim 0.5\%$  phosphorylated glucose residues compared to  $\sim 0.05\%$  for cereal starches. Usually the phosphate is linked to carbon 6 of glucose molecule. The difference in the phosphorylation imparts tuber starches with properties significantly different to cereal starches in terms of viscosity and flow properties.

The lipid content of cereal starches is low,  $\sim 1\%$ . However, even this can be divided into three operational classes: nonstarch, surface, and internal lipids. The internal lipids of wheat consist entirely of lysophospholipids which are complexed inside linear glucan chains. The fatty acid composition of lipids differs slightly between wheat and rice, with wheat being richer in 18:2 (linoleic acid) and rice having more 16:0 (palmitic acid) fatty acids. How lipids accumulate in cereal starches is not yet clear but the hydrophobic environment in the interior of the glucan chain leads to complexes of lipids with amylose.

## **Pathway from Sucrose to** Starch Granule

#### **Pathway Preceding Starch Biosynthesis**

Starch synthesis requires a hexose phosphate supply. Plants produce sugars in photosynthetic tissue (e.g.,

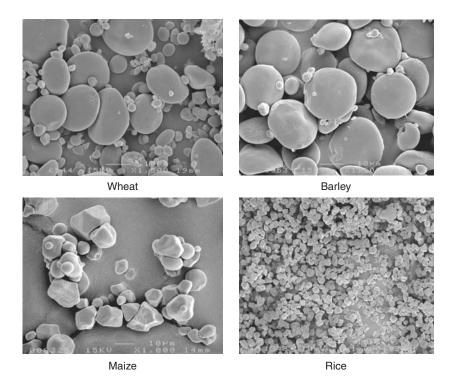


Figure 1 Starch granules from common cereals. (Scanning electron micrographs by CSIRO Plant Industry by scanning electron microscopy.)

leaves, pericarp of the grain) where they can support leaf starch synthesis. In the endosperm, incoming sucrose is either converted into uridine diphosphate (UDP)-glucose and fructose by a UDP-dependent sucrose synthase or converted to hexoses by invertase, as observed in maize. The UDP-glucose or hexose phosphate derived from fructose is inter-converted to glucose-1-phosphate by other enzymes such as UDP-glucose pyrophosphorylase, hexokinase, and phosphoglucomutase. This glucose-1-phosphate can be used in various metabolic pathways; however, once the glucose-1-phosphate is utilized to form adenosine diphosphate (ADP)-glucose (ADPG), a commitment is made to the synthesis of starch.

#### **Biochemistry of Starch Biosynthesis**

A consensus view of starch biosynthesis in cereal endosperm is shown in Figure 2. Starch biosynthesis requires four types of enzymes, which are as follows.

- 1. ADPG pyrophosphorylase which produces ADP-glucose from glucose-1-phosphate and ATP.
- Starch synthases which elongate glucan chains by using ADP-glucose to add a glucose residue at the nonreducing end.
- 3. Starch branching enzymes which introduce  $\alpha$ -1,6 branches to the glucan chain. These branches can then be extended by starch synthases.
- 4. De-branching enzymes remove branches from starch molecules. The role of de-branching enzymes in starch synthesis is still being debated, although the genetic evidence for a role for these enzymes is unambiguous.

Other enzymes are also capable of influencing the properties of the starch produced and these will be discussed later.

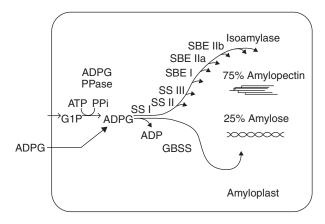


Figure 2 A consensus view of starch biosynthetic pathway in cereals.

Maize has been the most extensively characterized model system for the isolation of mutations affecting starch biosynthesis. Analysis of these mutants (along with those from other model systems such as Arabidopsis) has greatly contributed to the exploration of the key enzymes and their isoforms involved in starch biosynthesis. In maize, mutations in genes affecting starch synthesis such as the waxy and amylose extender mutations are brought about by the insertion of transposable elements, a DNA sequence capable of moving from one location to another within a genome. Such mutations often lead to alterations in the structure and properties of starch. Specialty maize varieties such as waxy (granule bound starch synthase 1 mutant), amylose extender (starch branching enzyme IIb mutant), sugary 2 (starch synthase IIa mutant), sweet corn (sugary-1, isoamylase mutant), and dull (starch synthase III mutant) find applications for specific food and industrial use.

## **ADPG Pyrophosphorylase**

ADPG pyrophosphorylase is believed to be the key enzyme which determines the rate of starch synthesis in leaves. Genetic engineering has been used to introduce the ADPG pyrophosphorylase from *Escherichia coli* (which is not subjected to feedback inhibition) into potatoes, and tubers with a higher content of starch were obtained. It is not clear, however, whether ADPG pyrophosphorylase is also the rate-limiting enzyme for starch biosynthesis in the grain as the purified enzyme from leaves and grains show differences in properties.

ADPG pyrophosphorylase from both leaves and endosperm is composed of four subunits, two of each type, large and small. The large subunit has a mass of  $\sim 55 \, \mathrm{kDa}$ , whereas the small subunit is slightly smaller,  $\sim 50 \, \mathrm{kDa}$ . The sequence of amino acids of these subunits is  $\sim 50 \, \%$  identical. Mutations in the sequence of either of these subunits can destroy or reduce the activity of the enzyme and then grains or seeds with a shrunken appearance (due to decrease in starch synthesis) are produced. Such mutations in ADPG pyrophosphorylase were given the names brittle-2 (mutation in small subunit) and shrunken-2 (mutation in large subunit) when they were discovered in maize (Table 1).

Recent evidence shows that ADPG pyrophosphorylase in the cereal grain exists both in the cytoplasm and amyloplast of endosperm cells; however, most of it is in the cytoplasm. This enzyme in the cereal grain differs from that in the seeds of dicots and leaves of all the plants investigated so far, where ADPG pyrophosphorylase appears to exist exclusively in the plastids. Evidence from both monocots (such as barley and

maize) and dicots (such as peas) suggests that there is more than one gene for the large and small subunits of ADPG pyrophosphorylase and different versions of these may code for the forms that are expressed in the cytoplasm and in the plastid.

The transfer of ADP-glucose from the cytoplasm of endosperm cells to the amyloplast is important as the other enzymes in the process are located there. Mutations are known where this transfer is affected and this too produces grain where starch synthesis is affected (Table 2).

ADPG pyrophosphorylases exhibit complex regulatory properties which vary depending on the species and tissue source. The enzyme from the leaves of all plants examined, maize endosperm, and potato tubers are allosterically activated by 3-phosphoglyceric acid (3-PGA) and inhibited by inorganic phosphate. Pea and bean embryo, and wheat and barley endosperm ADPG pyrophosphorylases, on the other hand, are much less sensitive, or insensitive, to modulators compared to their leaf counterparts. The allosteric properties of ADPG pyrophosphorylases are of potential importance in determining its role in controlling the rate of starch synthesis. Altered starch biosynthetic rates resulting from altered allosteric properties due to mutations in ADPG pyrophosphorvlase subunits are demonstrated in maize and the green algae, Chlamydomonas.

Table 2 List of selected starch mutants<sup>a</sup>

Mutant	Phenotype	Plant species	Causal mutations/only enzyme affected
Brittle-1 (bt 1)	Low starch	Maize	Adenylate transporter
Shrunken-1 (sh 1)	Low starch	Maize	Sucrose synthase
Rugosus 4 (rug 4)	Low starch	Pea	Sucrose synthase
Rugosus 3 (rug 3)	No starch	Pea	Phosphoglucomutase
Rb	Decreased starch content and increased levels of sucrose	Pea	ADPG pyrophosphorylase
Brittle-2 (bt 2)	Low starch	Maize	Small subunit of ADPG pyrophosphorylase
Shrunken-2 (sh 2)	High levels of sucrose and low levels of starch	Maize	Large subunit of ADPG pyrophosphorylase
Waxy (wx)	Zero amylose	Maize, wheat	GBSS 1
Low amylose (lam)	Low level of amylose	Pea	GBSS
Shrunken (shx)	Reduced starch content, altered granule size distribution	Barley	SS-I (not known to be casual)
Sugary-2 (su 2)	Altered granules, high sugar	Maize	Starch synthase IIa (?)
Rugosus 5 (rug 5)	Granules with deeply divided lobes, reduced amylopectin synthesis, altered amylopectin chain length	Pea	SS II
Dull (du1)	Mature kernels with tarnished, glassy and dull appearance. High apparent amylose content	Maize	Mutation at SS III, SBE IIa affected secondarily
Amylose extender (ae)	High levels of amylose	Maize, rice	SBE IIb, RBE III
High amylose (amo1)	Higher level of amylose	Barley	Unknown
Floury-2 (flo 2)	Soft, white endosperm which crumbles easily into powder. High amylose content	Rice	RBE I RBE III, and GBSS (not known to be casual)
Sbella::mu	No endosperm phenotype, altered leaf starch	Maize	BE IIa
Sbe1::mu	No known phenotype	Maize	BE I
Rugosus (r)	Wrinkled seed, lowered starch, enhanced amylose, sucrose and lipid levels	Pea	SBE I
Sugary-1 (su 1)	Low starch, phytoglycogen accumulation	Maize, rice	Isoamylase
Soft starch (h)	Loosely packed, large granules	Maize	Unknown
SGP-1	Abnormal starch granule morphology. High amylose content	Wheat	SS II
Isoamylase mutant	Phytoglycogen accumulation. No B granule initiation	Barley	Isoamylase
Sex6	Decreased amylopectin synthesis, shortened amylopectin chain length distribution, reduced gelatinisation temperature	Barley	SS IIa

<sup>&</sup>lt;sup>a</sup>Data compiled from the references in "Further Reading" section.

## **Starch Synthases**

Starch synthases elongate pre-existing glucan chains in the amyloplast by adding the glucosyl moiety from ADP-glucose to the nonreducing end of an existing  $\alpha$ -1,4 glucan.

Two classes of starch synthases have been identified in plants. One class of starch synthase is only found bound to starch granules and these are known as granule-bound starch synthase (GBSS). The other class of starch synthase is present either in the amyloplast stroma alone or distributed between the stroma and granular fraction and these are known as soluble starch synthases. Four types of soluble starch synthase (SS) have been reported in crop plants: SS I, SS II, SS III, and SS IV. In monocots the SS II has been further subdivided into the IIa and IIb forms. The primary structures of soluble starch synthases from different sources share a distinguishing feature, an N-terminal extension with little homology between sequences from different sources. Comparison of the deduced amino acid sequences of starch synthases revealed that maize SSI, SSIIa, and SSIIb contained an N-terminal extension of 93, 176, and 144 amino acids, respectively, compared to GBSS. Pea SS II also contains a "flexible" 162 amino acid N-terminal arm. It has been shown through expression analysis using Nterminally truncated SSII that the N-terminal extension is not essential for the catalytic activity of the enzyme but is probably related to substrate binding.

There are two types of GBSSs, I and II. GBSS II is similar in amino acid sequence to GBSS I but is not expressed in the grain. GBSS I is a single polypeptide,  $\sim$ 60 kDa in mass. If the grain lacks an active GBSS I, then starch is composed almost entirely of amylopectin. Such starch is called waxy starch (Table 2). Clearly then GBSS I is essential for the synthesis of amylose and the other starch synthases cannot substitute for it. The hexaploid nature of wheat allows a range of starches of intermediate amylose content to be developed, an opportunity not possible in diploids such as barley. In wheat the GBSS I gene has been mapped as a triplicate set of single-copy homoeoloci on chromosome arms 7AS, 4AS, and 7DS. A reduction in the proportion of amylose is observed when the GBSS I activity is reduced and these starches are known as low amylose or partial waxy starches. The loss of only one of the three isoforms, the 4A form of GBSS, has clearly been shown to yield a starch with increased ability to swell in water on heating. This property is desirable for Udon noodle production and such low amylose starches from wheat are preferred for this end use.

The soluble starch synthases are also all single polypeptide enzymes. SS I is  $\sim$ 75 kDa in mass and

is found both free in the amyloplast and bound to the starch granule. The importance of this enzyme is not clear as no mutants have been discovered which lack this enzyme. This may mean that either the lack of this enzyme has no effect on starch synthesis in the grain or that it is so important that no grain forms. Further research is needed in this area.

SS II has been subdivided into IIa and IIb forms on the basis of gene sequence comparisons; however, the IIb form does not seem to be present as an expressed protein in the cereal endosperm. The IIa form is  $\sim$ 85 kDa in mass although the wheat enzyme appears to be over 100 kDa by electrophoresis. Barley and wheat lines lacking SS IIa have been produced and the starch from these lines is clearly different from normal. The barley mutants have high amylose (70%); the wheat mutants less so (35%). It is not clear if there are differences in the structure of the amylose. The starch granules are of distorted appearance and have a lower gelatinization temperature. The proportion of branches of 7-25 glucose units in length is decreased and the proportion of branches of 4-6 glucose units in length is increased. There are also effects on the abundance of other starch biosynthetic enzymes so it is difficult to know how many of the effects on starch properties are the direct result of the lack of this enzyme and how many are due to the other enzymes affected. The differences in the starch between indica and japonica rice cultivars appear to be due to a combination of alterations in SS IIa and GBSS.

SS III is a polypeptide of  $\sim$ 180 kDa in length. The absence of this enzyme in maize leads to a slightly higher amylose content and the starch looks dull compared to normal. An intermediate glucan fraction ( $\sim$ 15%), which is distinguished from amylose and amylopectin, occurs in starch from maize lines missing SS III.

The importance of SS IV for starch synthesis in the endosperm is at present unknown.

#### **Starch Branching Enzymes**

Starch branching enzymes (SBEs), as their name suggests, are required for the addition of branches to linear glucans. Their impact is clearly more on the synthesis of amylopectin than amylose, although branching enzymes may also have a subtle role in amylose synthesis.

There are two broad types of SBEs: SBE I and SBE II. Expression of these enzymes in bacteria has shown that SBE I adds longer branches than SBE II. They are both single polypeptide chains of  $\sim$ 85 kDa. SBE I and SBE II share  $\sim$ 60% sequence identity over the middle third of the molecules.

The importance of SBE I for starch biosynthesis in the grain is not clear. A mutation in SBE I in maize did not produce a clear phenotype. A number of isoforms of SBE I may exist in wheat.

SBE II is divided into two isoforms in cereals: IIa and IIb. The amino acid sequences of IIa and IIb isoforms are very similar (over 80% identity within a species). In maize the IIb form makes up ~90% of the starch branching II activity in the endosperm but the proportion of the two isoforms is nearly equal in wheat. Maize lines lacking branching enzyme IIb produce a very high (70% and higher) proportion of amylose. Such high amylose starches are linked to the formation of resistant starches in products. Resistant starch has been shown to have very beneficial effects if present in the diet. In contrast, the role of branching enzyme IIa in the cereal endosperm is not clear and may vary from cereal to cereal.

## **De-branching Enzymes**

De-branching enzymes remove branches from branched glucans. Two types of de-branching activities, pullulanase and isoamylase, have been described from developing endosperm of rice and maize. The role and importance of these enzymes for starch biosynthesis is still being debated.

Pullulanase is a single polypeptide enzyme of  $\sim 100 \, \mathrm{kDa}$  in mass. No mutants are known in which only pullulanase is affected and the role of this enzyme in starch biosynthesis is still to be determined.

Isoamylase is also a single polypeptide enzyme of  $\sim 90 \, \mathrm{kDa}$  in mass. It can de-branch highly branched structures like amylopectin and glycogen. The lack of this enzyme in rice and maize is associated with the production of a very highly branched and unusual polysaccharide called phytoglycogen. Such lines also contain high levels of free sugars and are known as sugary mutants. However, there is also a reduction in the level of pullulanase in these sugary mutants so it is difficult to know which of the effects are directly due to the lack of isoamylase.

There are differences between the isoamylase mutants described in rice, maize, and barley. In rice, different phenotypes were observed for the sugary mutants ranging from no starch and only phytoglycogen to starch in the outer layer of the endosperm and phytoglycogen internally. In contrast in barley, both phytoglycogen and starch were reported in the same endosperm cells. The maize sugary mutants also contain both phytoglycogen and starch. In barley it has been reported that the lack of isoamylase leads to an alteration in the initiation pattern of starch granules.

While it is established that isoamylase plays a significant role in starch biosynthesis, the mechanism by which it acts is not resolved. Two mechanisms have been proposed. One proposal known as the "glucan trimming" mechanism suggests that isoamylase removes moderately and loosely spaced branches from a soluble pre-amylopectin structure that interfere with crystallization of amylopectin, and promotes the formation of stable amylopectin structure with regularly packed glucose units. In the absence of isoamylase activity, the pre-amylopectin structure is further branched by branching enzymes and results in the production of phytoglycogen. The second model is based on the assumption that a competition exists between polysaccharide aggregation into starch and the nonproductive formation of water-soluble polysaccharides (WSPs), in turn resulting in a competition for both carbon source and for soluble enzymes involved in amylopectin synthesis. The role of isoamylase here would be to clear the stroma of WSP and prevent phytoglycogen formation. A role in starch granule initiation and growth, apart from phytoglycogen suppression, for isoamylase is also suggested based on analysis of barley isoamylase mutants.

# Other Enzymes with Potential Roles in Synthesis

Much of the new insights into starch synthesis has come from the study of simpler systems, such as Chlamydomonas and Arabidopsis. Such studies have indicated other loci that are important in starch synthesis in these organisms. It is possible (although by no means certain) that genes encoded by such loci are also important for starch synthesis in the grains of cereals.

D-enzyme or disproportionating enzyme has the property of breaking a glucan chain and adding one portion to the nonreducing end of a pre-existing chain. In this way one chain is made shorter but the other one is made longer. In Chlamydomonas mutants where the gene for D-enzyme is affected, the starch produced appears to have somewhat more amylose. These mutants are required to be grown in constant light. Genes for D-enzyme have been described from Arabidopsis and rice, but the importance of D-enzyme to starch biosynthesis in the cereal grain is yet to be clarified.

Starch phosphorylase is another enzyme that could have an impact on starch biosynthesis in the grain. This enzyme breaks down starch and was originally considered to be important for degradation. There are at least two isoforms in the plant — a cytosolic form

and a plastidic form; however, Chlamydomonas appears to contain three isoforms. Lack of one of these isoforms leads to a starch excess phenotype and the production of larger starch granules but the evidence for cereals is yet to be presented.

The initiator protein for glycogen is called glycogenin. Glycogenin is an autocatalytic glycoprotein and the glucose residues provide the starting point for glycogen synthesis. By analogy with this a number of glycogenin-like proteins have been described from plants but again, the importance of these proteins to starch biosynthesis in the grain is yet to be elucidated.

The R1 protein is important for starch phosphorylation in potatoes. If this protein is down-regulated in potatoes, then the starch produced is sharply reduced in the phosphate content and increased in amylose content. The peak viscosity was also reduced. As cereal starch contains naturally far less phosphate, the importance of this protein for cereal starch biosynthesis needs to be ascertained. However, sequences similar to the gene for R1 protein are found in the cereal genome. In Arabidopsis and potato, absence of the R1 protein leads to a decrease in starch breakdown and the consequent production of the starch excess phenotype.

#### **Genes and Genetics in Wheat**

The structure of the genes for the principal starch biosynthetic enzymes mentioned above has been described from wheat (Figure 3). The genes are all complex in intron/exon structure. The genes differ considerably in length, varying from 6 to 11 kb.

The processing of the initial transcript to the final RNA is clearly a complex process. Many of the mutants in GBSS I are due to errors or inefficiencies in the processing of the RNA. In barley, the waxy mutations have been reported that produce low amylose rather than zero amylose. In these mutant starch granules, the outer cell layers of the endosperm contain more amylose than the inner layers. Investigation of these mutants revealed that the change in expression of GBSS I in these lines is due to a 413 bp deletion of part of the promoter and 5' untranslated region of the gene. Two other barley waxy families analyzed have no detectable level of amylose – due to a 1 bp alteration in the GBSS gene that completely eliminates GBSS activity. In rice a single nucleotide polymorphism at the leader intron 5' splice site of GBSS gene has been shown to be associated with changes in amylose content due to altered efficiency of GBSS mRNA processing. A sequence of AGGTATA at this site is found to be associated with cultivars having an amylose content of above 18% as against a sequence

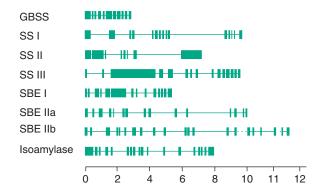


Figure 3 Structure of wheat starch biosynthetic genes. (Data from authors' laboratory.) Exon sequences are illustrated by the thick lines, intron sequences by the narrow line.

of AGTTATA that is present in cultivars having lower amylose content.

The locations of most of the starch biosynthetic enzyme genes are known in wheat and shown in Figure 4. Many of these genes are on chromosome 7.

#### **Future Work**

Genomics has essentially provided the whole genome sequence of Arabidopsis and rice. As rice is also an attractive model system for investigating reserve starch synthesis, it should be possible to identify all putative starch biosynthetic enzyme genes in the genome.

The function of such putative candidate genes can be analyzed by performing conditional knockouts of selected genes, and powerful RNAi technology is available for such experiments. Conversely, gene inactivation through tagging will also yield information about the role of candidate genes. This can arise either through the identification of the tagged gene and analysis of phenotype or by observing a phenotype and then identifying the gene inactivated. Both approaches will be highly informative.

Analysis of expression of all of the genes in a tissue by means of microarrays can also be performed. The ability to study the pattern of transcription of the whole genome by means of "chips" will facilitate the study of linked genes and pathways. If tagged mutants are compared with wild-type, then the total changes in transcription can be identified and pathways can begin to be deciphered. For untagged mutants or natural phenotypes, all the genes affected in a particular phenotype can be identified from microarray analysis of the expressed genes in bulked segregants differing in the phenotype studied.

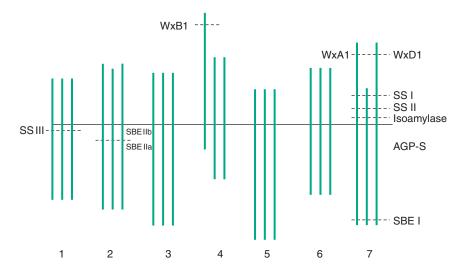


Figure 4 Chromosomal location of wheat starch biosynthetic genes. (Data from authors' laboratory observations and from the suggested readings.)

#### **New Starches in Cereals**

It is clear that with increasing knowledge of the enzymes involved in starch biosynthesis, the ability to alter the starch produced is also gained. New products and processes will need to be developed to capture any advantages occasioned by these new starches. Although such new phenotypes can be produced most easily by using transgenic technology, current public opinion would make it prudent to also produce starches by conventional breeding. The development of molecular markers and rapid breeding technologies (such as the production of doubled haploids, embryo culture and the ability to quickly extract DNA from small samples) will undoubtedly also speed up the production of novel types of starch by conventional breeding strategies.

#### Conclusion

The involvement of four classes of enzymes – ADPG pyrophosphorylase, starch synthases, starch branching enzymes, and de-branching enzymes - in starch biosynthesis is clearly established, although the precise roles of isoforms in many cases are not clear. Other enzymes may also have roles in starch biosynthesis. Starch phosphorylase and disproportionating enzyme (D-enzyme) are examples. The role in starch degradation of both of these enzymes is well known. However, their involvement in starch synthesis, although suggested from a few studies, is a matter requiring further research. The priming of polysaccharide synthesis and granule formation during starch biosynthesis is still unresolved.

Although it is evident that mutations in the core genes lead to specific starch phenotypes over

a variety of species, species-specific characteristics are observed that are often significant and of practical importance. Understanding the complex nature of starch biosynthesis has always been facilitated and accelerated through investigations on model systems such as Chlamydomonas and Arabidopsis and increasingly rice. However, attempts should also be focused on individual species to be able to precisely manipulate the starch structure and functionality to suit specific end uses.

See also: Cereals: Chemistry of Nonstarch Polysaccharides. Grains Other than Cereals, Nonstarch Polysaccharides. Noodles: Starch Noodles. Starch: Uses of Native Starch; Analysis of Quality; Chemistry.

## **Further Reading**

*Plant Biology* 6: 215–222.

Buleon A, Gallant DJ, Bouchet B, et al. (1997) Starches from A to C: Chlamydomonas reinhardtii a model system to investigate the biosynthesis of the plant amylopectin structure. Plant Physiology 115: 949–957. James MG, Denyer K, and Myers AM (2003) Starch synthesis in the cereal endosperm. Current Opinion in

Jane J, Chen YY, Lee LF, et al. (1999) Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. Cereal Chemistry 76: 629-637.

Morrison WR (1988) Lipids in cereal starches: a review. *Journal of Cereal Sciences* 8: 1–15.

Morrison WR (1993) Seed storage compounds: Biosynthesis, interactions and manipulation. 12. Cereal starch granule development and composition. In: Shewry PR and Stobart K (eds.) Seed Storage Compounds-Biosynthesis, Interaction and Manipulation. Proceedings

of the Phytochemical Society of Europe, pp. 175–190. Oxford: Oxford University Press.

Nakamura Y (2002) Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: rice endosperm as a model tissue. Plant Cell Physiology

Rahman S, Li Z, Batey I, Cochrane MP, Appels R, and Morell M (2000) Genetic alteration of starch functionality in wheat. Journal of Cereal Science 31: 91-110.

Shewry PR and Morell M (2001) Manipulating cereal endosperm structure, development and composition to improve end-use properties. In: Shewry PR, Lazzeri PA, and Edwards KJ (eds.) Advances in Botanical Research Incorporating Advances in Plant Pathology Biotechnology of Cereals, vol. 34, pp. 165-236. San Diego: Academic Press.

Smith AM (2001) The biosynthesis of starch granules. Biomacromolecules 2: 335-341.

Topping DL and Clifton PM (2001) Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiological Reviews 3: 1031-1064.

Yu TS, Kofler H, Hausler RE, et al. (2001) The arabidopsis sex 1 mutant is defective in the R1 protein, a general regulator of starch degradation in plants, and not in the chloroplast hexose transporter. Plant Cell 13: 1907-1918.

### **Relevant Websites**

http://www.tigr.org/tdb - Provides databases containing DNA and protein sequences and gene expression, protein family and taxonomic data for microbes, plants and animals.

http://www.ncbi.nml.nih.gov - A resource for molecular biology information. The site provides public databases and software tools for genome analysis.

Starch see Noodles: Starch Noodles.

# STORED GRAIN

Contents

Handling from Farm to Storage Terminal **Invertebrate Pests Pest Management Physico-Chemical Treatment** 

# **Handling from Farm to** Storage Terminal

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## Introduction

Traditionally, grain production has occurred close to the site of its utilization, so the logistics of grain

transport and storage have been relatively simple. However, with the advent of large-scale production in major grain-producing regions of the world, it is a very large undertaking to move the grain from the site of production via stages of transitional storage to the final destinations. This complex process may involve the movement of the grain from one side of the world to the other. Conservation of grain quality throughout its transport and storage is of critical importance. The technologies for doing so have developed enormously from the traditional times of subsistence farming.

Historical accounts from sixteenth-century Europe explain the importance, even then, of grain drying and

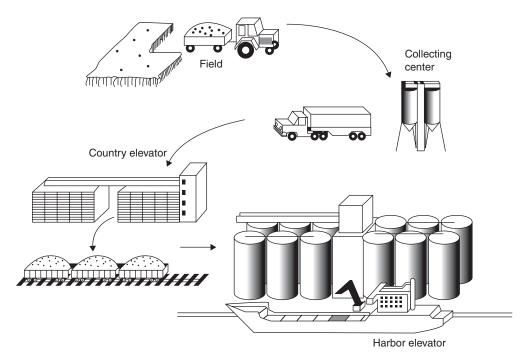


Figure 1 Grain collection, from field to terminal elevator, a long trip for the grain. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 1019, Elsevier Ltd.)

ventilation, and the use of modified atmospheres for the good conservation of grains. However, these texts did not explain the scientific bases for these practices. Today, with the development of scientific knowledge on the grain ecosystem and progress in the fields of building materials, sensors, and measurement techniques, grain storage technologies have been mastered.

New innovative technologies continue to be applied to the increasing demands of world trade in grains, particularly to the requirements for technological and sanitary quality. These are summarized in this article, with the degree of sophistication ranging from simple on-farm storage of seed for next season to an enormous terminal elevator, capable of handing and storing vast quantities of grain under hygienic conditions (Figure 1).

# Types of Storage Needed at All Stages of Handling

The farm is the first stage of handling and storage. At its simplest level, on-farm storage may involve a pile of unprotected grain on the floor of a farm building. At the farm level, grain may be stored temporarily in small concrete or round steel bins of small capacity (25–100 t), which receive the crop immediately after harvest and before transport to a better long-term storage facility or in order to wait for the best market situation.

Grains may also be taken from the farm directly to a collecting center, a small storage installation with several small bins providing an average capacity of  $\sim 1000 \, \text{t}$ . This does not generally have machinery for grain cleaning or drying, since the grain is usually transferred soon after to an elevator (Figure 1).

Country elevators (5000–50000 t), so-called because they are filled with grain elevated into them by rolling belts with buckets, receive grain from the individual producer or from small collecting centers. Their capacity is adapted to the seasonal production of the area, and their main role is to keep crops in good condition before and during storage, and to reload it into trucks or rail cars for transportation to terminal silos, export elevators, or industrial users.

There is no general rule for determining the best dimensions of a farm bin or a country elevator. Usually, the cost of storage increases sharply as the size of the silo decreases, so there is a tendency to build large structures. However, the grains are managed in multiple silos, where grains of different grades or water contents can be separated easily.

The terminal elevators are generally located close to trade centers and/or transportation terminals, such as harbors (Figure 2). Terminal elevators commonly offer storage capacities of 5000–500 000 t in the USA or northern Europe.

Equipment for grain handling and control is often basic at the farm level, with only very simple devices for grain elevation and ventilation. By contrast,



**Figure 2** Harbor elevator in Rouen, France, with a total storage capacity of 300 000 t. This is able to load grains into vessels at a rate of 5000 t h<sup>-1</sup>. (Photo courtesy of J Pfeiffer.)

country and terminal elevators have handling capacities adapted to their transportation facilities and usually have sufficient capacity for peak load handling during the short period just following harvest. Today, most are fully equipped with remote control and automation, so that grain can be automatically transferred from one silo to another or to ships and railway cars. Weighing, cleaning, sampling, and even several grain sample tests are now electronically controlled.

When possible, gravity discharge from grain bins is ideal, but the cost of the corresponding structures and equipment is high. Consequently, flat storage of cereals is also commonly carried out in many situations, both at the farm level and in terminal elevators, to provide additional storage capacities when necessary. Such flat-bottomed silos are emptied through a central outlet, where a rotating screw-conveyor draws the grain away beneath the floor. Many other sophisticated devices have been developed for moving the grain using air blown through metallic networks of ducts below the bins, or with suction blowers as back-up devices.

## **Maintaining Grain Quality during Storage**

The freshly harvested grains must be tested on receival to determine the appropriate treatment for safe storage, such as cleaning, dust removal to avoid very dangerous dust explosions, drying, cooling, chemical treatments against insects, etc.

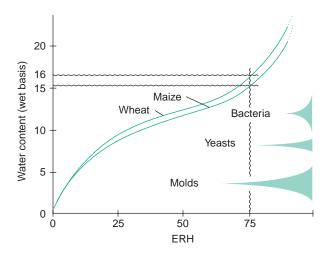
Grain moisture is the major attribute to be controlled during storage. Even with mature dry grain harvested at  $\sim$ 12–13% moisture content, serious problems will be encountered within a few months

if moisture cannot be properly managed to prevent the heat and moisture transfer that can occur in bulk during storage and transportation. It is, therefore, important to store grains with a sufficiently low initial moisture content to prevent mold development and biochemical changes, and to maintain this low temperature as constant as possible everywhere in the silo throughout the storage period. This can be achieved by artificially drying grains with excessive humidity at harvest, like maize in Europe, and by periodic forced ventilation during storage to remove excess water where condensation has occurred.

#### **Monitoring Moisture during Storage**

It is difficult to confidently determine the correct moisture level for a specific grain type in a given situation. Very general and theoretical rules can be given, but one should bear in mind that a large mass of cereal grain is never homogeneous in temperature, moisture, apparent density, thermal conductivity, etc., and so continuous monitoring with modern techniques, such as silothermometry, and periodical sample examination is highly recommended in every situation.

The thermodynamic activity of water, which represents the availability of water in the grain, is the most important physical parameter governing grain stability during storage, determining both biochemical changes and microbial growth. The availability can be estimated (and measured) by the equilibrium relative humidity (ERH) of the intergranular air in equilibrium with the grain. A nonlinear relation relates ERH to moisture content, as shown in Figure 3 for wheat and maize. This curve is named a "sorption"



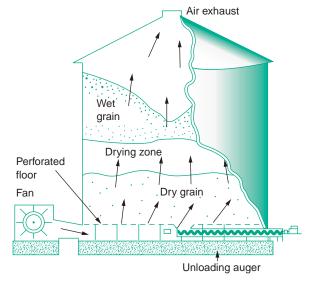
**Figure 3** Sorption isotherms for wheat and maize at 20°C and zones of activity for the main causes of grain degradation during storage (the wider the zone representing a cause of spoilage, the more intense and rapid the spoilage). (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 1020, Elsevier Ltd.)

isotherm" and depends on both the average temperature and the biochemical composition of the grain. It differs from one cereal to another, but its relationship to the level of deterioration by different causes, such as microorganisms or enzymes, remains constant to a first approximation. Wheat stored at 16% moisture content is in equilibrium with an ERH of  $\sim 80\%$  and will undergo the same kind of degradation at the same rate as maize with 15% moisture content, which also has an ERH of 80% at the same temperature. As the temperature increases, the sorption isotherm is shifted to the left, i.e., at the same water content, there is a higher ERH, and spoilage is faster.

Silothermometry is a sophisticated technique, utilizing thermocouples placed in the silo able to detect slight variations in grain temperature, which indicate the beginning of deterioration through an aerobic process, resulting from a localized increase in humidity. Modern sensors can detect temperature changes of less than 0.5°C. In most situations, this gives sufficient sensitivity and overcomes the problem of the very low thermal conductivity of cereal grains, which allows temperature differences to be transmitted only over short distances in the bulk. With this method it is also possible to determine the best moment for drying and ensure the completion of grain cooling by nocturnal ventilation.

## **Drying Methods and Alternatives**

The use of adequately dried grains for storage in silos is a mandatory condition (Figure 3). Many types of driers, such as full-bin driers, layer driers, continuous-flow driers, etc., are presently used on



**Figure 4** Farm bin for low-temperature drying. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 1021, Elsevier Ltd.)

farms or in country elevators, but all utilize the same basic principle: heated dry air is blown through the grain to remove excess water. Figure 4 represents a farm bin equipped with a low-heat drier capable of drying grains with moisture contents of less than 20% (wet basis).

When large quantities of moist grain are to be dried rapidly, high-temperature drying is necessary. This gives a higher extractive capacity but can also cause thermal degradation of the grain, if used improperly, as often happens with maize, which is sometimes harvested with a water content as high as 35% (wet basis) in Europe. If the air for drying is too hot, the functional proteins are denatured, and the value of the crop is decreased. However, high drying temperatures destroy all forms of insects in the bulk.

When possible, a combination of different drying and cooling practices often gives the best technical and economic results. The essential principle is to decrease the moisture content rapidly to a level, thus decreasing the rate of mold development, i.e., to  $\sim 18\%$  moisture in a first step, then to decrease the residual humidity to 15-16% by forced aeration when the ambient air is cool and dry enough to be efficient.

## **Natural Drying Methods**

Alternative natural methods for grain drying have often been used, and at least two must be mentioned. The first, used mostly for maize, uses gentle drying by the wind of cobs stored outside in cribs or other forms of mesh silos. This is satisfactory, provided that the temperature remains sufficiently low to inhibit the growth of such toxinogenic fungi as *Fusarium* species.

The second type of natural drying method uses solar driers. These have been tested in efforts to save energy, but their use can be recommended only in regions where sufficient energy is provided by the sun to achieve drying in a time short enough to avoid spoilage by microorganisms. Unless drying to 80-82% ERH can be achieved in less than 7-10 days, the grain will certainly be damaged.

## **Modified-Atmosphere Storage**

When cereals are not intended for uses requiring particular properties, such as a high germination ability for malt production or functional properties of proteins for breadmaking, it is possible to store them with a higher water content than usual, provided the oxygen is removed from the system.

Underground storage and storage in anoxic atmospheres in hermetically sealed silos, or silos continuously flushed with nitrogen, for example, are quite possible. It was recently shown that the nutritional value of wheat for animal feeding could be successfully maintained over several months by storing it, after grinding, in airtight conditions at 21% water content and 15-20°C. Storage of wet (35% water content or more) maize for pig feed in hermetic silos is now popular in several countries. Because of the important demand for intergranular oxygen at such a high humidity, especially through respiration of yeasts and lactic acid bacteria, the concentration of available oxygen remains extremely low in the silo. The growth of molds is inhibited and, at least until the silos are nearly empty, that is to say when free oxygen again appears and concentrations of carbon dioxide are decreasing, no mycotoxins can be produced. With such hermetic silos, it is not necessary to grind the humid grain in order to produce silage, and the commodity can be handled more easily than with traditional practices.

#### **Control of Insects**

Storage of dry or wet grains under modified atmospheres is an excellent way to kill insects, and modern techniques for storage of dry wheat under high concentrations of carbon dioxide have yielded very good results in Australia in recent years. Most often, if all living insects are to be killed, chemicals need to be used when infestations are detected in a silo or a ship.

Insecticides can be divided into two main classes: contact insecticides, which kill insects and prevent reinfestation owing to their remanent effect, and fumigants, which destroy insects without leaving any significant residues. Organochlorine insecticides, which belong to the first class, were extensively used in the past but are now forbidden because the toxicity of their residues is very high for humans.

Pyrethroids like deltamethrin, permethrin bifenthrin, and organophospates like malathion, dichlorvos or methyl pirimifos, are also contact insecticides, used at concentrations of  $\sim 4-8$  g of active substance per tonne of grain. They are very fast-acting and highly toxic by contact and ingestion, but their residues necessitate careful consideration of the number and timing of treatments. Their relative inefficiency against the hidden forms of insect (eggs, larvae) that live within the kernels should also be mentioned.

The only current fumigant is hydrogen phosphide, which is used to rapidly kill all live insect stages, including adults. It is used in airtight structures equipped with systems that allow the gas to be introduced and removed safely.

Apart from modified-atmosphere storage under nitrogen or carbon dioxide, only persistent insecticides can provide good long-term protection for grain. It is then of primary importance to ensure that there is no residue in by-products like flour and brans used for human and animal food. At the present time, there is a general tendency to decrease the concentrations of toxic substances used against insect infestations. A possible approach would be to combine the use of carbon dioxide with an active substance, such as methyl bromide or hydrogen phosphide, which would permit a considerable reduction in the dose of fumigant used because of synergism with carbon dioxide, which increases the penetration of the active substance in the respiring stages.

#### The Future

All over the world, safe storage of cereal grains is a vital but expensive activity. Too many cereal grains are still used in the food industry despite the poor quality resulting from inappropriate conditions. Current scientific and technical knowledge in this domain is sufficient to answer the main questions about handling and storage of grain, but new trends are appearing: consumer demand for safe products, i.e., free of chemical residues, is growing fast. This probably means that the use of contact insecticides and even fumigants should be replaced by more acceptable techniques like physical techniques in the coming years.

Another trend seems to be a clear demand for identified grains, nongenetically modified grains, and grains from a precise geographic origin or a wellspecified technological quality, for example. Such an evolution would certainly introduce very important changes in the way in which the grains are harvested, collected, stored, and distributed. Loading speeds as

high as  $5000 \, \text{t h}^{-1}$  are often reached today in harbor elevators, but this is not the most convenient technology if small bulks of  $50-100 \, \text{t}$  of well-certified grains are to be prepared.

See also: Barley: Harvesting, Storage, and Transport. Canola: Harvest, Transport, and Storage. Chemicals for Grain Production and Protection. Stored Grain: Invertebrate Pests; Pest Management; Physico-Chemical Treatment. Wheat: Harvesting, Transport, and Storage; Grading and Segregation.

#### **Further Reading**

Christensen CM (ed.) (1982) Storage of Cereal Grains and their Products, 3rd edn. St. Paul, MN: American Association of Cereal Chemists.

Navarro S and Donahaye E (eds.) (1993) Controlled Atmosphere and Fumigation in Grain Storages. Proceedings of the International CAF Conference, Winnipeg, Canada, June 1992. Jerusalem: Caapit Press. Richard-Molard D (1990) Conservation of humid grains in

controlled atmosphere storage. In: Calderon M and BarkaiGolan R (eds.) Food Preservation by Controlled Atmosphere, pp. 57–82. Boca Raton, FL: CRC Press.

Sauer DB (ed.) (1992) Storage of Cereal Grains and Their Products, 4th edn. St. Paul, MN: American Association of Cereal Chemists.

#### **Invertebrate Pests**

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#### Introduction

A storage filled with grain represents a large and delimited resource of biologically available energy. Under many conditions this resource will become the home for a range of invertebrate pests unless some positive action is taken.

Much of the basic information on these pests was obtained prior to the early 1980s and is consequently not accessible electronically. Two series of international conferences – "The International Working Conference on Stored Products Protection" and "The Conference on Controlled Atmosphere and Fumigation in Stored Products" – are useful to

keep track of modern thought on the entomological aspects of stored product science.

There remain many gaps in the scientific study of invertebrate grain-storage pests. Furthermore, the type of studies needed to fill in these gaps are usually time consuming and require a considerable number of person hours to complete. This means that they are unlikely to take place in contemporary circumstances where both time and money are scarce resources. This article aims to give a broad overview of what is known and what can be implied from this knowledge and to direct the reader to some of the less-referenced but important sources of information.

#### The Stored Grain Ecosystem

The stored grain ecosystem is characterized by copious food, limited water, and a stable thermal and humidity environment. It can often remain predator free and can remain undisturbed for significant periods of time. These conditions can be ideal for the development of large infestations of a number of specialist invertebrate pests. The vast majority of these pests are either insects, Coleoptera (beetles), Lepidoptera (moths), and Psocoptera (booklice), or Acarina (mites).

#### **Grain Invertebrates**

Detection and identification of stored-product pests is not always easy. Many of the insects that infest stored products are small, less than 5 mm in length and only 1 mm wide. Some are much smaller, particularly psocoptera and mites, which may be less than 2 mm long and easily mistaken for dust, were it not for the fact that they move.

The patterns of development of stored-product pests also complicate detection. Some species go through their early developmental stages inside the grain kernel with little or no external evidence of their existence. Others complete their life cycles between or on the surface layers of the grain. Others periodically move in and out of the grain and its storage structures. More details on the identification and biology of individual species can be found in the specialist literature referenced at the end of this article.

The most widely used method of detection of insects within a bulk of grain is sampling and sieving. Samples are taken at random (but sometimes targeted) and then sieved through an appropriate mesh, which allows the pest and dust to pass through, while the grain is retained. Samples are taken either from moving grain or probed from static grain. This is an ideal method for sampling free-living adults but of no use for immature stages developing in the grain,

or eggs attached to the grain. Sieving can be automated and this is often done at large grain terminals. Assessment, counting, and identification however remain a manual operation. Probing and sieving of grain bulks is a very labor-intensive operation, especially if a high level of detection is required.

Trapping is also used as a means of detection, and it can detect very low infestation levels for some species. The traps can vary from a simple pitfall trap, through refuge traps to pheromone-baited traps. The major problem of trapping is to understand the significance of the numbers trapped in terms of the numbers actually present in the grain and surrounding areas. Not all species are equally trapped so the significance of low numbers in a trap may range from very low for an easily trapped species, to very high for a hard-to-trap species.

#### Why Are Some Invertebrate Pests Important?

The magnitude of almost all the problems created by invertebrate pests in grain is more or less proportional to the number of insects present. The only notable exceptions to this are when detection of a single insect of quarantine interest may have the same impact as detecting many hundreds. A similar circumstance applies to exporters who ship grain to a standard that specifies no live insects.

The number of invertebrates present in any parcel of stored grain depends first on the number in the initial population. This population may come in with the grain, it may have been a resident population in the empty storage, or it may have entered the storage after the grain had been in storage for some time.

The next determinant of population size is the rate of increase in the population. This is determined by the often-complex demographic attributes of the species. Rate of increase can be approximated to the time it takes to develop from egg to reproductive adult and the number of surviving progeny from one set of parents. Both of these demographic parameters are strongly influenced by the grain type, density of

insects, moisture, and temperature, and can vary widely between species. Even more simply it can be expressed as the multiplication factor per unit time (Table 1).

# The Consequences of Invertebrate Infestation

#### **Biomass Consumption**

This is the first and most obvious consequence of invertebrate infestation. An uncontrolled coleopteran infestation in favorable environmental conditions can change a pile of grain to a pile of dust in about four generations (where development time takes 25– 50 days from egg to adult). This is a worst-case scenario and assumes no action is taken to reduce or remove the infestation. In reality, the biomass-loss rates due to invertebrates are usually much less even in primitive storage conditions. However, figures up to 40% are sometimes reported and losses of 10– 20% are common. In well-managed grain-handling systems, the losses are so small that they are hardly assessable (e.g., much less than 0.1% in the Australian bulk-handling system). This range in the amounts of biomass consumption is almost entirely due to the numbers of insects and their multiplication with generations (modified by species, temperature, and relative humidity of the intergranular air or the associated grain-moisture content).

There is a reasonable body of literature on the energetics of a range of stored products coleoptera. A single beetle requires in the range of  $40-250 \, \mathrm{J}$  (depending on species, temperature, and relative humidity) to develop from egg to mature adult while a single grain of wheat ( $\sim 0.035 \, \mathrm{g}$ ) is able to provide 530 J on average. Using simple arithmetic, it is easy to estimate loss rates under a worst-case scenario when each female beetle produces 100-300 viable progeny in a lifetime under optimal conditions. In the case of stored product, Lepidoptera *Ephestia cautella* and

Table 1 Rate of population increase, development time, and longevity of a range of common pests of stored grain

Common name of group (scientific name of group)	Species	Multiplier of increase in 4 weeks	Development time (days)	Average adult longevity (weeks)
Beetles (Coleoptera)	Sitophilus oryzae	24	25	5
	Rhyzopertha dominica	29	25	4
	Tribolium confusum	60	20	11
	Cryptolestes ferrugineus	60	21	7
Booklice (Psocoptera)	Liposcelis bostrychophilus	22	21	2
Moth (Lepidoptera)	Ephestia cautella	50	25	1

*Plodia interpunctella* consume higher amounts of energy, ~600 J per individual (34 mg), during their lifetime.

There is little energetics or demographic data for the Psocoptera of stored grain, but it is likely that their rate of respiration on a body-weight basis is at the high end of the beetle range. Their mass is  $\sim$ 1% of that of a typical stored grain beetles which would imply a lifetime energy requirement of  $\sim$ 2 J per insect or 0.1 mg biomass consumption per insect.

There have been two energy studies on mites infesting stored grains. *Tyrophagus putrescentiae* and *Acarus siro* have a lifetime energy consumption of 2.5 and 2.8 J, respectively. This translates to a biomass consumption of  $\sim$ 0.1mg per individual. Mites in general are capable of explosive population growth in favorable conditions with an increase of 2500 times in one month, as quoted in several references.

Grain pests are sometimes classified as primary and secondary pests. In general, primary pests are ones that can be found on their own. Secondary pests tend to appear after the primary pest. It is often claimed that only "primary" grain pests are important in grain consumption, but the energy requirements of "secondary" pests are little different to those of primary pests, and in a large infestation the only source for most of that energy will be the stored grain.

#### **Quality Damage**

In addition to biomass loss, invertebrate pests feeding on grain may cause a wide range of quality damage. This damage may be generalized or specific depending on the size of the infestation, the species involved, and the grain being attacked. General damage results from insect-induced heating, unselective consumption of biomass, and webbing of grains. Specific damage occurs when the pest consumes critical components of the grain.

Reduction in germination of seeds is a common consequence of germ-consuming insects or infestations that cause significant heating. Insects consuming the endosperm will cause considerable reduction in seedling vigor together with some reduction of germination. It follows that there is a commensurate loss of nutrients associated with consumption of specific parts of the grain rich in those nutrients.

Generally, the results of infestation at a chemical level have been likened to an apparent acceleration of those types of effects seen in the natural aging of grain. This may be due to any or all of the following: heating, consumption of specific energy-rich components, removal of protection against oxidative changes, odor, and discoloration.

#### Contamination

The presence of a few insects, alive or dead, is rarely a direct problem to users or consumers of grain. Large infestations may create problems with feed rejection where grain is used as stock feed. Large infestations of *Tribolium* species and of the mite *Acarus siro* may give rise to objectionable odors related to specific metabolic by-products of these species.

Downstream food-processing industries are often required to produce a product containing less than a defined number of insect fragments per gram. Meeting this requirement is greatly assisted by low numbers of insects and insect fragments in the raw materials.

The real influence of contamination of grain by insects and insect fragments is through their role as quality criteria in the trading of grain. For example, grain cannot be exported from Australia if it contains detectable live insects. In other parts of the world, different standards may apply but there is usually an insect-related quality criterion associated with traded grain.

#### **Indicators of Poor Storage**

The presence of insects in grain is a useful indicator of how well the grain has been managed during storage and the extent to which deterioration has occurred. Large infestations rarely occur in well-managed storages and are often associated with poor storage hygiene, the presence of birds and rodents in and around the grain, and with hotter and wetter grain than is optimal for storage.

#### **Allergy**

Allergic reaction to living insects and mites is a common phenomenon, although only a few stored grain insects feature as commonly reported causes of allergic reactions, while an allergic response to insect fragments has occasionally occurred in humans consuming or handling infested grain. Amongst the coleopterans *Sitophilus granarius* and *Rhyzopertha dominica* have been known to induce asthma and similar respiratory problems, psocids of the *Liposcelis* group have caused similar problems, and the hairs of dermestids cause skin irritation. Handling Lepidoptera, in general, has been known to cause dermatitis and allergies. Skin irritation and allergy to a wide range of mite species is very well known.

#### **Collateral Problems of Control**

An important consequence of infestation is the need to control the infestation. This is frequently achieved by fumigation (the use of a toxic gas) or by

using a contact insecticide (a grain protectant). The essential difference between these two is that the active phase of fumigants is a gas or vapor while that of grain protectants is a solid or liquid. The major operational difference between the two types of chemical is when and how they are used.

Fumigants can be applied at anytime during storage as long as the gas concentration can be retained or made up. Fumigants can be purged from the storage rapidly and the grain can become available for use in days. Fumigants tend to leave low levels of residues and alteration products. They do not offer any protection to the grain from invading insects once the gas is removed.

Grain protectants can only be applied to moving grain and are most commonly applied as grain enters storage or is moved between storages. Chemicals are effective as protectants because they stay in an active form for significant periods, and therefore residues are an important consideration.

Besides national regulation concerning what chemicals (and what rates of application) may be used on grain, there are international agreements set down by the Codex Alimentarius Commission. Despite the national and international regulations and agreements individual customers may have their own standards for treatments and residues depending on the end use of particular parcels of grain. The organic grain market requires grain that has not been treated with any fumigants and grain-protectant chemicals.

#### **Insects and Mites as Vectors**

There are many reports of invertebrate pests and mites in particular, acting as vectors for fungal spores. If the environmental conditions are then conducive (water activity greater than 0.7), active fungal infection can result. Some stored-product pests can be vectors for parasites and food-poisoning organisms, but only under conditions of poor hygiene.

#### **Insect and Mite Control**

Effective and economic control of insect and mite infestation requires a combination of several different types of action all aimed at ensuring that the grain remains effectively free of insects during the storage period. These types of actions are: hygiene, inspection, disinfestation, and protection.

#### Hygiene

Hygiene is fundamental to good pest control, and a properly implemented hygiene operation goes a long way towards complete pest control. The aim of hygiene is to ensure that the starting population for a potential infestation is small and that the risk of infestation from outside the stored material is very much reduced. Hygiene starts with structure and plant design and, to a large extent, good hygiene can only be as good as the construction of the storage and handling structure will allow. Details of designing for hygiene are well beyond the scope of this article and are poorly documented in the literature. However, the general principles are simple. They include making sure that there are no places where grain (or grain dust) can accumulate and remain undetected and undisturbed for long periods. For insect and mite control, dust, spills, and accumulations which can be seen and easily removed are not a problem. It is residues that remain hidden from easy inspection and removal that are the cause of many infestations. A second feature of a well-designed storage is a physical barrier to infestation from outside the storage. Such a barrier should also restrict the access of birds and rodents whose presence is a problem but may also help establish sites of infestation in nest materials. A barrier that provides complete sealing will also improve the efficacy of fumigation.

Where a storage has not been designed with some consideration to hygiene, it may be possible to modify problem-causing features. This is not without its own problems since it can produce even more inaccessible spaces that entrap residual grain and dust.

#### Work Procedures and Skill

The day-to-day practice of hygiene is a routine and dull job that can be labor intensive. It is, therefore, essential that hygiene tasks are carried out thoroughly; otherwise the resources invested are wasted. A hygiene checklist is an essential tool for good hygiene management. This list ensures that all the important places are drawn to the operators' attention.

Once a storage and its surrounds have been cleared of all detectable grain and grain residues, there may be some advantage to treating these areas with a surface structural treatment of a contact insecticide or an inert dust.

#### Inspection

There are three generic times when inspections are carried out: on intake; during storage; and at outturn. Outturn is often the most important as far as the next user of the grain is concerned, but in pest control terms the least useful. Disinfestation of grain at outturn is impossible and the options for the rapid disinfestation (<24 h) of detained grain almost nonexistent (see methyl bromide fumigation).

In a well-managed grain handling and storage facility, the infestation status of grain at intake largely

determines the starting population of insects. In cases where grain with infestation is taken into storage, it is important that it is disinfested before mixing with uninfested grain. A thorough cleanup of the grain path should be carried out before the path is used for uninfested grain.

Inspection during storage is strategically the most important inspection. There should be an insect-free period of about three generations, i.e., ~3 months at or above 25°C for the majority of species. In situations where hygiene has been adequately carried out, intake inspection was effective, and any infested grain disinfested. After three months any minute residual initial infestation (or invasion) is likely to have reached detectable levels. After three months postintake, or following disinfestation, a monthly inspection is recommended.

#### **Chemical Control**

#### **Fumigation**

Fumigation for control of infestation can be employed in two ways: prophylactic, where a treatment is carried out routinely, usually shortly after intake, even if no insects or mites are found on inspection; or tactically when it is carried out at the first signs of infestation. Good fumigation used with good hygiene and adequate sealing should not need repeating as the recommended application procedures are designed to give a total kill of all insects. Where a reinfestation appears after fumigation, it is most probable that either the fumigation was not carried out adequately or reinfestation pressures are high. Fumigation only works properly if adequate concentration can be maintained for an adequate exposure period. These conditions can only be achieved in a well-sealed system for a single application of fumigant or by "continuous fumigant addition" in an adequately sealed

Currently, phosphine and methyl bromide are the only grain fumigants in widespread use for fumigation of grain.

#### **Phosphine**

Phosphine is applied in two physical forms, as a metallic phosphide (usually aluminum phosphide) or as gaseous phosphine from cylinders. Phosphine requires a significant time to be efficacious and is not able to kill all pest species at every stage below a nominal threshold concentration, no matter what the exposure period (within commercial reality). These approximate conditions are shown in Table 2 for both phosphine-resistant and nonresistant pests.

The main issues around the continued use of phosphine are the worldwide occurrence of resistant populations and concerns about its safe use.

#### **Metal Phosphide**

The most common type of phosphine treatment is where a metal phosphine-based formulation is applied to the grain in a variety of physical forms: pellets, tablets, plates, bags, blankets, and chains. All these forms are designed to allow a controlled release of phosphine as the phosphide reacts with water contained in the air surrounding the commodity or on the surface of the commodity. The rate of release depends on a number of factors: the metallic component of the phosphide (magnesium is faster than aluminum); the surface area to volume ratio of the physical form; the degree of compression (powders are faster than compressed formulations); and other components mixed with the phosphide, temperature, and water vapor availability.

The rate of release and the level of sealing of the fumigation enclosure are the principal determinants of the concentration profile over a period of time. The magnitude of the concentrations (but not the shape of the profile) is modified by the presence of commodity and the amount of phosphide added.

#### **Gaseous Phosphine**

A more recent application technique for phosphine involves the use of gaseous phosphine. This requires careful control as phosphine can be explosive in air (1.8%, 18 000 ppm) and the gas is usually supplied as phosphine mixed with a nonreactive gas, such as carbon dioxide or nitrogen, to assist in eliminating the explosion risks. The gas is then further diluted with air to give the required concentration. This is done on a continuous basis for flow-through fumigation or as a single application for a sealed fumigation.

Table 2 Exposure period in days required at various threshold concentrations to ensure disinfestation with phosphine

Concentration	PPM	10 000	2500	250	100	25	20
	(g m <sup>-3</sup> )	(14)	(3.5)	(0.35)	(0.14)	(0.035)	(0.027)
Exposure time (days)	Susceptible	1.5	2	8	10	25	30
	Resistant	1.5	2	10	>30	>30	>30

#### **Methyl Bromide**

Methyl bromide has been the mainstay of rapid disinfestation for many years. Unfortunately it has been shown to be a potent stratospheric ozone depleting substance. This means that its use has been severely curtailed, and under international treaty it will only be available for genuine quarantine and related preshipment uses.

#### **Fumigants under Development**

Attempts are being made to find replacements for methyl bromide and alternatives to phosphine. Carbonyl sulfide, ethyl formate, and sulfuryl fluoride all show some promise at the research level but are not yet available for commercial use on grain. Even if they are found to be satisfactory, there are commercial and regulatory issues still to be resolved. There is a chance of registration of all these products in Australia in the next 2-3 years.

#### **Controlled Atmospheres**

Controlled atmospheres can be thought of as a special case of fumigation, where the gases used are normal components of the atmosphere and biological systems. The gases most commonly used are nitrogen to displace oxygen, and carbon dioxide. There are only a few places around the world where controlled atmospheres are used routinely. The main problems with controlled atmospheres are long exposure times (typically 15 days or longer), the need for a high level of sealing, and the large volumes of gas required to establish or maintain concentration of > 40% for carbon dioxide and 99% for nitrogen (<1% oxygen). Despite these limitations both types of controlled atmospheres are used where these constraints are not limiting, or where special requirements exist.

For example, nitrogen is used in Australia at the GrainCorp shipping terminal at Newcastle, New South Wales where a large grain-storage capacity allows grain to be held for treatment. There is a large industrial source of liquid nitrogen close by, and the storage has been constructed to a suitable level of gastightness. Carbon dioxide is used to disinfest organic grains exported in shipping containers from Australia to many parts of the world.

#### **Grain Protectant Chemicals**

Grain protectants (i.e., insecticides approved for direct application on grain) can be a very effective way of controlling invertebrate infestation. In the past they have been widely used and have resulted in spectacular reductions in insect infestation. For example, the introduction of malathion treatment in the 1960s resulted in a fall in the level of infestation in Australian wheat imported into UK from ~90% of cargoes to well under 20% over a period of 5 years. The use of grain protectants has lost popularity over the last few years as a direct and indirect response to a perception of concerns about residues. This has led to it being strategically easier to store, handle, and market grain with "no residues" as it is more versatile at the moment of outturn (i.e., if it has no insects, it will suit most markets). Improved fumigation technology has helped this movement away from protectants in many places.

#### **Physical Control**

Invertebrate control by manipulation of the physical environment has been widely discussed. The use of heat, cold, aridity, shock, radiation, and separation appears in the literature. None are widely used in modern handling systems, due largely to their higher capital cost relative to chemical treatments such as protectants and fumigants. The only exception is the use of ambient air aeration, which is rarely used directly for insect control but slows the rate of population increase by reducing the grain temperature (although it is not necessarily effective for mites).

#### **Future Prospects**

Control of invertebrate pests in storage is likely to remain an important part of grain storage and handling for as long as the storage of grain remains a vital component of feeding the human population. Controlling these pests is likely to become more challenging as economic, environmental, and biological pressure increase.

Pressures to reduce the cost of storage and handling mean that labor-intensive activities such as routine hygiene, sampling, and pest detection are seen as costs that have to be reduced. Pressures for justin-time delivery mean that reliable but slow-acting pesticidal activities are becoming more difficult to accommodate. International competition means that commodity prices are critical and any additional on-costs must be avoided where possible.

On the environmental side, there are continuing concerns of emissions of fumigants to the atmosphere, chemical residues on treated grain, worker safety, and the energy costs (dollars and greenhouse gas emissions) associated with pest control. All these reduce the number of readily available options.

The final pressures are the biological ones including the inevitable move of the pest gene pool towards resistance to chemical and possibly physical treatments; the ability of invertebrate pests to capitalize on new niches created by new crops, new storage strategies, and any other opportunities created as systems change.

The prospects for registration of new commercially viable grain-protectant materials are not good. However, there is a possibility of some new fumigants becoming available over the next few years. Large-scale physical and biocontrol remain promising at the research level but have massive cost and logistical barriers to overcome before they can become commercial realities.

See also: Cereals: Grain Defects. Chemicals for Grain Production and Protection. Contaminants of Grain. Food Safety through the Production Chain. Organic Growing of Grains. Plants: Diseases and Pests. Stored Grain: Handling from Farm to Storage Terminal; Pest Management; Physico-Chemical Treatment.

#### **Further Reading**

Banks J and Fields P (1995) Physical methods for insect control. In: Jayas DS, White NDG, and Muir WE (eds.) *Stored Grain Ecosystem*, pp. 353–409. New York: Marcel Decker.

Bell CH, Price N, and Chakrabarti B (eds.) (1996) *The Methyl Bromine Issue*. Chichester: Wiley.

Donahaye EJ, Navarro S, and Leesch JG (eds.) (2001) *Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products.* Fresno, USA, 19 Oct-3 Nov. 2000, Executive Printing Services, Clovis, CA, USA.

Howe RW (1965) Losses caused by insects and mites in stored foods and feeding stuffs. *Nutrition Abstract Reviews* 35: 285–293.

Howe RW (1973) Losses of viability of seed in storage attributable to infestations of insects and mites. *Seed Technology* 1: 563–586.

Mound L (ed.) (1989) Common Insect Pests of Stored Food Products: A Guide to their Identification, 7th edn. Economic series, (British Museum (Natural History)); no. 15.

Lauhoff Grain Company (1991) A Guide to Good Manufacturing Practices for the Food Industry. Danville Ill.

Proceeding of the 7th International Working Conference on Stored Products Protection (1999) Chengdu, PR China: Sichuan Publishing House of Science and Technology.

Rees DP (2004) A Field Guide to Insects of Stored Products. Melbourne: CSIRO Publishing.

Snelson JT (1987) Grain Protectants. ACIAR Monograph No 3. Canberra, Australia: Australian Centre for International Agricultural Research.

Stepien ZA, Goszcynski W, and Boczek J (1973) The Energy Budget of Tyrophagus putrescentiae (Schr.)

(Acaridae). Proceedings of the 3rd International Congress of Acarology, Prague, 1971, pp. 373–380.

The e-UK Pesticide Guide 2003, gives details of pesticides actively marketed in the UK and is designed to ensure the correct pesticide for the job in hand. CABI Publishing Wallingford.

White NDG (1995) Insects, mites and insecticides in stored grain ecosystems. In: Jayas DS, White NDG, and Muir WEM (eds.) *Stored Grain Ecosystem*, pp. 123–167. New York: Marcel Decker.

#### **Relevant Websites**

http://www.sgrl.csiro.au — This website reports current research by one of the few laboratories working on the science of stored grain, the Stored Grain Research Laboratory of CSIRO Entomology. This site also contains links to many sites of interest. http://www.ento.csiro.au — A listing of insect species known to cause allergic reaction due to inhalation (includes several invertebrates of grain storage).

http://www.fao.org — The FAO Inpho website which deals with postharvest matters. Reader should use the built-in search engine to find "fumigation." This will provide a variety of topics around the subject of fumigation.

http://www.pesticides.gov.uk — This website provides information on registered pesticides in the UK. http://www.cdpr.ca.gov — This website contains information on registered pesticides in the USA. It is maintained by the California Environmental Protection Authority and accesses the USEPA pesticide database.

http://www.apvma.gov.au — This website from the Australian Pesticides and Veterinary Medicines Authority contains information on all registered pesticides available for use in Australia.

http://res2.agr.ca — This website is a collaborative product from the Storage Group at the Cereal Research Centre with Agriculture and Agri-Food Canada, the Department of Biosystems Engineering at the University of Manitoba and the Canadian Grain Commission. Its objective is to bring together in one place a variety of resources useful for the sound management of stored products on the farm, in grain elevators, processing facilities, warehouses and retail outlets.

http://www.dpi.qld.gov.au/infopest — Infopest AG-VET (Mar-2003, but updated three times a year). The complete reference of Australian registered insecticide and miticide chemicals and their uses. Available from Animal and Plant Health Services, Department of Primary Industries, GPO Box 46 Brisbane Queensland 4001 Australia.

## **Pest Management**

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#### Introduction

Insect infestation is one of the major causes of rejection of grain deliveries to grain stores, terminal elevators or processors, or at least a cause of downgrading of a grain delivery. Yet, there is little information available about the real rate of rejection or downgrading in domestic uses or at export. The level of 3-5% rejection is considered a realistic estimate for grain deliveries to processing industries in developed countries. The first threat with insect infestation in grain is related to the great power of multiplication of the majority of insect pest species that infest grain. In optimal conditions, the multiplication rate of the rice weevil, S. oryzae, is 25-fold in a month (Table 1). For the very common grain beetle O. surinamensis, this rate climbs to 50-fold. Insect detectable density in a grain bulk with an ISO standard method is rather high (not less than one insect per kg). Nevertheless, no detectable insect infestation is acceptable (and accepted) in the grain trade. This rule is applied worldwide, whatever the grain final destination and the level of infestation in domestic grain stocks. Consequently, the eradication of insect infestation of grain lots destined to a long period of storage is of prime importance for sanitary quality retention.

### **Insect Pest Control by Chemical Means**

#### **Chemical Insecticides**

The most popular method to control grain infestation relates to the use of chemical pesticides. There are two main kinds of insecticides that can be applied on grain: contact chemical insecticides and fumigants. Each of them has a very different scope of use.

Grain can be protected by chemicals during binning or during handling, which are applied onto moving grain, conveyors, or at the elevator. Considering the low cost of such chemicals, a large proportion of cereal grain held in commercial stores is protected against insect infestation by chemical protectants. Grain protectants generally are insecticides registered

Table 1 Ecophysiological characteristics of the various insect and mites species living in stored grain – developmental temperature and rh threshold, natural rate of increase, and multiplication factor in optimal conditions

	Grains more often affected		Conditions for develo	opment
		Temperature range (°C)	rh range (%)	Population increase rate (28 d)
Insect species				
Corcyra cephalonica	Rice	17-35	15-90	10
Cryptolestes ferrugineus	Wheat/barley	20-40	40-95	60
Cryptolestes pusillus	Cereals/pulses	18-38	45-100	10
Ephestia kuehniella	Cereals/by-products	12-30	0-80	50
Lasioderma serricorne	Cereals/by-products	20-37	22-100	20
Nemapogon granella	Corn/by-products	7–27	65-95	
Oryzaephilus surinamensis	All cereals	18-37.5	10-90	50
Plodia interpunctella	Corn/oilseeds/cereals	18-33	25-95	30
Prostephanus truncatus	Corn/sorghum	18-37	40-90	25
Rhyzopertha dominica	Wheat/corn/sorghum	18-39	25-70	20
Sitophilus granarius	Wheat/rye/barley	13-33	55-100	15
Sitophilus oryzae	Wheat/barley/rice	17-34	45-100	25
Sitophilus zeamais	Corn/sorghum/wheat	17-34	45-100	25
Sitotroga cerealella	Corn/sorghum/rice	16-35	25-80	50
Stegobium paniceum	Cereal/by-products	15-35	30-100	7.5
Tenebroides mauritanicus	Corn/cereal products	18-37	25-100	2.5
Tribolium castaneum	All cereals/oilseeds	20-40	10-95	70
Tribolium confusum	Cereal products	20-38	10-100	60
Trogoderma granarium	Durum/oilseeds/pulses	24-31	1-73	12.5
Mites species				
Acarus siro	Wheat/oilseeds	7-30	65-95	2500
Tyrophagus putrescentiae	Oilseeds/cereals	12-35	65-95	20 000

for application on to whole grain to protect it against insect attack. There are basically two types of insecticides: (i) residual (i.e., long-term persistence substances that leave active residues on grain during several months to more than a year after the treatment) and (ii) nonresidual (rapid killing substances related to a high vapor pressure and a high liability of residues). The first group is mainly composed by organophosphates, pyrethroids, and insect growth regulators (IGRs) that are the active ingredients of the formulations. Only the dichlorvos (also an organophosphate) enters the second category.

Grain protectants are applied on grain mainly as a liquid formulation by simple spraying with airpowered applicators that can automatically be driven in well-equipped storage installations. However, powder formulations are still available and are useful for complementary treatments of the regions of a grain bulk that are favorable for insect development such as the surface layers in flat-bed storage that must be protected from external reinfestation after fumigation or cooling.

The use of grain protectants varies widely with the country, market preference, and local regulations (Table 2). Contact insecticide admixture in stored grain is widely used in grain-exporting countries and especially in France where four organophosphates and two pyrethroids are registered. In contrast, there is a marginal use of fumigation or controlled atmospheres (CAs) in France, unlike in Australia, North America, or in Germany. Because of heat degradation, insect resistance, increased buyer's concern, and more stringent requirements for registration of new molecules, the use of chemical protectants is declining in almost all the developed countries. Nevertheless, the maximum residues limit (MRL) for any active substance registered for direct application on grain forms the object of a worldwide approval by the Codex Alimentarius (joint commission WHO and FAO). The World Trade Organization had adopted these approved MRLs in cereal grain international trading.

The IGRs have been registered and used on a small scale in Australia. They are considered as substances with a poor efficacy during the weeks after the treatment and, even if they have a high lethal effect on insects, the populations are reduced to nothing only after weeks or even months after the application. These slow-active substances may be considered not to be useful by many grain handlers who expect a rapid effect in destroying existing infestations.

Today, more than 100 strains of stored grain insect species have been described resistant to organophosphates (chlorpyryfos-methyl, pyrimiphos-methyl, malathion, etc.) in different countries. The lesser grain borer, Rhyzopertha dominica, is certainly the most tolerant species to organophosphate treatment and, thus for this particular species, pyrethroids (deltamethrin, bifenthrin, or bioresmethrin) are preferred. This "adaptation" of insecticide treatment

Table 2 Chemicals (active substances) registered for direct application on cereal grain with their main characteristics and the MRL level fixed by the Codex Alimentarius Commission

Insecticide	MRL Codex Alimentarius (mg kg <sup>-1</sup> grain)	Oral LD <sub>50</sub> for rats (mg kg <sup>-1</sup> body weight)	ADI for human consumption (mg kg <sup>-1</sup> body weight)
Bioresmethrin	1	7070-8000	а
Bromophos	b	3750-8000	0.04
Carbaryl	b	850	0.01
Chlorpyrifos-methyl	10	1630-2140	0.01
Deltamethrin	1	135-5000	0.01
Dichlorvos	2	56-108	0.004
Etrimfos	5	1800	0.003
Fenitrothion	10	800	0.003
Fenvalerate	2	451	0.02
Lindane	0.5	88-270	0.01
Malathion	8	2800	0.02
Methacrifos	10	678	0.0003
Methoprene	b	> 34 600	0.1
Permethrin	2	430-4000	0.05
Pirimiphos-methyl	10	2050	0.01
Pyrethrins	3	584-900	0.04
Piperonyl butoxide	20	7500	0.03

 $MRL = maximum residue limit; LD_{50} = lethal dose 50%; ADI = acceptable daily intake.$ 

<sup>&</sup>lt;sup>a</sup>No value currently assigned.

<sup>&</sup>lt;sup>b</sup>Not registered for application on grain.

to the characteristics and the threat of a target insect species is one of the principles of Integrated Pest Management (IPM) strategies (see Stored Grain: Invertebrate Pests).

#### **Fumigation with Gaseous Fumigants**

Preliminary considerations Fumigants are volatile chemicals that are under a gaseous state at normal atmospheric pressure and ambient temperature, and which have toxic properties against insects. Once in gaseous form, the fumigants readily penetrate into infested kernels, thereby eliminating all insect life stages if applied properly. Numerous fumigants have been evaluated since the first discovery of the insecticidal properties of methyl bromide by Le Goupil in 1932. Although methyl bromide has played a major role in quarantine fumigation for international trading and for structural fumigation, there are few examples of its systematic use for grain fumigation. Recently, it was incorporated to the list of compounds possibly contributing to the depletion of the upper atmospheric ozone layer. Consequently, the use and production of methyl bromide will be forbidden in 2005 in all the developed countries. Two other fumigants that have been used for grain disinfestation were registered after methyl bromide: hydrogen cyanide (HCN) and phosphine (PH<sub>3</sub>). The first one is only used rather exclusively for the treatment of empty holds of ships prior to loading. Only one fumigant is currently used worldwide for grain disinfestation: phosphine. Phosphine gas is generated from solid formulations of aluminum or magnesium phosphides. With its low molecular weight and low boiling point, phosphine is easily penetrating into grain bulks and inside grain kernels and diffuses through permeable packaging material. In addition, the adsorbed gas is quickly eliminated after fumigation by aeration. One disadvantage is the long exposure period needed to completely eliminate the target insect species population. The time to reach maximal concentration in the fumigation enclosure varies with the formulation, magnesium phosphide generators releasing phosphine more rapidly than aluminum phosphide. Nevertheless, complete efficacy of phosphine fumigation needs at least 3-7 days and even more with temperature levels between 10°C and 15°C. Below 10°C, phosphine fumigation is not recommended and can lead to efficacy failures very easily. Consequently, phosphine fumigations are performed during the warm season on grain at a temperature above 10°C.

Implementation of phosphine fumigation Phosphine gas is released from a solid generator through the action of the water vapor of air (occupying the intergranular space in a grain bulk). In these conditions of slow release of the gas during time, a specific unit of exposure was defined in 1984 by a Canadian researcher. He described the use of a "ct" term, in which the concentration of the fumigant and the time of exposure (usually in hours) are multiplied to quantify the "dose" for fumigation conditions. Other conditions of the fumigation influence the penetration and the efficacy of the fumigant for the killing of the target insects: temperature, relative humidity (rh), insect species, developmental stage, airtightness of the enclosure, or of the grain bin sealed for fumigation purpose. Modeling of the release of phosphine gas and the level of observed "ct" with fumigation conditions may allow the monitoring of the entire process and standardization of procedures in order to minimize the risk for workers.

An important aspect to consider with a gaseous treatment is the lack of residual protection, so reinfestation can occur immediately the grain has been aired and the gas concentration fallen below the safe concentration for workers. Fumigation with phosphine is used in two different manners: as a preventive or as a corrective treatment. In most cases, fumigation is applied when a grain bulk is obviously infested with insects and when the term of storage is not achieved. However, in the two countries, Australia and the USA, where it is the major method for insect control, it is preferably used as a preventive method for pest control. In these two countries, the policy of long-term storage of large quantities of grain in low-cost structures (flatbed storage, semi-underground pits or "grain bunkers," hemispheric storage bins, etc.) renders the use of fumigation indispensable. The difficulty to move grain to another bin equipped with different control means (cooling or liquid insecticide treatments) is also a major issue that can be solved by this technique. In this particular case, the storage structures dedicated to fumigation are built with permanent sealing during the whole period of storage in order to achieve several fumigations per year. In other cases, where fumigation is used for a complete disinfestation of infested grain, this operation can be carried out in specialized airtight bins called "hospital bins." The most popular technique which has the agreement of regulation authorities of many countries is called the "I system" (Figure 1).

Safety use considerations Very strict safety procedures are associated with the use of fumigants in developed countries such as specialized training, capability to monitor the concentration during the whole operation, and the use of safety equipment.

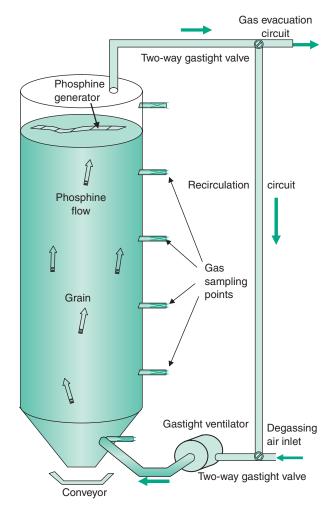


Figure 1 Design of an airtight grain bin devoted to phosphine fumigation operations ("hospital bin") - This bin is equipped with a gas re-circulating duct, an open aeration circuit and airtight ventilator and valves ("J system" principle).

So, this job is done by trained, reputable professionals. In developing countries, fumigation of durable food products is used as a complementary or marginal technique and most often regarded as a measure of last resort, also for safety reasons. Where regulations permit, in-transit fumigation of bulked and bagged grain on board ship can be carried out. This technology is widely used for grain export mainly in the USA. Other countries that require grain to be free of infestation at the point of export cannot use in-transit fumigation.

Phosphine – more recent issues In order to limit the main drawbacks of phosphine treatments - i.e., the (long) time needed for the natural release of hydrogen phosphide, and the difficulty to accurately deliver the expected dose from the solid generators – several

methods have been developed to directly release phosphine gas. Generally, these gaseous formulations of phosphine compressed in gas cylinders contain 2% phosphine mixed to 98% carbon dioxide. Various systems have been patented and are currently used, especially in Australia (Siroflo<sup>®</sup>, Phosfume<sup>®</sup>, Profume<sup>®</sup>, etc.).

Probably because phosphine is of worldwide use today, several strains of grain insect species (e.g., Rhyzopertha dominica and psocids) have been shown resistant to phosphine fumigation in several countries. Consequently, there is recent enhancement in fumigation technology. In addition, active research has been launched for a decade in order to find new fumigants either for the replacement of methyl bromide or to overcome problems with target insect resistant strains. Today, there is very active research on sulfuryl fluoride and on carbonyl sulfide as the two major candidates for the registration of new fumigants that should replace methyl bromide.

#### **Insect Pest Control by Physical Means**

#### **Temperature Control of Insects**

Effect of temperature on insects Most of the insect species living in stored grain are of tropical and subtropical origin and have fairly high temperatures for optimal development (Table 1). Cooling grain below 20°C greatly reduces the rate of increase of the most noxious species called "the primary feeders": Sitophilus spp. (the weevil species complex), Rhyzopertha dominica and Prostephanus truncatus (the lesser and larger grain borers, respectively), and the juvenile instars of Sitotroga cerealella (the Angoumois grain moth). The population growth of all grain insect species is inhibited (natural rate of increase reduced to zero) when temperature falls down below 10°C (lower limit of developmental temperature for the more tolerant species of primary feeder to cold: S. granarius) (see Stored Grain: Invertebrate Pests). Accordingly, the lowering of grain temperature to this level of 10°C is the ultimate objective of grain cooling in order to control the insects. In most cases, grain temperature in a range 15-18°C will reduce insect damage to acceptable levels. Thus, the safe temperature level to reach is 15°C or less considering that in this condition the rate of increase of insect populations remains very low or is nil. But, the cooling process being slow due to insulating properties of grains, reducing grain temperature to this low level requires several aeration periods. These cooling steps are generally obtained by 1-2 weeks of aeration, mainly achieved during the night. The most important cooling step is the immediate period after grain harvest. In addition, secondary insect species such as the saw-toothed grain beetle, Oryzaephilus surinamensis, the rust-red grain beetle, Cryptolestes ferrugineus, or the flour beetles of the *Tribolium* genus may move in great numbers during the aeration time to reach the opening at the surface where air flow goes out the grain bin. The more rapidly the first cooling step is achieved, the longer is the safe storage period. This first step has the objective to reduce the temperature to ~20°C. Such a lowering of temperature from 30°C to 20°C reduces the natural rate of increase of the majority of noxious species of  $\sim 50\%$ . The immediate cooling of harvested grain is generally feasible in all countries with a temperate or Mediterranean climate during the month following the harvest. Thus, in the United Kingdom cooling by aeration is used on 60% of the farms and in over 90% of the commercial stores. This proportion is also high in France (50% of commercial wheat and malting-barley storage facilities are equipped for cooling aeration assisted by a temperature monitoring system). It has been proven that grain aeration is also appropriate for warm countries such as Israel, Australia, and South USA provided that the air rh and moisture content of grain will be sufficiently low (e.g., 40% and 12%, respectively) to reduce the natural rate of increase of the main insect species. With an air-flow rate of 10 m<sup>3</sup> h<sup>-1</sup>, the total cumulative time of aeration is  $\sim$ 100–120 h, i.e., 10–15 nights at the rate of eight "favorable hours" per day. Automated control systems for automatic running of the aeration process are available to control fan operation, some of them being now associated to software packages in decision support systems. These systems can be used to perform the calculations and to produce an optimal design for fans, ducts, etc.

**Heat disinfestation** Temperature plays a major role in the number of physiological functions of insects that are "cold-blood" animals (poikilotherms). Heat was used to kill insects 80 years ago: temperature of 52-55°C maintained during 10-12 h were found effective for flour mill disinfestation. Several methods of heating grain in bulk are now available. A recent method is the use of fluidized-bed or spoutedbed heating, where heated air is used as the heat transfer medium. Wheat grain treated in a fluidized bed by hot air can be disinfested through the heating of grain to a temperature of 65°C that is maintained only a few minutes before a rapid cooling to ambient temperature. In these conditions of heat shock, it was found that this treatment does not adversely affect the viability, the moisture content, and the baking quality of wheat provided that grain is immediately cooled after the heating phase and that its moisture is at a normal level. At a temperature of  $62-65^{\circ}$ C inside the kernels, all developmental stages of insect internal feeders are killed. Several different approaches have been used to adapt heat rapid disinfestation for use with modern systems of grain storage. They all heat the grain during the conveyance. The final temperature to reach in the whole kernel required to kill insects is slightly above  $60^{\circ}$ C ( $62-65^{\circ}$ C).

Fluidized-bed or spouted-bed heating is the first means of heat disinfestation that has been implemented at the practical stage (150 T h<sup>-1</sup>). With the fluidized-bed process, cooling grain after heating is very easy with optimization of energy input by heat exchange between the hot and the cold parts of the equipment. Since heat disinfestation technology in a fluidized bed is compatible with the high grain transfer rates found in large storage facilities, a mathematical model of the process could be formulated. The prediction of the temperature at which grain is heated internally has been approached by simple asymptotic regressions using only the measurable variables: air inlet and outlet temperature, airflow velocity, and specific rate (volume of air per unit of grain mass). The simplification of the calculation for the model regression is as follows (eqn [1]):

$$1/t_{x\%} = T + C$$
 and  $T_{\text{max}} - T = ab^{\exp[t_{x\%}]}$  [1]

where  $t_{x\%}$  is time to give x% mortality, T is grain temperature (°C),  $T_{\text{max}}$  is air inlet temperature, and C, a, and b are constants.

In the recent years, a general modeling approach of heat and mass transfer in any system of grain drying, cooling, or aeration was obtained using numerical methods. From a starting point of a model describing moisture migration arising from natural convection currents in two-dimensional systems, an extension of the diffusion equation for moisture and water vapor through hygroscopic models has been proposed. Then, this model for the diffusion process was generalized to simulate moisture migration in grain bulks of arbitrary shapes. The actual model based on the finite element method allows the description of heat and moisture associated with both natural and forced convection aeration in arbitrary shaped grain bulks. Thus, real-time changes in moisture and temperature can be predicted enabling to detect air-flow conditions that may generate temperature or moisture accumulation or redistribution in the regions of the bin. This tool combining heat and moisture transfer can also be used to visualize, in two-dimensions, the effect of the spacing and the size of aeration ducts on the cooling or drying efficacy in all regions of the bin.

#### Microwave (MW) and Radiofrequency (RF) Heating

Infrared, radiofrequencies (RFs), microwaves (MWs), and light radiation are nonionizing electromagnetic waves that can be used for the direct transfer of radiant energy to solid matter such as grain (a dielectric material). Among these means, only RF and MW heating present an interest for heat disinfestation of food commodities, provided their qualities are not impaired by heating. Electromagnetic waves without ionizing effect such as MW and RF transfer energy from a source to a target without a need for an energy transfer fluid. In the case of MW and RF, their energy can be absorbed by inducing vibration of electrically charged particles within the matter, thus increasing temperature because of internal friction.

**RF** heating RF heating involves frequencies between 3 and 300 MHz, i.e., wavelength between 100 and 1 m, respectively. Commonly used frequencies for energetic applications in Industry, Science and Medicine (ISM use) have been fixed by regulations authorities at 13.56, 27.12, and 40.34 MHz. RF dielectric heating is accomplished with the product placed between two electrodes in an oven. Rapid heating of the exposed material is obtained in applying a high electrode voltage that induces a high alternative field intensity inside the material. Field intensities of 1.4–1.5 kV cm<sup>-1</sup> are currently applied for cereal grain disinfestation. The field intensity is quite uniform if the material is homogeneous in presentation between the electrodes.

MW heating MW heating involves a higher frequency range than RF heating, expanding from 300 MHz to more than 50 GHz. The main MW frequencies allowed for ISM applications are 434, 915, and 2450 MHz. The technology of MW was developed later than that of RF after the discovery of the magnetron and other MW sources in the years since the 1970s. MW heating is obtained with lower electric field intensities than RF heating. But, partly because of the lower wavelength of MW, attenuation with the depth of penetration in the material is high. The result is a certain heterogeneity if the material remains static under the MW source. To minimize this heterogeneity risk, the product is generally rotated or mixed during exposure to achieve a better homogeneity.

Even if interesting results have been obtained in disinfestation and, to some extent, in final-step dehydration of cereal grain either by RF or MW, this method has not been used in current practice for grain treatment because of the high capital cost of installations, the relatively high running costs for electric energy consumption (even if its transfer rate into

heat is generally better than 60%), and the low product flow rate that can be disinfested by this treatment (pilot scale machines have reached 4 T h<sup>-1</sup> with an RF generator delivering 60 kW energetic electric field). There are many drawbacks for the current use of dielectric heating for grain disinfestation and vet this method has not been shown to be superior to air-based systems. Provided that there is a good monitoring of the heating process, the eventual damage to the end-use qualities of treated cereals at levels of temperature required to eliminate insects is limited and generally acceptable. These heat-sensitive qualities include bread-making quality of wheat, rice color and taste, and viability of malting barley and seeds.

#### Irradiation or Ionization

Irradiation represents either the exposure to  $\gamma$ -rays (radioactive isotopes) or to X-rays (accelerated electrons). Insects are not immediately killed after an irradiation but they are all sterilized at a low dose. Thus, irradiation of bulk grains may theoretically be used in order to eliminate insects provided that the presence of live but sterile arthropods is tolerable. Two different types of equipments correspond the two kinds of radiation: the radioactive sources (<sup>60</sup>Co or <sup>137</sup>Cs) and the accelerated electrons emitted from a heated cathode. Gamma rays are penetrating deeply into the food products, whereas accelerated electrons have a limited depth of penetration limited to 2-5 cm. Consequently, the accelerated-electrons plant may only treat thin layer of the product conveyed under the irradiation "gun." A plant dedicated to the disinfestation of imported grain was used a long time ago (in the USSR) but is no longer operational. The technology of radioisotopes was tested in Indonesia recently for the disinfestation of bagged rice. This was observed an effective treatment against S. oryzae at a dose of 0.40 kGy.

Although there are internationally recognized agreements for the use of irradiation for disinfestation of a range of foodstuffs, including grain, there are public acceptance problems with irradiated food products. Consequently, the use of irradiation for grain disinfestation purposes is greatly affected by this negative perception of retailers and consumers.

#### **Controlled Atmospheres**

The process of controlled atmosphere (CA) disinfestation involves the modification or the replacement of intergranular atmospheric composition of grain bulks by inert gases CO<sub>2</sub>, N<sub>2</sub>, or their mixtures in association with a low level of residual O2. The use of CA is an adaptation of the age-old principle of hermetic storage. In simple airtight or hermetic storage

systems, the progressive depletion of oxygen by the natural respiration of grain, microorganisms, and insects themselves can also be lethal for insects after long-term exposure. The lower the moisture content of grain, the longer the delay to kill the insects. An anaerobic atmosphere can be achieved quickly (several hours to several days) with the injection of inert atmospheres inside airtight grain storage structures. There are two different ways to eradicate insects in infested grain stored in an airtight enclosure by using CAs: the "high-CO<sub>2</sub>" CA, often called "modified atmosphere" and the "low-O<sub>2</sub>" CA.

High-CO<sub>2</sub> CA Adding carbon dioxide (more often under its gaseous form) to an airtight grain bin has an effect similar to that of fumigation. Carbon dioxide has a specific lethal effect on insects when its concentration is above 40% (v/v) in air. Atmospheres containing ~60% carbon dioxide rapidly kill storedproduct insects. As an example, at a temperature of 26°C, ∼4-day exposure would be sufficient to kill all stages of most stored-product insects. However, there are many drawbacks in using "high-CO<sub>2</sub>" CA to control grain insects: the amount of CO2 required to fumigate 1 ton (t) of grain is  $\sim 2 \,\mathrm{m}^3$ , i.e., close to 4 kg of CO<sub>2</sub> in liquid form (to be compared to 2 g of phosphine gas). Carbon dioxide sorption on grain is important and may induce negative pressure in well-airtight enclosures. This phenomenon renders the practical management of CO<sub>2</sub> fumigation more difficult to achieve than a phosphine fumigation and the use of pressure safety valves is required.

Low-O<sub>2</sub> CA The oxygen depletion in an airtight enclosure may be principally achieved by adding oxygen-free gas such as pure nitrogen, or by adding low-oxygen content gas such as the output of an hydrocarbon burner (also called exothermic inert gas generator). In contrast to "high-CO<sub>2</sub>" fumigation, it is considered that nitrogen is only active in producing a progressive hypoxia or anoxia without other particular effect on living organisms. Consequently, in pure nitrogen atmospheres the grain beetles can survive for more than 1 month at low-temperature levels of the grain during the winter season (e.g.,  $10-12^{\circ}$ C). The rule of oxygen deficit being correlated with the killing dose-effect relationship has a classical exception with the weevil, S. oryzae, which is less tolerant to 1% O2 residual concentration than to almost anaerobic atmospheres with only 0.1–0.2% residual oxygen. Pure nitrogen generation by a separation process of atmospheric nitrogen from air is now available with modern units useable on small capacity storages as it exists in cereal processing plants. The technology of generating nitrogen from air on-site (by separation and purification of the atmospheric nitrogen) is progressing rapidly and becoming cheaper.

Low-oxygen atmosphere can also be obtained by exothermic gas burners. Several such items of equipment were built in Australia, USA, and UK during the years 1980–95. No significant advantage could be proved between the use of a burner compared to the use of pure nitrogen neither in terms of insecticidal efficacy nor on an economical basis. There are very few grain stores equipped with fixed installations with either pure nitrogen or gas burners, except for certain high-grade cereals such as aromatic rice, or premium malting barley. The strategy that is most commonly used for CA is the permanent preservation under a low-O<sub>2</sub> atmosphere of grain in an airtight bin equipped with relief valves absorbing the differences in pressure in the airtight bin when temperature changes.

The future of CA fumigations CA treatments based on either nitrogen or carbon dioxide atmosphere storage provide technical alternatives to methyl bromide uses for disinfestation of bulk or bagged grain. But their use is constrained by the cost of the implementation of CO<sub>2</sub> fumigation or long-term generation of low-O<sub>2</sub> atmosphere by a burner or an atmospheric-nitrogen purifier. The slow speed of action of CA atmospheres is not really an inconvenience in tropical countries and several strategic reserves of cereals are preserved from deterioration by this technique in specific situations (e.g., in Indonesia or Singapore).

#### **Other Miscellaneous Treatments**

# Mechanical Impact, Turning, and Pneumatic Conveying

During pneumatic conveying of grain in fluid-lift handling (e.g., when vessels and barges are out-loaded), the high-speed impaction of grains against the walls of the transportation ducts eliminates a significant proportion of adults and well-developed hidden stages of primary feeders. Adult stage of grain insects being particularly sensitive to a mechanical impact, this sensibility is exploited in impacting machines used on grain entering flour mills. This device eliminates a great part of infested grain and removes broken grains and their insect content by forced ventilation before the conditioning of grain prior to milling. In this way the content of flour and semolina in impurities of entomological origin is reduced to an acceptable level for the miller. This is not really a stored grain treatment but one can consider that it is the last tool available by the grain user to eliminate an insect infestation just before grain milling. Disinfestation of wheat grain prior to milling by impacting machines

("entoleter") has been routinely used for more than 20 years in the flour and semolina industries to destroy all insect stages.

#### **Inert and Abrasive Dusts**

For two decades, several inert dust formulations have been registered for structural treatment and to protect stored grain. Diatomaceous earth (DE) and silica aerogels (and their mixture) are used in several countries as an alternative to the use of chemical protectants, especially when grain is not treated with chemicals (e.g., organic grain or grain intended for uses in baby food). DE is classified as a "generally recognized as safe" (GRAS) food additive by the US Environmental Protection Agency. It provides a good protection against insect infestation in dry grain stored for a long term in flat bed stores. Liquid formulations of DE are used in Australia and North America for grain protection in sprays applied to the storage fabrics to minimize residual infestation in grain store buildings and to limit the migration of pests into the bulks of stored grain. DE is an effective protectant when applied to the entire grain mass at a dose of 200 mg kg<sup>-1</sup>. However, DE on grain is also considered as poorly effective with grain at an elevated moisture content as it can be found in humid temperate countries (northern Europe, Canada, and northern Asia) where the moisture content of stored cereal grain can be as high as 15%. Problems associated to the use of DE in large-scale operations are: (1) machine abrasion; (2) a reduction in the bulk density (test weight), which is a criterion of grain quality used worldwide; (3) grain fluidity change; (4) decrease in qualities such as color and presence of foreign material; and (5) health hazards (respiratory disease hazard for the applicators).

#### **Combined Methods**

Physical methods can be used simultaneously or in sequence, especially if there are synergistic effects. For instance, low-O<sub>2</sub> and high-CO<sub>2</sub> (hypercarbic) atmospheres in airtight enclosures have a higher efficacy rate when temperature is elevated to levels that cause hyperactivity of target insects. Consequently, the reduction of time needed for disinfestation process is significant even for species that are rather concentration insensitive to carbon dioxide such as Tribolium confusum.

#### **Decontamination of Spoiled Grain**

#### **Preliminary Considerations**

Stored grain molds may grow in grain with an  $a_{\rm w}$  level more than 0.65 (see Stored Grain: Physico-Chemical

Treatment), i.e., for the majority of cereal grain, when moisture content is more than  $\sim 14.5\%$  (wet basis). Some of the grain mold species produce mycotoxins which are potentially hazardous to man and animals. Mycotoxins are a worldwide important issue in terms of public health, agro-food industry concern, and economics. The optimum temperature for growth of stored grain molds is  $\sim 25-30^{\circ}$ C, but some grow well at 35–37°C or above, like Aspergillus spp. Grains are contaminated by spores of storage fungi during harvest, transport, and handling operations. In storage, provided that temperature and moisture content  $(a_w)$ meet their minimum requirements, they germinate and fungal growth occurs. The rate of growth is correlated with the levels of  $a_{\rm w}$  and temperature.

#### **Mechanisms of Grain Spoilage**

Mycotoxins are secondary metabolites of fungi. Many storage fungal species may produce mycotoxins. The most toxigenic storage fungi include members of the genera Aspergillus and Penicillium. Formation of mycotoxins is closely related to mold growth. Without mold growth, mycotoxin production will not occur. Competition between storage fungi influences mycotoxin production by the most hazardous toxigenic species such as Aspergillus flavus or *Penicillium verrucosum*, the species producing aflatoxin B1 and ochratoxin A, respectively. An infestation of stored grain by insects can also enhance the production of mycotoxins. The presence of molds on the grains does not mean the presence of mycotoxins but that the potential for mycotoxin production exists. However, the absence of storage fungi on grain stored on a long-term period does not guarantee that the grain is free of mycotoxins. The fungal growth can be related to the fungal biomass produced by storage molds in grain. The best criterion that can be used to quantify the fungal biomass produced by the storage microflora is the ergosterol content. Thus, ergosterol content is considered as a good biochemical marker of fungal growth and represents a permanent index of mold spoilage, with or without mycotoxin production, that persists with time.

#### **Decontamination Methods**

Once grain is contaminated by toxic fungi due to prevention measures not being applied or incorrectly applied, only a few practices are available. Any decontamination process has its own limitations, since the treated products must be health-safe from the chemicals used and their nutritive value should not be diminished. So, unless the contaminated grain batch is of considerable value for a potential user, the first recommendation with mycotoxin-contaminated

grain is its complete destruction. If the conditions for a decontamination of grain are favorable, it may be carried out in two different ways: (1) separation of the contaminated kernels from the sound grain, and (2) inactivation of mycotoxins by physical, chemical, or biological means. Mechanical automatic separation of heavily contaminated kernels with an abnormal dark color can be removed by specific photoelectric detection machine based on imaging and robotics (electronic color sorting). The screening and discarding of small shriveled kernels by density-based separation equipment may also significantly reduce the amounts of mycotoxins in grain because the most heavily infested kernels are less dense than the sound ones. Mycotoxins in contaminated grain may be detoxified or converted into a nontoxic derivative using a limited number of practices. Aflatoxin being the most dangerous mycotoxin, the majority of decontamination treatments preferentially affect this mycotoxin. Heating and cooking under pressure can destroy a great part of aflatoxin in grain but this process cannot be applied to whole grain intended for food uses. Detoxification of oilseed cakes and corn using ammonia is a marginal method. It may be useful only for the decontamination of cereal by-products such as bran (this grain tissue is the most heavily contaminated) extracted from contaminated grain and that need a decontamination before utilization for feed making. The ozone treatment combined with heating (100°C) has a potential to inactivate aflatoxin toxicity. Nevertheless, several hours of exposure are needed and this treatment may decrease protein efficiency ratio and available lysine (observed only on peanut meal).

## Future Prospects of Stored Grain Preservation

#### **Stored Grain Quality Maintenance**

The quality maintenance of stored grain has traditionally been the responsibility of grain handlers who rely on measurements achieved on samples of grain or its milled products and on implicit knowledge gained through science, common sense, and job experience. Today, treatments achieved for quality preservation may be integrated into operational systems if they are to be effectively applied. This basic principle connects with the modern approach of integrated quality management (IQM). Integrated quality management of stored grain can be defined as the acceptable use of practicable treatments to minimize, cost-effectively, the losses and damages caused by deteriorative forces (insects, mites, and microorganisms), or the consequences of a poor control of grain condition

(temperature manipulation,  $a_{\rm w}$  control by drying, dockage level, insect infestation). Thus, decision support systems (DSSs) are indispensable to solve stored grain preservation issues that require experience, knowledge, judgment, as well as a knowledge base to explain the complex deterioration processes occurring in stored grain bulks. DSSs are considered valuable support to promote the implementation of the IQM strategy at the practical level.

#### The Potentialities of Computer-Aided Decision Systems

The potentialities of computer-aided decision systems have continuously evolved since their first application to stored grain pest management. Actually, the DSSs are considered as very useful tools for grain handlers to take optimal management decisions. Several DSSs are in use for many purposes in stored grain management: (1) diagnosis of grain condition; (2) prediction of risks for long-term storage of grain lots; (3) as an aid in insect identification; (4) to monitor stored grain condition and support problem solving; (5) to support the implementation of drying or cooling operations; and (6) to predict the storability prior to storage. Until recently, the majority of the systems under current use were devoted to pest management and to the training and education of extension workers. A new approach of total quality management of stored grain was tackled only recently. It is based on the computing of an array of causal relations between all variables of the stored grain ecosystem (whatever their nature and the form of causal relation). This approach consists in combining: (1) the models of complex interactions between control and state variables of the stored-grain "ecosystem" and (2) the human experts' subjective knowledge on the changes that will occur in grain quality criteria during storage in constant conditions. The safe storage period (grain "storability") as well as the effects of different treatments (such as cooling, drying, separation of impurities, insecticide admixture, etc.) may be predicted by such a knowledge-based system. For each described situation (grain initial condition), an output is delivered under the form of storage options susceptible to increase the safe storage period. The data collected on stored grain condition by sensors and probes (temperature, aw or moisture content and storage time) can also be computed to see if the stored grain observed quality state is identical to the prediction by the DSSs at any time during storage. The remote control of the drying or cooling processes may also be entirely assumed by DSSs. Tools for automatic detection and identification of insect species at very low population density levels are now available to predict storage—insect population dynamics.

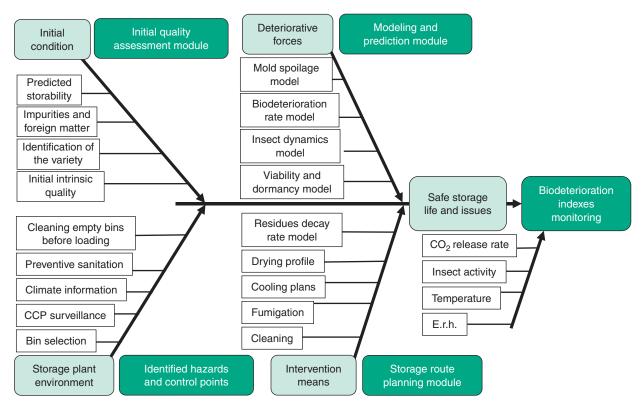


Figure 2 Decision-tree diagram associated to the knowledge base of a decision support system enabling the integrated management of quality criteria of stored malting barley in relation to storage time, intervention means, and grain condition.

#### The Scientific Approach to **Grain-Quality Retention**

The scientific approach to grain quality retention during storage can entirely refer to computer-assisted DSSs. Thus, such artificial intelligence tools enable the grain handler: (1) to understand the causes and economical consequences of quality changes in grain bulks; (2) to forecast quality changes; (3) to provide support for problem solving; and (4) to propose optimal storage strategies (Figure 2). The methodology already applied for malting-barley quality computation should be expanded to quality management and control of any type of cereal grain in the near future.

See also: Cereals: Grain Defects. Chemicals for Grain Production and Protection. Contaminants of Grain. Food Safety through the Production Chain. Organic Growing of Grains. Plants: Diseases and Pests. Stored Grain: Handling from Farm to Storage Terminal; Invertebrate Pests; Physico-Chemical Treatment.

#### **Further Reading**

Baur FJ (ed.) (1984) Insect Management for Food Storage and Processing. St. Paul, MN: American Association of Cereal Chemists.

Baur FJ (ed.) (1992) Insect Management for Food Storage and Processing. St. Paul, MN: American Association of Cereal Chemists.

Bell CH, Price N, and Chakrabarti B (eds.) (1996) The Methyl Bromide Issue. Chichester, UK: Wiley.

Bond EJ (1998) Manual of Fumigation for Insect Control. Plant Production and Protection Paper No. 54. Rome, Italy: FAO.

Calderon M and Barkai-Golan R (eds.) (1990) Food Preservation by Modified Atmospheres. Boca Raton, FL: CRC Press.

Credland PF, Armitage DM, Bell CH, and Cogan PM (eds.) (2003) Advances in Stored Product Protection. Wallingford, UK: CABI Publishing.

Donahaye EJ, Navarro S, and Leesch J (eds.) (2001) Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products. Clovis, CA: Executive Printing

Fleurat-Lessard F and Ducom P (eds.) (1991) Proceedings 5th Int. Working Conf. Stored Product Protection, vol. 3. Bordeaux, France.

Jayas DS, White NDG, and Muir WE (eds.) (1995) Stored-Grain Ecosystems. New York: Marcel Dekker.

Koe WJ de, Samson RA, van Egmond HP, Gilbert J, and Sabino M (eds.) (2001) Mycotoxins and Phycotoxins in Perspective at the Turn of the Millenium. Wageningen, The Netherlands: WJ de Koe.

Le Goupil M (1932) Les proprietes insecticides du bromure de methyle. Revue de Pathologie Vegetale et d'Entomologie Agricole 19: 169–172.

Navarro S and Donahaye E (eds.) (1993) CAF International Conference on Controlled Atmosphere and Fumigation in Grain Storages. Jerusalem. Israel: Caspit Press Ltd.

Rechcigl JE and Rechcigl NA (eds.) (2000) Insect Pest Management. Boca Raton, FL: Lewis.

Subramanyam B and Hagstrum DW (eds.) (1996) *Integrated Management of Insects in Stored Products*. New York: Marcel Dekker.

Vincent C, Panneton B, and Fleurat-Lessard F (eds.) (2001) *Physical Control Methods in Plant Protection*, 329pp. Berlin: Springer and Paris: INRA.

Vincent C, Hallman G, Panneton B, and Fleurat-Lessard F (2003) Mangement of agricultural insects with physical control methods. *Annual Review of Entomology* 48: 261–281.

#### **Relevant Websites**

http://www.org.teap.org — UNEP (1998) Methyl bromide technical options committee — 1998 assessment of alternatives to methyl bromide. UNEP, ozone secretariat, Nairobi, 184pp.

http://www.fao.org — Problems of pesticide residues in stored grain. Ed. ACIAR, Canberra (Australia). Executive seminar Series No. 3 (1989).

http://vm.cfsan.fda.gov — US Food and Drug Administration Center for Food Safety and Applied Nutrition Pesticide Program: Residue Monitoring 1999 (2000).

http://www.fao.org - Manual of fumigation for insect control FAO (Ed.).

http://apps1.fao.org – Pesticide Servlet Pesticide Residues in Food (MRLs/EMRLs).

# **Physico-Chemical Treatment**

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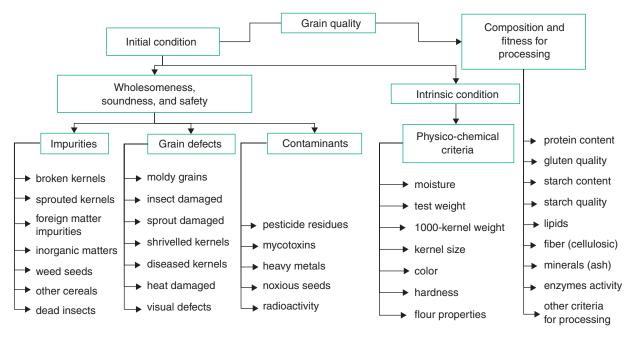
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#### Introduction

The storage of cereal grains is achieved for extended periods of time in order to supply the domestic and export needs. The storage period may last several months to a year or even more when grain is stored for market regulation objectives or as strategic reserve. At a central position along the processing chain, the storage stage has to play three essential roles: (1) the assessment of the quality of each grain load delivered by the farmers in order to identify quality grades; (2) the preservation of original properties and composition of freshly harvested grain; and (3) the permanent supply of the first processing industries with homogeneous grain batches of a specific quality grade.

Postharvest losses of grain are a significant factor in the world food supply and may represent as much as 5-10% of the world production of cereal grains and oilseeds. On a qualitative basis, there is a constant risk of grain quality deterioration in storage. The quality of grain is specified with different attributes closely related to the making of a specific end product. It can be defined as the combination of an intrinsic and an extrinsic component with complex relationships. The intrinsic component of grain quality may be considered as both the initial condition and the biochemical composition of grain at the harvest (**Figure 1**). The extrinsic component – which includes the soundness, the purity, the sanitary, and the safety condition - is dependent on the action of deterioration factors. The two major biological causes of deterioration of properties and quality of stored grain are microorganisms (storage fungi) and invertebrate pests (insects and mites).

The deterioration of stored grain quality being irreversible, the prevention of quality losses is of prime importance for any grain store manager. To face the storage issues, he disposes of a range of equipment, tools, materials, and techniques to prevent (or reduce) the grain quality deterioration process. The stored grain treatment includes the elimination or the inhibition of the main causes of loss such as infestation by insects or mites, and contamination by microorganisms. However, minor causes of quality losses such as grain respiration, gradual deterioration of viability, nutritive quality, and end-use properties are also concerned by preventive actions. The preservation of stored grain from adverse storage conditions that may endanger its marketing value depends on different means that can be considered either preventive or corrective. The preventive means include cleaning, drying, aeration, cooling, pest control treatment, kernel breakage prevention, and controlled atmosphere storage. The corrective actions have rather recourse to quick-action treatments such as high flow-rate cleaning, fluidized-bed drying, temperature shock, fumigation, and some other grain sanitation treatments. This review of the means of stored grain preservation complements the previous works dealing with good storage practices of grain stocks that have been published in books listed under the Further Reading section.



**Figure 1** Basic criteria and parameters involved in the definition of grain commercial quality – four components are identified: intrinsic and physico-chemical condition; sanitary and safety condition; biochemical composition and nutritional value; and properties for processing. (Adapted from Fleurat-Lessard 2002.)

#### **Grain Cleaning**

Cleaning grain is usually done before storage of grain lots in bins or granaries. Freshly harvested grain contains a small proportion of impurities, also called "dockage." Dockage components include small or large seeds of weeds or other grains, chaff, straw, dust, soil pellets, small stones and boulders, cracked kernels, and other trash (see Contaminants of Grain). In other respects, harvested grain may contain shrivelled kernels, smaller than those of healthy grain and being in most cases heavily contaminated by pathogen fungi and their related mycotoxins. A maximum percentage of impurities is acceptable in grain trade, according to fluctuations of the respective limits for each kind of impurities fixed by each country regulations (see Defects of Cereal Grain). When this limit is exceeded, some quality criteria are affected (e.g., the test weight). Moreover, the presence of a significant amount of larger-sized impurities such as ear fragments, plant stalks, green weeds parts, grain husks, and chaff, etc., represent a serious risk for safe storage because they are a source of microbiological spoilage and lead to favorable conditions of multiplication for insects and mites. In the case of high percentage of wet impurities, grain heating may start in some parts of the grain bulk a short time after binning (humid hot spots). In addition, these regions with heated grains are liable to become compact enough to disrupt airflow distribution and hinder an eventual beneficial effect of drying or cooling later operations. Thus, the grain cleaning through the separation of a significant amount of impurities may improve grain condition before storage.

Grain cleaning operation can be carried out with a large range of equipment, ranging from the simple sieves used in developing countries to remove light impurities (thanks to the wind) to very sophisticated high-speed industrial equipment found in grain terminal elevators. Some of these high-flow-rate cleaners may clean 10–12 t of grain per hour with a work input of 1 kWh.

Some other cleaning equipment combines grain cleaning and calibrating operations. It has a much lower output than specific cleaning machines and it is mainly used by the seed-producing companies before conditioning perfectly cleaned and calibrated seed grains. Winnowing machines can also be included in grain cleaning equipment even if they are mainly used to clean the part of the grain harvest kept by small farmers as their own seed reserves.

From an economical point of view, the financial profitability of removing dockage and foreign material of grain devoted to export depends on the initial dockage level. Some export markets will pay a premium for dockage-free grain while other markets do not. Consequently, grain cleaning is generally not practiced in all cereal-producing and -exporting countries. In USA and France, grain operators have little economic incentive to provide dockage-free

grain. The delivery of cleaned grain is marginally profitable in Australia, a cereal exporting country that supports a policy of exporting high-quality grain. In Australia, the grain cleaning costs are deducted by the grain handling company from the value of the raw grain delivered by the farmers to commercial grain stores.

#### **In-Bin Drying**

#### **Preliminary Considerations**

Seeds being hygroscopic, grain either absorbs moisture from the environment (under high relative humidity (rh) conditions) or loses moisture (under low rh conditions). This relationship is generally represented graphically by two typical curves, respectively, when grain is adsorbing or desorbing moisture. These curves represent the correlation between the surrounding air rh and the water activity  $(a_{w})$  potential in the kernel at a constant temperature (so-called isothermal sorption-desorption-equilibrium curves). When this thermodynamic equilibrium is established, the temperature and pressure being constant and equal in the two phases (gaseous atmosphere and grain), the grain  $a_{\rm w}$  can be numerically identified with the ratio of the partial pressure of water in grain surrounding atmosphere (in "empty" space of the grain mass) (eqn [1]):

$$a_{\rm w} = \frac{\text{equilibrium rh (\%)}}{100}$$
 [1]

In this equilibrium condition, there is a close relation between the moisture content of the grain, and the  $a_{\rm w}$  (or the rh inside the grain bulk). Consequently, grain  $a_{\rm w}$  can be related to the moisture content of grain by mathematical models. Numerous mathematical models of these sorption—desorption equilibrium curves have been built up in dependence with the temperature level for almost any type of seed or food matrix. Several researchers have regularly refined the equations that are now available for the majority of cereals and are accurate enough to be used for the monitoring of aeration or moisture migration changes in a stored grain bulk through rh sensors (eqns [2a] and [2b]):

$$rh = \exp\left[-\frac{C1}{T + C2}\exp(-C3M)\right]$$
 [2a]

$$M = \frac{1}{-C3} \ln \left[ \frac{(T+C2) \ln(rh)}{-C1} \right]$$
 [2b]

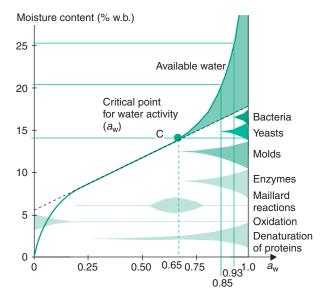
where rh is the relative humidity, *M* the moisture content, *T* the temperature, and *C*1, *C*2, *C*3 the coefficients.

The modified Chung—Pfost eqn [2a] gives a good fit with experimental sorption curve of cereal grain, especially for wheat and it can be used for the accurate conversion of moisture content into grain  $a_{\rm w}$  for several cereals.

# Moisture Content and Allowable Safe Storage Times

The importance of water in grain can be deduced directly from the sorption curve. From a critical  $a_{\rm w}$ corresponding to the end of the linear portion of the desorption curve (Figure 2), a less tightly bound water appears that becomes available for the feeding biodeterioration processes. Thus, above a limit of  $a_{\rm w}$  in grain the respiration becomes active. Consequently, the grain moisture content (or the  $a_{\rm w}$ ) is the most important factor determining the intensity of grain respiration. The heat produced by the respiration of organisms living in the grain bulk increases the temperature of the grain that indirectly favored the fungal growth. Grain respiration results from the aerobic consumption of complex carbohydrates (starch) by living organisms. This oxidation of energetic nutrients of grain also generates liquid water (eqn [3]):

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 2817 \, kJ$$
 [3]



**Figure 2** Grain desorption curve (wheat at 25°C) focusing on the zone in which water adsorbed in grain becomes available to support various processes of quality deterioration within specific limits of grain moisture content and water activity level. (Reproduced with permission from Multon JL (ed.) (1988) *Preservation and Storage of Grains, Seeds and Their By-Products.* New York: Lavoisier Tec and Doc. © Technique et Documentation.)

In airtight storage, when carbon dioxide level exceeds 10% and the oxygen levels falls down below 1%, the respiration process is inhibited and the anaerobic fermentation that occurs produces less heat (eqn [4]):

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + 209 \text{ kJ}$$
 [4]

In addition, nonuniform temperatures in the grain bulk generate air convection currents and lead to moisture migration, especially when large changes in external air temperature occur. These moisture migrations may induce the "top crusting" phenomenon that develops at or near the grain surface in metallic bins or in flat storage during the grain natural cooling (Figure 3).

Among the various living organisms in the stored grain ecosystem, the storage fungi represent the major causes of deterioration in grain and seeds. The main deleterious effects of fungi on stored grain quality are: (1) decrease in germinability; (2) induction of changes in kernel color and external aspect; (3) induction of hot spots where grain is heating; (4) inducing a mustiness odor that may be detectable by smelling; (5) induction of various biochemical changes; (6) production of mycotoxins that, if consumed, may be injurious to man and to animals; and (7) loss in weight and decrease of specific weight.

The water activity  $(a_{\rm w})$  of grains is closely related with the growth and the metabolic rate of storage molds. Therefore, the safe storage life of a grain bulk is dependent on the level of moisture content of grain at the beginning of storage. However,

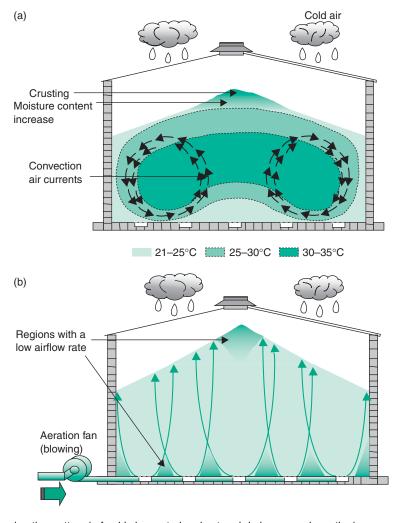


Figure 3 (a) Moisture migration pattern in freshly harvested grain stored during several months in a nonaerated metallic bin when outdoor temperature is lower than grain temperature showing the "surface crusting" phenomenon (adapted from various sources). (b) Grain cooling of a flat-bed grain bulk by a blowing system of aeration with multiple longitudinal air ducts - progress in cooling and observed distribution of cooled and less-cooled regions of the bulk.

below the  $a_{\rm w}$  threshold of "available" water presence, both the germination of fungal spores and the growth of storage fungi are inhibited. Today, the different thresholds of  $a_{\rm w}$  allowing the development of fungal species that may grow in stored grain are well known, especially for the species that are potential mycotoxin producers such as Aspergillus flavus, A. ochraceus, Penicillium cyclopium, P. verrucosum, and P. viridicatum (Table 1). This knowledge has been regularly refined, permitting the development of predictive models of "safe storage life" of stored grain from indexes of deterioration such as the decrease in germinability or from composite indexes combining relevant quality criteria. These quantitative models take into account the original quality of grain and the expected storage conditions (temperature and moisture). These models permit the prediction of the quality status of stored grain at any stage or the determination of the time left before to reach the minimum acceptable level of quality. The provision of models to predict safe storage life of stored grain offers the possibility of maximizing and guaranteeing market value in terms of grain-specific utilization by the processor or the consumer. As an example, an equation applicable to the determination of the safe storage period for malting barley, before the limit of 95% viability level is reached, was recently validated (eqn [5]):

$$M_t = \frac{\ln(35/T)}{\exp(C4 + C5 \times a_{\rm w})}$$
 [5]

where  $M_t$  is the safe storage time, T the temperature, and C4 and C5 the coefficients of the equation, variable with the type of cereal (e.g., for malting barley, C4 = -21.22 and C5 = 20.33).

In generalizing the previous equation for malting barley, a model of calculation of sorption equilibrium was derived. It can be useful in monitoring moisture content changes during drying or aeration operations. This predictive model may be included in the knowledge base of software packages aimed at the management of stored grain treatment or they can also be used as prediction tools (Figure 4).

To properly stored grain, both the grain moisture content and the temperature level must be compatible with the expected period of time the grain will be stored and with the grain intended use. The commercial upper limit of moisture content corresponds generally to critical moisture level for grain respiration activity, lowered with a safety margin of  $\sim$ 2%. Thus, the lower limit of grain moisture content allowing the growth of storage molds at 20°C in malting barley is 16.2% and the limit recommended for safe storage conditions was recently fixed at 14% in Europe. The recommended moisture contents for stored grain are listed in Table 2 in the two common situations: local short-term storage (less than 6 months) and regional or terminal long-term storage (for more than 6 months).

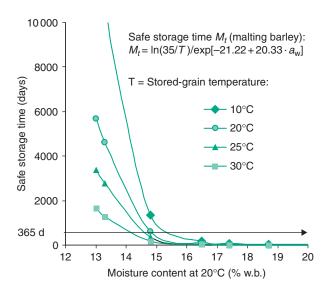
#### The Main Objectives of Drying

In regions where the climate is humid, mature grain is harvested at moisture content levels incompatible

List of storage (xerophilic) fungi harmful for stored cereal grain quality and the associated mycotoxin production (when it exists)

Fungus species	Mycotoxins	Grains more often affected	Minimal a <sub>w</sub> for growth	Corresponding grain moisture at 20° C
Scopulariopsis brevicaulis		Oilseeds/rice/peanuts	0.90	22.5
Aspergillus parasiticus	Aflatoxin	Corn/peanuts/cottonseed	0.86	19.8
Penicillium brevicompactum		Peanuts/rice/corn	0.86	19.8
Aspergillus ochraceus	Ochratoxin Penicillic acid	Corn/wheat/barley/oats	0.84	19.0
Aspergillus flavus	Aflatoxin	Corn/peanuts/cottonseed	0.82	18.0
Aspergillus fumigatus	Gliotoxin	Oilseeds/cereals	0.82	18.0
Penicillium aurantiogriseum	Penicillic acid	Cereals/cereal products Corn/peanuts/rice	0.81	17.3
Penicillium verrucosum	Ochratoxin Citrinin	Corn/wheat/barley/oats	0.80	16.8
Aspergillus versicolor	Sterigmatocystin	Wheat/barley/corn	0.80	16.8
Wallemia sebi	Walleminol	Pulses/cereals/by-products	0.78	15.8
Aspergillus restrictus		Wheat/rice/corn/beans	0.75	15.5
Aspergillus candidus	Kojic acid	Wheat/corn/by-products	0.75	15.5
Eurotium amstelodami	-	All cereals/by-products	0.75	15.5
Eurotium chevalieri		Pulses/wheat/corn	0.74	15.1

with safe storage for a long period of time. The removal of the excess of moisture from grain can be achieved by grain drying. Thus, the prime objective of grain drying involves reducing the moisture content in harvested grain in order to minimize mold-spoilage hazard during long-term storage. Thus, drying grain to the equilibrium with an air rh of less than 70% is a necessary prerequisite for safe storage. Additionally, low moisture content levels are less favorable conditions for the growth and buildup of populations of insects and mites.



**Figure 4** Quantitative model predicting the safe storage life of malting barley (95% germinability) in relation to moisture content and temperature in stored grain (constant conditions).

**Table 2** Maximum recommended moisture contents of various grains for short-term (6 months) or long-term (more than 6 months) storage

Grain species	Short-term storage (less than 6 months)	Long-term storage (more than 6 months)
Corn	15.5	13.5
Durum wheat	14	13
Edible pulse seeds	16	13.5
Malting barley	14.5	13
Oats	14.5	13
Rapeseeds (canola)	9.5	8
Rye	14	12.5
Sorghum	14.5	13
Soybeans	13	11
Sunflower seeds	10	8
Wheat	14.5	13

Adapted from several sources.

#### **Grain-Drying Technologies**

There are two different grain-drying techniques.

- 1. High-temperature drying which is performed from specialized equipment with a high grain-flow rate and mainly devoted to the drying of wet grain that cannot be stored in its initial condition at the harvest (maize, sorghum, paddy, oilseeds, and all small-grain cereals grown under wet climates). This method involves an initial high capital investment and high running costs for a drying operation achieved in a few hours in continuous-flow grain dryers.
- 2. Low-temperature or natural air drying, which concerns grain batches needing a moderate reduction of moisture content at the harvest. This kind of drying can be carried out with minimal specific equipment in grain stored in bins specifically equipped for drying purpose. The complete drying can last over a period of several weeks.

Only the second type of drying corresponding to "in-bin" grain drying will be described below.

#### In-Bin Grain Drying in Practice

Drying has two basic stages: (1) diffusion of internal moisture to the surface of the kernel and (2) removal of external moisture by an airflow around the kernel. Water-vapor pressure is increased inside the kernel which causes moisture to diffuse through the micropores of the seed pericarp layers. The grain temperature is related to the rate of moisture diffusion and therefore can be monitored and controlled in order not to exceed a maximum rate of diffusion that could produce grain damage. The rate of evaporation of external moisture is also dependent on the air temperature (at a fixed airflow). These two stages are in constant interaction and in unstable equilibrium. The drying rate is also affected by atmospheric conditions. In this situation of complex interactions between several variables, the initial setting of the parameters of the drying system cannot be predicted, and it is necessary to test the drying process with several batches of grain before determining the exact setting of the dryer on a specific storage site. The main parameters that must be taken into account in determining a grain-dryer setting are given as follows:

- 1. the evaporation capacity, which is a fundamental characteristic of any dryer (expressed in kilogram of water evaporated per hour: kg h<sup>-1</sup>);
- 2. the heating capacity, which is the quantity of heat produced by the dryer per hour (kJ h<sup>-1</sup>);
- 3. the drying yield, which is the percentage of effectively used drying power (corresponding to the

- amount of water removed from grain) on the delivered power (energy consumed by the dryer);
- 4. the net calorific power of the dryer burner fuel, which is the heat quantity effectively delivered by the combustion of fuel (in kJ m<sup>-3</sup>);
- 5. the specific airflow rate through the grain mass which is the rate of the airflow per unit of grain mass (in m<sup>3</sup> h<sup>-1</sup> of air per m<sup>3</sup> of grain); and
- 6. the specific heat consumption, which represents the heat quantity required for the evaporation of 1 kg of grain water. This value is closely related to the fuel consumption and is generally minimized by the grain-dryer manufacturer.

In addition, the evaporation capacity and the rate of dried grain produced per hour in defined conditions of initial moisture content of grain and blown air temperature level in the dryer are also taken into account.

In static dryers operating in a bin equipped with air ducts in the bottom or with a perforated floor, a close relation between the temperature of the air blown through the grain mass and the renewal rate of the air per unit of grain bulk volume must be respected in order to obtain a homogeneous profile of moisture content throughout the dried grain bulk.

Different types of static driers for stored grain are used in developed countries, especially at the farm level. The original in-bin high-temperature drying system is initially loaded with a batch of grain, which remains in the bin until the drying operation is achieved. During the first phase of drying, grain is heated and in the second phase grain is cooled by ambient air aeration in order to recover its initial temperature. During the drying/hot-air stage, the grain mass is crossed from bottom to top or from side to side by the "drying front." The eventual heterogeneity that may occur at the end of the drying process, when the drying front comes out the grain mass, is generally limited by mechanical stirring-up devices. In these systems, the air temperature is  $\sim 50-60^{\circ}$ C with a specific flow rate of 140 m<sup>3</sup> h<sup>-1</sup> of air per m<sup>3</sup> of grain. During the drying operation, the grain of the bottom of the bulk is systematically drawn up at the top of the bulk by several vertical augers (Archimedean screw).

Another type of dryer built on the same principle provides a continuous grain flow within the drying bin. The dried layer of grain at the bottom is regularly extracted and moist grain is automatically added above the previous batch. In this configuration, the maximum thickness of the grain layer should be limited at 2-4 m, and the specific airflow rate at  $300 \, \text{m}^3 \, \text{h}^{-1}$  of air per m³ of grain, and air temperature can be set in the range  $40-80^{\circ}\text{C}$ ,

depending on the sensibility to heat injury of the processed grain.

Storage bins equipped with aeration systems can also be used in a specific process of drying called "dryeration." In this process, the grain is partly dried in a conventional continuous-flow dryer and then the partially dried batch of warm grain is aerated by ambient air in a resting bin during 2 weeks. This type of process is more economical and involves less labor input compared to a continuous-flow drier. However, the aeration of semi-dry grain requires specific aeration equipment. The specific flow rate must be maintained between 40 and 60 m<sup>3</sup> h<sup>-1</sup> of air per m<sup>3</sup> of grain. Nevertheless, after the cooling and the end of the drying operation, the grain must be transferred in a long-term storage bin. These additional handlings of the grain batch may increase the amount of broken grain. The dryeration system is used only to dry wet grain at the harvest such as maize or sunflower seeds. There is a risk for a slight loss of dry matter during the aeration of warm grain (coming from the drier at a temperature levels of 50-60°C) and the cooling bin must be equipped with air extraction fans in the headspace above the grain mass in order to minimize the water condensation problems that may occur in any metallic bin.

The temperature level reached by grain during conventional or dryeration drying, which is more than 60°C, is lethal for most of the hidden stages of insect primary feeders that may be present inside maize, rice, or sorghum kernels at harvest. This disinfestation effect of heating grain during the drying process will be developed further with the heat-shock treatment.

The drying of maize cobs in a bin equipped with an inclined perforated floor has also been used in order to limit the development of fungal microflora on humid grain (especially maize and sorghum) and the consecutive production of various mycotoxins, either produced by field fungi (when growing conditions remaining favorable for the field microflora) or by the most competitive strains of storage molds (on partially dried grain).

#### Aeration and Cooling

#### **Preliminary Considerations**

The stored grain bulk is slowly influenced by environmental temperature due to its low thermal conductivity. Although temperature has little direct influence on grain condition, it greatly influences the development of insect and mites and microorganisms, and it affects the viability of seeds. The first objective of aeration is to reduce the temperature of grain at the start of the

storage period. Harvested grain typically comes into store at average outside temperature in summer, i.e., often above 25°C or more in Mediterranean or subtropical cropping areas. At such temperature levels even dry grain is at risk from insects and moisture migration within regions of the grain bulk. Grains harvested or dried at commercial moisture levels (12–14% moisture) and at temperature levels as high as 25–35°C cannot be preserved during a long period of time if they are not cooled at lower temperature levels, thereby inhibiting quality-deterioration processes. Moreover, the viability of seeds decreases rapidly in a few weeks in grain stored at 30°C or more.

#### **Purpose and Benefit of Aeration**

Aeration is the process of forcing air through grain to reduce its temperature. The main beneficial effects of grain cooling are given in the following:

- 1. preservation of the technological properties of stored grain at a grade level as close as possible as its initial grade at the harvest time (e.g., baking quality of wheat, viability of malting barley);
- 2. limitation of the moisture migration phenomenon in bulked grain storage and related mold development, which is the principal cause of damage to several grain quality parameters (e.g., reduction of seeds viability and increase in fat acidity);
- reduction of the dry matter loss consecutive to the natural respiration of grains remaining active in high-temperature conditions. The associated risks of release of metabolic water and of hot spot forming are also reduced; and
- 4. prevention of the insect multiplication at a high rate of increase when temperature can be lowered below 12–14°C, the level at which the rate of increase of the most cold-tolerant species falls to zero.

The use of cooling aeration was first developed to reduce problems of moisture translocation with warm grain when stored in metallic bins. But, the potential of cooling grain by forced air aeration is currently used to combat arthropods as pest and to unify temperatures, thus preventing moisture migration and hot spot formation. The theoretical basis of temperature transfer to grain by aeration either with ambient or with refrigerated air has been investigated by a large number of authors since the 1980s that lead to the production of many reference reviews. More recently, several simulation models for temperature migration during a cooling/aeration process have been produced and they may become useful supports for automatic control of aeration and for an adequate design of air ducts and ventilator characteristics. Heat and

moisture migration within regions of an aerated grain bulk can now be visualized in real time for each distance element of the bulk and at each time increment using finite element/difference methods. With modern computers, real-time calculation can be carried out fast enough to produce moving images.

#### **Practical Implementation of Cooling Aeration**

Stored grain aeration requires the provision of an airexhaust ventilator associated to an adequate storage bin design. Grain bins devoted to aeration have to be equipped with perforated ducting on the floor through which air is blown into (or sucked through) the grain. The design and the dimension of the ducts, as well as the optimal characteristics of the ventilator are described in previous reviews. The air-feeding network (pipes and ducts) has to be properly calculated to minimize the pressure drop for an appropriate airflow rate  $(5-15 \text{ m}^3 \text{ h}^{-1} \text{ m}^{-3})$ . A centrifugal fan is not appropriate for aeration of bin higher than 15 m, when fan static pressure exceeds 2 kPa (e.g., for wheat aerated in a 15 m high bin with an airflow rate of  $10 \,\mathrm{m}^3 \,\mathrm{h}^{-1} \,\mathrm{m}^{-3}$ ). When air is blown, the fan compresses and heats the cooling air (e.g., 2°C in the conditions of the previous example). This disadvantage is overcome when air is sucked from top to bottom by a fan at ground level.

When air is forced through the grain mass, it carries both a "cooling front" and a "moisture front." The temperature front moves rapidly, this speed being governed by the rate of airflow and the temperature of the aeration air. The cooling power of this front is rather independent of the initial temperature of the grain. In well-designed aeration installations, the speed of the moisture front is so slow that wetting problems seldom occur or they are localized in very small regions of the grain bulk.

There are many storage situations where ambient air conditions are not sufficient to cool grain. Nevertheless, aeration with refrigerated air achieves much lower temperatures when ambient conditions are warm. In warm climates, or when very warm grain (35–40°C) is stored immediately after the harvest, aeration with ambient air may not be sufficient to control fungi on moist grain, or to preserve the germination capacity and quality of stored grain. Grain chilling through refrigerated air ventilation is regarded as an expensive method if used only for insect control purposes, but it can be justified for storage of fragile grains such as malting barley and seed grains in hot conditions, when retention of viability is required. Refrigerated air units for chilling grain have been developed to enable aeration to be carried out in summer for temperate regions of Europe and North

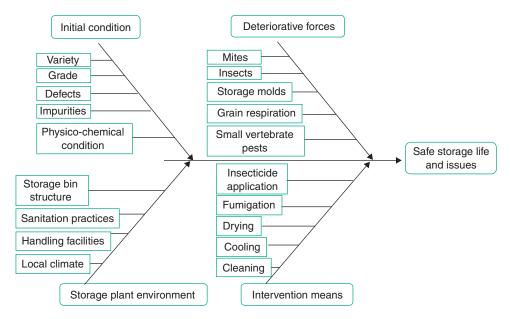


Figure 5 Cause-and-effect diagram (Ishikawa fishbone chart) applied to the stored-grain ecosystem: description of the changes with time and storage conditions of quality criteria of malting barley.

America and in tropical climates where aeration with ambient air is totally impractical.

#### Other Miscellaneous Treatments

#### Mechanical Impact, Turning, and **Pneumatic Conveying**

When grain is warming in a bin, it can be turned (e.g., out-loaded and returned to storage) to help maintain a homogeneous temperature and, if any, to eliminate the "hot spots" or "caked grain" during turning bulk grain from one bin to another. However, at each time grain is moved, there may be a loss due to additional breakage. In addition, when "hot spots" are effectively formed, the fungal spoilage and contaminated grain with mycotoxins can be mixed with the sound part of the grain batch during moving. There is only a slight decrease in the average temperature of grain during turning from bin to bin.

#### **Limitation of Kernel Breakage**

The free fall of grain during empty bin filling or throwing grain through grain thrower in loading vessel holds or flat-bed storage compartments presents some serious risks of kernel breakage. This increase of the percentage of broken kernels, a part of the dockage, can lower the grade and the commercial value of an entire grain batch. The maximum breakage is obtained from drops on a concrete surface inclined 45°, and minimum breakage is observed from drops onto

a grain surface. Grain handling through a bucket elevator is also a source of significant breakage. Reducing grain velocity and impact by different systems reduces physical damage. Drops of less than 12 m, or on a layer of grain reduces the breakage. Increasing the size of the grain stream is also beneficial. Reduced bin height, additional equipment to reduce velocity, and slower handling of grain can reduce kernel breakage hazards.

#### **Future Prospects**

The grain bulk is an ecosystem and, in most cases, stored grain treatments are used as preventive methods in order to prevent the occurrence of biodeterioration processes during the expected storage time (Figure 5). The stored grain quality management more often requires the combination of different methods and approaches to give optimal results. This combination needs to take into account the wholeness of the user's requirements in order to meet his demand, e.g., for residue-free grain or viability-guaranteed seeds after a long storage time. Recently, a qualitative reasoning approach was developed to support storage treatments combination and their logical chaining (see Stored Grain: Pest Management). This new approach should allow the grain handlers to more easily fulfill the buyer's quality expectations and to minimize the costs of stored grain preservation.

See also: Cereals: Grain Defects. Chemicals for Grain Production and Protection. Contaminants of Grain. Food Safety through the Production Chain, Organic Growing of Grains. Plants: Diseases and Pests. Stored Grain: Handling from Farm to Storage Terminal; Invertebrate Pests; Pest Management.

#### **Further Reading**

- Christensen CM (ed.) (1982) Storage of Cereal Grains and their Products. St. Paul, MN: American Association of Cereal Chemists.
- CIRAD-CEEMAT (1988) Conservation des grains en régions chaudes, 546p. Paris, France: La Documentation Française.
- Credland PF, Armitage DM, Bell CH, and Cogan PM (eds.) (2003) Advances in Stored Product Protection. Wallingford, UK: CABI Publishing.
- FAO (1982) Aeration of grain in subtropical climates. Agricultural Services Bulletin No. 51. Rome: FAO (http://www.fao.org/inpho).
- FAO (1983) Processing and storage of food grains by rural families. Agricultural Services Bulletin No. 53. Rome:
- FAO (1985) Manual of Pest Control for Food Security Reserve Grain Stocks. Plant Production and Protection Paper No. 63. Rome: FAO (http://www.fao.org/
- Fleurat-Lessard F (2002) Qualitative reasoning and integrated management of quality of stored grain: a new promising approach. Journal of Stored Products Research 38: 191-218.
- Highley E, Wright EJ, Banks HJ, and Champ BR (eds.) (1994) Proceedings of the 6th International Working Conference on Stored Product Protection, vol. 2, 1274p. Wallingford: CAB international.
- Holman LE (1960) Aeration of Grain in Commercial Storage. US Department of Marketing Service, Research Division, Marketing Research Report No. 178 (http:// www.ext.nodak.edu).
- Jayas DS, White NDG, and Muir WE (eds.) (1995) Stored-Grain Ecosystems, 757p. New York: Marcel Dekker.
- Multon JL (ed.) (1988) Preservation and Storage of Grains, Seeds and Their By-Products. New York: Lavoisier Tec
- Navarro S and Noyes R (eds.) (2001) The Mechanics and Physics of Modern Grain Aeration Management. Boca Raton, FL: CRC Press.
- Niquet G and Lasseran JC (1991) Guide pratique stockage et conservation des grains à la ferme (http:// www.fao.org/Wairdocs/X5163F/X5163F00.htm).

- Pfost HB, Maurer SG, Chung DS, and Milliken GA (1976) Summarizing and reporting equilibrium moisture data for grains. ASAE Paper No. 76-3520.
- Proctor DL (ed.) (1994) Grain Storage Techniques -Evolution and Trends in Developing Countries. FAO Agricultural Services Bulletin No. 109. Rome: FAO.
- Sauer DB (ed.) (1992) Storage of Cereal Grains and their Products, 4th edn. St. Paul, MN: American Association of Cereal Chemists.
- Shejbal J (ed.) (1980) Controlled Atmosphere Storage of Grains, 608p. Amsterdam, The Netherlands: Elsevier.
- Sinha RN (ed.) (1973) Grain Storage: Part of a System, 482p. Westport, NY: The AVI Publishing.
- Sun Da-Wen and Woods JL (1994) The selection of sorption isotherm equations for wheat based on the fitting of available data. Journal of Stored Products Research 30: 27-43.

#### **Relevant Websites**

http://www.fao.org - This website gives technical information on the management of storage operations such as cleaning, drying, and aerating stored grain. It gives online access to a reference book on mycotoxin prevention and control in food grains. It also gives access to a reference book edited by the Group for Assistance on Systems relating to Grain after harvest (GASGA). This website of the Food and Agriculture Organization of United Nations is accessible either in English, French, or Spanish.

http://www.ag.ndsu.nodak.edu - This website of the Extension Service of the North Dakota University (USA) is dealing with the management of grain: drying, handling, and storage.

http://lancaster.unl.edu - This website of the Nebraska University provides updated information about grain storage management (strategies, equipment, engineering, and practices) in the North American environment.

http://www.gov.on.ca - This website of the Ontario Ministry of Agriculture and Food (OMAFRA, Canada) is dealing with stored grain management, and especially insect pests management at the farm grain-store level.

http://www.extension.umn.edu - This website of the Extension Service of the University of Minnesota (USA) gives details on the management of stored grain with aeration.

## **SUNFLOWER**

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Published by Elsevier Ltd.

#### **Overview**

Sunflower (Helianthus annuus) is grown as an oilseed crop worldwide in temperate and subtropical climates. Among oilseeds, sunflower generally ranks fifth behind soybeans, rapeseed, cottonseed, and peanuts, with an average annual world production of 21-27 million metric tons (Mt) (Table 1). Unlike soybean, sunflower is primarily an oil crop, with high protein meal being a by-product. Sunflower is grown on every continent, with Argentina, the former USSR, Eastern Europe, the European Union, and the United States being the largest producers (Table 2). US production accounts for 7–9% of the world output. Nutritionally, sunflower oil has the greater proportion of the unsaturated fatty acids (oleic, linoleic, linolenic), than many other vegetable oils, especially in the recently developed high oleic content NuSun<sup>TM</sup> varieties (Table 3).

#### **History**

Sunflower was domesticated by Native Americans in the eastern US  $\sim 3000$  BC. They used the seeds directly as food and crudely extracted the oil. Native Americans had selected a tall, single-headed variety by the time European explorers reached North America in the sixteenth century. While sunflower was not the staple that the "Three Sisters" (maize, beans, and squash) were, it, nonetheless, was cultivated by many tribes from eastern North America through the Midwest and as far as northern Mexico. The Native American also used sunflower hulls as a source of dye, leaves for herbal medicines, and pollen in religious ceremonies.

Historical records indicate that Spanish were the first to introduce sunflower to Europe in the early 1500s. Sunflower was initially grown as an ornamental plant. Early English and French explorers also introduced it to their respective countries. From western Europe, sunflower spread along the trade routes to Egypt, Afghanistan, India, China, and Russia. By the early 1700s, sunflower seeds were eaten as a snack, and in 1716, the first patent for the use of sunflower oil (for industrial purposes) was filed in England. The most significant

Table 1 World production (Mt) of major oilseed crops since 1997

Commodity	1997/98	1998/99	1999/2000	2000/01	2001/02
Soybean	155	157	155	172	184
Rapeseed	34	37	42	37	36
Cottonseed	34	33	34	33	37
Peanut	29	29	29	31	34
Sunflower	24	26	27	23	21
Palm Kernel	6	6	6	7	7
Coconut	5	5	5	6	5
Total	286	298	298	308	324

Source: www.unitedsoybean.org

Table 2 World sunflower seed production (in 1000 t) since 1997

Country	1997/98	1998/99	1999/2000	2000/01	2001/02
Argentina	5680	7130	5800	2950	3650
Eastern Europe	2179	2594	2754	1657	1767
European Union	4078	3438	3105	3333	3019
China	1176	1465	1765	1954	1750
Former USSR	5412	5737	6890	7368	4979
United States	1668	2393	1970	1608	1551
India	1160	1170	870	730	870
Total	23 891	27 604	26 957	23 110	21 276

Source: www.sunflowernsa.org.

**Table 3** Fatty acid composition of traditional, high oleic, and NuSun<sup>TM</sup> sunflower oil in comparison with other vegetable oils

Сгор	Saturated fatty acids	Unsaturated fatty acids		
	Palmitic and Stearic	Oleic	Linoleic	Linolenic
Sunflower, traditional	12.5	20	66	0.1
Sunflower, high oleic	6.7	80	12	0.1
Sunflower, NuSun	9.5	60	30	0.1
Olive	15	75	9	1
Rapeseed	6	63	20	8.6
Soybean	14	28	50	7
Corn	13	29	57	1

Sources: http://www.who.int/fsf/Documents/Biotech\_Consult\_May2000/Biotech\_00\_10\_tables.pdf and http://www.sunflowernsa.com/pdfs/12.pdf.



**Figure 1** Sunflower field in bloom. (Courtesy of Dr. Brady Vick, USDA Sunflower Unit, Fargo, ND, USA.)

boost for sunflower as a crop, however, came from the Russian Orthodox Church. Lenten regulations prohibited the consumption of many oily foods, but since sunflower was not specifically listed, the seed and oil became a staple diet item in Russia.

Efforts by Russian scientists led to significant crop improvements with oil contents soon exceeding 40%. While sunflower was grown throughout western and eastern Europe, Russia historically was the largest producer with production in excess of 3 million hectares (Mha) in the early twentieth century, compared to 1.5 Mha for the rest of Europe. Russian immigrants are credited with introducing sunflower to North America. In fact, the open-pollinated variety "Russian Mammoth," still sold by garden seed firms, traces its lineage back to the same-named variety initially introduced in the 1880s. Early cultivation of sunflower in North America was primarily for livestock silage and seed for poultry. By the 1950s, improved Russian varieties with oil levels of 45-55% were available. Increased US production of these high-oil sunflower varieties spurred interest by oil crushers, which led to expanded US plantings, especially in the northern Great Plains (North Dakota and Minnesota). The discovery of cytoplasmic male sterility (CMS) by French scientists laid the foundation for the development of sunflower hybrids in the early 1970s. Hybrid sunflower, with higher yields and oil content and more uniformity, in comparison with open-pollinated varieties, provided the last great impetus in establishing sunflower was a worldwide crop (Figure 1).

#### **Botany**

Domesticated sunflower, *Helianthus annuus* L., is the same species as the wild, multiheaded, common annual sunflower found throughout the contiguous



**Figure 2** Common annual sunflower, *Helianthus annuus*, the progenitor of cultivated sunflower, with multiple flowers on a branched stem. (Courtesy of Dr. Brady Vick, USDA Sunflower Unit, Fargo, ND, USA.)

United States, northern Mexico, and southern Canada (Figure 2). The genus *Helianthus* is comprised of  $\sim 50$ species within the tribe Helianthinae of the family Asteraceae. The only other Helianthus species grown commercially is the perennial H. tuberosus, commonly known as Jerusalem artichoke. There are 13 annual *Helianthus* species, all with 2n = 34chromosomes, and 37 perennial species, which may be diploid, tetraploid, or hexaploid. Many species freely hybridize with each other in nature, giving rise to intermediate forms, which has led to confusion in taxonomy. While H. annuus is a geographically diverse species occupying a wide range of habitats, other Helianthus species are very habitat specific and thus are endemic in a limited number of locations. For example, *H. niveus* ssp. *tephrodes* is only found in active sand dunes in southern California and in northern Sonora, Mexico. Helianthus paradoxus is only found in saline, marshy sites in western Texas and New Mexico, and H. exilis is only found in serpentine sites in northern California whose soils are nearly toxic to many nonadapted plants. Wild Helianthus species, both annual and perennial, have contributed many useful traits to domesticated sunflower, including disease resistance, drought and salt tolerance, and improved oil quality. All annual species, except H. agrestis, can be crossed with H. annuus. Of the perennial species, there has been more success in crossing tetraploid and hexaploid species with H. annuus than with the diploid perennial species.

#### **Production Practices in North America**

Sunflower production in the United States is concentrated in the northern Great Plains (North Dakota, South Dakota, Minnesota) with a secondary production area in the central Great Plains (western Kansas

and Nebraska, eastern Colorado) (Table 4), but there is some amount of sunflower grown in each of the 48 contiguous states (especially for birdseed). Most of the production recommendations have come from research conducted in North Dakota, but as interest in sunflower increases in other areas, new research generated from other states addresses these different environments.

Sunflower is traditionally planted between May 1 and the middle of June in the northern Great Plains, with hybrid maturities of 100–120 days to fit the short, 120-day average frost-free growing season of this region. Longer maturing hybrids are used where short growing seasons are not a restriction. Similarly, there are early maturing varieties that would be suitable for a double-crop situation. Double-cropped sunflowers are typically planted in July, either following the failure of an initial crop, or harvesting of a small grain crop. Yields of 2200–3500 kg ha<sup>-1</sup> are attainable under optimal, irrigated conditions, while the average yield of sunflower in the US ranges from 1275 to 1515 kg ha<sup>-1</sup>. The phenology of sunflower hybrid maturation is depicted in Table 5.

Sunflowers are typically planted when soil temperatures reach 10°C at the 10 cm depth. Below 10°C, germination is very slow. Sunflower is generally planted in row widths of 50–75 cm to match the available equipment, although solid seeding (widths <30 cm) is also practiced. Sunflower is planted 4–7 cm deep at rates of 37–62 000 seeds ha<sup>-1</sup> for oilseed, and 35–50 000 seeds ha<sup>-1</sup> for confection. Lower plant populations will produce larger head size and a greater proportion of large seed, which is desirable in confectionery sunflower. Conversely, high plant populations will result in smaller heads, which dry down faster, but also in thinner stalks which are more prone to lodging.

Sunflower has a root system characterized by both a deep taproot and an extensive fibrous root system, which makes it efficient at both water and nutrient uptake. While its water requirements are similar to that of corn, sunflower's greater efficiency can result in higher returns under moisture-limiting conditions. Sunflower responds well to nitrogen fertilization, with a general rule of 5 kg ha<sup>-1</sup> nitrogen required for 100 kg seed ha<sup>-1</sup>. Thus, for a yield target of 2000 kg seed ha<sup>-1</sup>, the soil nitrogen content plus added nitrogen would be 100 kg ha<sup>-1</sup>.

Early weed control is important in sunflower, and several preplant, pre-emergence herbicides are available for grass control, and to a lesser extent for broadleaf weed control. No post-emergence herbicides for broadleaf weed control are currently available. By 2004, hybrids developed through conventional breeding methods will be available with tolerance to both

**Table 4** Sunflower production (ha planted and yield) by state in the United States since 2000

US State	Production (× 1000 ha)		Yield (kg ha <sup>-1</sup> )			
	2000	2001	2002	2000	2001	2002
North Dakota	538	441	554	1543	1605	1493
South Dakota	291	289	259	1705	1582	976
Minnesota	38	24	28	1773	1487	1509
Kansas	101	136	87	1330	1363	1010
Colorado	89	79	53	1077	1285	707
Nebraska	36	33	23	909	1184	617
Texas	24	44	17	875	1313	1050
Other	30	28	24	1167	1414	1290
Total	1150	1074	1046	1503	1515	1273

Source: www.sunflowernsa.org

**Table 5** Sunflower growth stages and approximate time and growing degree days (GDDs) to reach each stage

Sunflower growth stage	Description	GDDs	Days to reach stage
VE	Emergence	93	10
V4	4 true leaves	194	20
V8	8 true leaves	303	28
V12	12 true leaves	383	34
V16	16 true leaves	429	38
V20	20 true leaves	484	44
R1	Miniature terminal bud	511	46
R2	Bud <2 cm from last leaf	695	61
R3	Bud >2 cm from last leaf	774	67
R4	Bud open, ray flowers visible	829	71
R5.1	Early flower	859	73
R5.5	50% flowered	902	77
R6	Flowering complete	989	84
R7	Back of head pale yellow	1140	86
R8	Bracts green, head back yellow	1228	104
R9	Bracts yellow, head back brown	1372	119

GDD = ((max temp in  $^{\circ}$ C) - (min temp in  $^{\circ}$ C)/2) - 10 $^{\circ}$ C Source: NDSU Ext. Bulletin 25. Sunflower Production.

imidazilinone and sulfonylurea herbicides. There are no glyphosphate-resistant hybrids in any country, nor are there currently any other traits introduced into sunflower from other organisms beyond the genus *Helianthus* (i.e., sunflower is currently still a non-GMO crop).

Sunflower in the northern Great Plains typically flowers in August,  $\sim 60-70$  days after planting. Four to five weeks postpollination are required for seed maturation, at which point, seed moisture is 30-40%. If the crop is killed by frost, seeds may be harvested without use of a chemical desiccant. Any conventional grain combine can be used to harvest sunflower, with a variety of attachments to facilitate catching the sunflower heads. Fields are

sometimes harvested with high moisture (i.e., 25%) seed to minimize losses due to birds and/or head rots. Seed moisture must be at 10% or less to retard fungal deterioration in storage, with 8% moisture preferable for storage over summer months.

#### **Pest Problems**

Cultivated sunflower has a number of pest problems, including diseases, insects, and birds. Since the genus Helianthus is indigenous to North America, there is a great diversity of insects and pathogens adapted to sunflower, plus a natural reservoir of these pests on wild Helianthus. In other continents, the diversity of sunflower pests and pathogens is generally less, although international seed movement has dispersed most sunflower pathogens around the globe.

#### **Diseases**

Sunflower is subject to a number of fungal, bacterial, and viral diseases, with fungi being the most numerous and economically serious. Diseases causing the most losses worldwide are Sclerotinia head and stalk rot, *Phomopsis* stem canker, rust, and downy mildew. Some diseases are serious in only a few countries, such as Verticillium wilt in Argentina or white rust (Albugo) in South Africa. Most sunflower diseases are caused by pathogens specific to sunflower, such as Phomopsis helianthi, Alternaria helianthi, and Plasmopara halstedii. Some of the most serious diseases, however, are caused by pathogens with wide host ranges, such as Sclerotinia sclerotiorum.

Disease organisms may be grouped based on plant parts affected. The only significant seedling disease is downy mildew (*Plasmopara halstedii*). Foliar diseases on sunflower include rust (*Puccinia helianthi*), blights caused by Alternaria (five species) and Septoria helianthi, powdery mildew (Erysiphe cichoracearum), bacterial blight (Pseudomonas syringae pv. helianthi), apical chlorosis (P. syringae pv. tagetis), sunflower mosaic virus, and sunflower chlorotic mottle virus. The main stem diseases of sunflower are Sclerotinia stalk rot (S. sclerotiorum), stalk rots caused by S. minor and Sclerotium rolfsii, Phomopsis stem canker (P. helianthi), charcoal rot (Macrophomina phaseolina), and bacterial stalk rot (Erwinia *carotovora*). Head rots are probably the most devastating because of their direct impact upon seed. The main pathogens responsible are Sclerotinia sclerotiorum, Rhizopus (three species), Botrytis cinerea, and Erwinnia carotovora.

Control of most diseases is accomplished primarily with resistant hybrids, and to a lesser extent with cultural practices. Single dominant genes are used to confer resistance to Vertillium wilt, and downy mildew. At least two dominant genes are required for resistance to *Phomopsis* stem canker, while resistance to Sclerotinia head rot and stalk rot is controlled polygenically, making it the most difficult disease to select resistance for. Very few diseases are managed with fungicides, primarily because of economics. Seed treatments for control of downy mildew have been very effective until the pathogen developed resistance to the fungicide metalaxyl. In some countries, foliar fungicides are used to manage Phomopsis and rust. The use of foliar fungicides is more practical with confection sunflower where the profit margin is greater than with oilseeds.

#### Insects

In the US, there is a wide variety of sunflower-specific, native insects that preferentially feed on either stems, leaves, roots, or seeds. Stem feeding insects on sunflower include at least four different cutworms (Euxoa species and Agrotis orthogonia), the sunflower stem weevil (Cylindrocopturus adspersus), the black sunflower stem weevil (Apion occidentale), the sunflower maggot (Strauzia longpennis), the longhorned sunflower stem girdler (Dectes texanus). Root feeding insects include the carrot beetle (Ligyrus gibbosus) and the sunflower root weevil (Baris strenua). Leaf feeding insects include grasshoppers (four Melanoplus species and Camnula pellucida), aphids (primarily Aphis helianthi and Masonaphis masoni), the painted lady caterpillar (Vanessa cardui), and the sunflower betel (Zygogramma exclamationis). Seed and head feeding insects include the sunflower moth (Homeosoma electellum), the sunflower midge (Contarinia schulzi), red sunflower seed weevil (Smicronyx fulvus), gray seed weevil (Smircronyx sordidus), banded sunflower moth (Cochylis hospes), sunflower head moth (Gymnocarena diffusa), sunflower seed maggot (Neotephritis finalis), and the sunflower headclipper weevil (Haplorhynchites aeneus). In other continents, insects are generally considered minimal or infrequent problems, and when they occur, they are generally caused by omnivorous insects such as aphids, plant bugs (Lygus spp.), and other nonsunflower-specific insects. Management of sunflower insect pests usually concentrates on cultural and chemical strategies. To date, no effective insect resistance has been used in cultivated sunflower.

## Birds

Ranging from tiny sparrows (Passeridae) to large parrots (Psittacidae), birds are a constant problem to sunflower on all continents. In commercial birdseed mixtures, sunflower is the preferred seed, so it is not surprising that the crop is beset by depredation by a wide variety of birds. In the US, the migratory red-winged blackbird (*Agelaius phoeniceus*) causes the most damage. In Europe, many different sparrows (*Passer* spp.) and doves (*Streptopelia* spp.) are the major problems, while in South America, parakeets (*Psitticidae*) and doves (*Columbidae*) predominate.

Seed losses due to bird feeding can easily exceed 10% in fields planted close to nesting areas, and losses of 100% are not unheard of. In the US, yield losses due to birds are significant enough to have necessitated the investigation of many different management strategies. There has been limited success in breeding for sunflower varieties that have physical barriers (long necks, tight bracts) to bird feeding. Most research efforts have explored cultural methods, habitat management, and mechanical frightening devices (noise makers). In the past, some growers have resorted to the use of avian poisons, now illegal, but even those efforts proved less than totally effective. One novel approach has resulted in a commercial product that contains the FDA-approved grape flavor component, which ironically many birds find repellant.

#### Sunflower Oil, Processing and Uses

Sunflower is grown worldwide, primarily as an edible oil crop. Sunflower oil is used mainly as a salad oil and frying oil, although it can be hydrogenated for use in margarines. In terms of world production, sunflower usually ranks fourth behind soybean, rapeseed or canola, and cottonseed. The oil content of oilseed sunflower varies from 40% to 50% by weight. There are currently two categories of sunflower oil: traditional and NuSun<sup>TM</sup>. Traditional sunflower oil is characterized by a high concentration of linoleic acid and a moderate amount of oleic acid. Plant breeders, using conventional methods, have selected varieties in which oleic acid is the major fatty acid component, and this oil has been given the tradename of NuSun<sup>TM</sup>. Oleic acid, a mono-unsaturated fatty acid, is considered by health and dietary experts to be better nutritionally than polyunsaturated fatty acids, like linoleic acid, and far superior than saturated fatty acids. While oleic acid levels of 80-90% are attainable, the frying industry prefers an oleic acid content of 55-60%, primarily because of the taste associated with the remnant linoleic acid. For a complete comparison of the fatty acid composition of various vegetable oils, see Table 1.

Oil extraction from sunflower seed is a relatively straightforward process. Seed is first thoroughly dried, de-hulled, and then flaked or rolled, and heated

to  $85-90^{\circ}$ C. The crushed seed is then subjected to one of three extraction methods:

- 1. a mechanical screw press,
- 2. extraction with an organic solvent, usually hexane followed by distillation to remove the solvent, and
- a combination of the screw press and solvent extraction.

In the US, the third method is the preferred method and generally recovers  $\sim$ 99% of the total seed oil. The crude oil is further processed to remove gums, waxes, free fatty acids, and odors. The refined oil is now suitable as a vegetable table oil, or can be hydrogenated and blended with soybean or canola oil for margarines.

Sunflower oil can be used as an alternative or additive to diesel fuel, and thus farmers could decrease their dependence upon petroleum fuels by substituting "farm-grown" fuel. For diesel engine use, sunflower oil requires more extensive purification including removal of waxes and gums. Minor engine modifications, including improved fuel filters, are also necessary to burn any vegetable oil. Since the energy content of sunflower oil is less than that of diesel, fuel consumption would be greater and power output less. As the price of diesel increases and the world supply diminishes, the feasibility of vegetable oil use in diesel engines becomes more of a reality.

#### **Sunflower By-Products**

#### **Sunflower Meal**

After sunflower seeds are crushed, the remaining meal is a high-protein product commonly used in livestock and poultry feeds. The meal contains  $260-500\,\mathrm{g\,kg^{-1}}$  protein,  $120-350\,\mathrm{g\,kg^{-1}}$  fiber (from remnant hulls), and  $10-90\,\mathrm{g\,kg^{-1}}$  fat. Sunflower meal is lower in lysine content and higher in methionine than soybean meal and, thus, is usually combined with soybean meal in livestock feeds. Feeding trials with both dairy and beef cattle have adequately demonstrated the utility of sunflower meal.

#### Hulls

Sunflower hulls comprise 21–30% of the total seed weight and are often a wasted by-product. In oil extraction factories, the hulls are often burned as a source of heat, both for the plant and for the distillation process to remove the hexane solvent. Hulls, mixed with appropriate binders and glue, have also been molded into cylinders and sold as ersatz fireplace logs. Public buildings in the vicinity of processing plants have also used sunflower hulls for heating, with only minor modifications of their boilers.

A red dye, suitable as a FDA-approved food colorant, can be extracted from the hulls of purple-seeded sunflower. With the trend away from the use of synthetic dyes, many foods high in anthocyanin content have been viewed as dye sources. The anthocyanin content of purple-hulled seed ranges from 6 to  $16\,\mathrm{g\,kg^{-1}}$  of hulls, which compares favorably with that of other sources (grape skins, beet pulp). Natural red dyes lack the chemical stability of synthetic dyes, and thus may only be usable in certain applications.

Pectin, used in jellies and as a general food thickener, can be extracted from sunflower heads. The pectin content, after seed removal, is from 150 to 250 g kg<sup>-1</sup>, and thus a hectare of sunflower could yield 200–350 kg pectin. Sunflower pectin is chemically a low-methoxyl pectin, as contrasted to high methoxyl pectins in apples and citrus. Low methoxyl pectins are used in making jellies low in sugar, and thus would be used primarily in diet food market.

Sunflower butter is a relatively new product intended to substitute for peanut butter in situations where peanut allergy is of concern. The product consists of ground, roasted sunflower seed mixed with sugar and other vegetable oils. It was introduced commercially in 2003 in the US.

#### **Confection Sunflower**

Sunflower varieties grown for human consumption are termed confectionery sunflower. The seeds generally roasted, salted, and eaten as a snack food, either "in-shell" or de-hulled to produce "nutmeats." The nutmeats are also used in breakfast cereals, "trail mixes," and in baked goods. Confectionery sunflower comprises 18–22% of US total sunflower production, with the US being the largest producer in the world market, followed by Argentina. Confectionery sunflower is characterized by its larger seed size and by having white or gray stripes on a black or brown background, as compared to uniform black color of oilseed sunflower.

Confectionery seed is graded on the basis of size. The largest seed, referred to as "in-shell," are those passing over a 7.9 mm round-hole screen, and generally make up 15-25% of the harvested seed. The medium-sized seed, destined for de-hulling for use as nutmeats, comprises 30-50% of the crop, and is that fraction recovered between a 7.9 and 7.1 mm round hole screen. The smallest seeds, passing through the 7.1 mm round hole screen, are used primarily as birdseed and comprise 15-20% of the crop.

Confectionery sunflower are genetically unique from oilseed sunflower, primarily because seed size and hull coloration are more important than oil content. As a group, they are much more susceptible to diseases and insects. Since the profit to the grower may be 25–100% greater than with oilseeds, extra management practices to control insects and diseases are more feasible.

#### **Future Trends**

Sunflower production in the US is in direct competition with soybean and rapeseed, which currently have the advantage of glyphosphate-resistance for weed management. Sunflower is also confronted by disease and insect problems that are of lesser concern in soybeans and rapeseed. The challenge to the sunflower industry is to make the crop as easy and profitable to grow as its competitors. The introduction of imidazilinone and sulfonylurea-resistant hybrids will offer growers a tool nearly as good as glyphospate resistance. Finding management schemes to control insects and diseases in sunflower will be the next challenge. Worldwide, Sclerotinia and Phomopsis are the two most devastating diseases. Both diseases can be managed with fungicides, but genetic resistance would be more economical and safer to the environment. While some seed companies are seeking Sclerotinia-tolerance via gene transfer from other crops, there are excellent sources of Sclerotinia-tolerance within the many Helianthus native to North America. Another option to make sunflower more competitive is to change its fatty acid composition to make the oil nutritionally better than other oils. A move in this direction is the introduction of Nusun<sup>TM</sup> hybrids, with oil nearly comparable with olive oil. As the world public becomes more health conscious, the benefits of NuSun<sup>TM</sup> oil should increase demand for sunflower oil and in turn generate more interest among growers. Commitments by large snack food processors and fast-food chain restaurants in the use of NuSun<sup>TM</sup> has been the first step in increasing domestic usage of sunflower oil.

See also: Grain Production and Consumption: Oilseeds in North America. Oilseeds, Overview.

#### **Further Reading**

Berglund DR (1994) Sunflower Production. North Dakota State University Extension Service Bulletin 25. Fargo, ND, 98pp.

Fick GN (1989) Sunflower. In: Robbelen G, Downey RK, and Shri A (eds.) *Oil Crops of the World*, pp. 301–318. New York: McGraw-Hill.

Heiser CB, Jr (1976) *The Sunflower*, 198pp. Norman: University Oklahoma Press.

Heiser CB, Jr, Smith DM, Clevenger SB, and Martin WC, Jr (1969) The North American sunflowers (*Helianthus*). *Memoirs of the Torrey Botanical Club* 22(3): 1–218.

Rogers CE, Thompson TE, and Seiler GJ (1982) Sunflower Species of the United States, 75pp. Bismarck, ND: National Sunflower Association.

Salunkhe DK, Chavan JK, Adsule RN, and Kadam SS (1992) Sunflower. In: World Oilseeds: Chemistry, Technology, and Utilization, pp. 97–139. New York: Van Nostrand.

Schneiter AA (1997) Sunflower Technology and Production. Monograph # 35, 834pp. Madison, WI: American Society of Agronomy.

Seiler GJ (ed.) (1992) Sunflower: special issue. *Field Crops Research* 30(3–4): 448.

Weiss EA (ed.) (2000) Sunflower. In: Oilseed Crops, pp. 205–243. Malden, MA: Blackwell.

#### **Relevant Websites**

http://www.unitedsovbean.org.

http://www.isa.cetiom.fr — International Sunflower Association, Paris, France.

http://www.ext.nodak.edu — North Dakota State University Extension Service, with individual bulletins on many aspects of sunflower production.

http://www.nsasunflower.com — National Sunflower Association, Bismarck, ND, US.

http://www.fargo.ars.usda.gov — US Department of Agriculture — Agricultural Research Service, Sunflower Research Unit. Fargo, ND, US.

http://www.unitedsoybean.org — United Soybean Board, Soybean Almanac.

http://www.who.int - World Health Organization.

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# **TAXONOMIC CLASSIFICATION OF GRAIN SPECIES**

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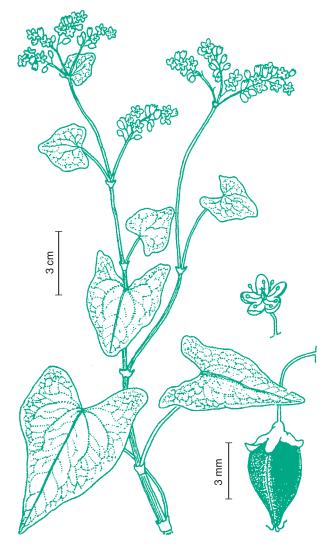
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#### Introduction

The common names of grain species often derive from localized, figurative expressions. They can lead to considerable confusion due to variations in region and language. In English, the problem is exacerbated by the adoption of common names from several languages, either as Anglicized words or direct translations. The grain legume *Cicer arietinum* offers a very good example. In languages of Europe and Asia, it is known as "cece" (Italian), "channa dhal" (Hindi), "garbanzo" (Spanish), "grão-de-bico" (Portuguese: beaked grain), "hummus" (Arabic), "Kichererbse" (German: "giggle peas"), and "pois chiche" (French: chickpea). In English, it is variously known as chickpea, gram, Bengal gram, chana, Egyptian pea, and garbanzo bean. Another problem is the changed usage of a common name from one region to another. In Europe, corn refers to cereal grains generally whereas in the Americas, it specifically refers to maize (*Zea mays*).

# Taxonomy — A System of Names to Avoid Confusion

Common names can often have multiple or misleading associations. If not qualified, pigweed can collectively refer to members of the genus *Amaranthus* without distinguishing between the weedy versus grain amaranth forms. The "wheat" in buckwheat (*Fagopyrum esculentum*; Figure 1) and Inca wheat (*Amaranthus caudatus*: Figure 2) creates the false impression of a relationship with wheat (*Triticum* species: Figure 3), when in fact wheat and these two pseudocereal species are far apart from each other in evolutionary relationships, in biology, and in grain attributes. A more reliable means of communicating is provided by



**Figure 1** Buckwheat (*Fagopyrum esculentum*). Its dried, triangular-shaped fruit, a nut, resembles the beechnut produced by beech trees (*Fagus* spp.). This similarity is the source for the genus name *Fagopyrum*, which translates to "beechwheat." It also explains the derivation of the English common name buckwheat. "Buck-" is the Anglicized version of the German word for beech, "Buche." (Adapted from Williams JT (ed.) (1995) *Cereals and Pseudocereals*. London: Chapman and Hall.)



Figure 2 A mature specimen of *Amaranthus caudatus*, the most important of the Andean grain amaranths. It is still known by the ancient Incan name "kiwicha." Seeds (left) are ∼1 mm in diameter and have a curved embryo. (Reproduced with permission from Vietmeyer ND (ed.) (1989) *Lost Crops of the Incas*. Washington, DC: National Academy Press.)

scientific names, which are assigned by a system known as taxonomy.

A taxonomic name consists of a genus and a species name which, when combined as a binomial, provide a unique identifier for the grain in question. This name is universally recognized in all scientific circles and removes the confusion created by common names, as the following example illustrates. *Amaranthus caudatus*, *A. creuntas*, and *A. hypochondriacus*, form the

group known as grain amaranths, major food staples in the pre-Columbian Americas, which have now regained importance and promise as new pseudocereal grains in international agriculture (*see AMARANTH* and **PSEUDOCEREALS, OVERVIEW**). Their taxonomic names distinguish these grain amaranths from other weedy *Amaranthus* species variously known as pigweed, amaranth, or water hemp.

Although some common names have been accepted into international usage, they can still create problems within a scientific context. For example, in large species groups such as the wheats, scientific names make distinctions where common names cannot. The term wheat does not differentiate between the wild and domesticated species of Triticum, nor between the species of Triticum and its sister genus Aegilops. The common name farro can collectively refer to any one of three hulled wheat species -T. monococcum, a diploid wheat which is a minor crop species; T. dicoccum, a primitive tetraploid wheat also known as emmer; and T. spelta (= T. aestivum ssp. spelta), a hexaploid wheat known as spelt which is still grown in Europe and Asia and now a specialty wheat in the USA and Canada. Common wheat and bread wheat are equivalent names that can encompass a complex of hexaploid wheats when they are classified under one species name, Triticum aestivum, thereby including forms that are neither common nor necessarily amenable to breadmaking (Figure 3) (see WHEAT: Genetics).

#### **Taxonomy – A System of Relationships**

Inherent in taxonomic groupings is an indication of evolutionary relationships. Thus, the taxonomy of grain species has some bearing on plant morphology, grain composition, and function, and many other attributes important to grain science. Unfortunately, taxonomy is not an exact science and is subject to conceptual disagreements and opposing interpretations. Currently, the debate primarily centers on taxonomy's ability to accurately represent phylogeny – the branching ancestor/descendent relationships that connect all plant species. On one side, are molecular systematists, who argue that classifications must reflect evolutionary relationships. On the other side, are traditionalists, who counter that the reliable order and structure of taxonomy is more important than an exact representation of phylogeny. Supporting the traditional view is the fact that phylogenetic classifications are continually subject to change due to the evolving interpretations of new evidence. Another key element of the argument is how to construct a better naming system than the one currently in use. Grain researchers will encounter the effects of



Figure 3 Variations in the appearance of heads of wheat species, one of many morphological characteristics used for their taxonomic classification. From left to right, the wheat species are (including their genome assignments and common names): *T. boeoticum* (2x: wild einkorn), *T. monococcum* (2x: einkorn), *T. dicoccoides* (4x: wild emmer), *T. dicoccoides* (4x: emmer), *T. dicoccoides* (4x: macaroni wheat), *T. carthlicum* (4x: Persian wheat), *T. turgidum* (4x: rivet wheat), *T. polonicum* (4x: Polish wheat), *T. timopheevi* (4x: Timopheevi's wheat), *T. aestivum* (6x: bread wheat), *T. spelta* (6x: spelt wheat), and *T. macha* (6x: macha wheat). The diploid A-genome species, *T. urartu*, is not shown here. 2x = diploid; 4x = tetraploid; 6x = hexaploid. (Adapted from Mangelsdorf PC (1953) Wheat. *Scientific American* 189: 50–59; © 1953 by Scientific American Inc.)

these conflicting views in different taxonomic nomenclature and classification schemes for the same grain species or species groups. While the debate rages, the taxonomic system developed in the eighteenth century by the Swedish scientist Carolus Linnaeus is still intact, flexible enough to have grown with the advances of modern genetics and evolutionary studies.

# Taxonomy – What It Is and How It Started

Taxonomy is a much-misunderstood discipline that is often cast as an outdated exercise. It provides a system by which plants are classified, named, and identified. Classification consists of the circumscription, grouping, and hierarchical ranking of a plant entity (taxon) or entities (taxa). Naming, or nomenclature, is governed by a set of rules laid out in the International Code of Botanical Nomenclature (ICBN) and in the more specialized rules for domesticated plants, the International Code of Nomenclature for Cultivated Plants (ICNCP). Identification, which usually goes handin-hand with nomenclature, is a determination based on similarity of a taxon's membership in an existing group or a new group.

Linnaeus initiated modern taxonomy with the publication of "Species Plantarum" in 1753, which classified every plant then known to him. The Linnaean system was not an isolated endeavor, but it was built on a long history of scientific attempts, beginning with the Greek Theophrastus (third century BC) and the Roman Pliny the Elder (first century AD), to construct universal naming and classification systems. The Linnaean binomial system, consisting of genus and species names, resolved the problem of concisely and uniquely naming a plant. Continuing with the scientific heritage that preceded him, Linnaeus used Latin as the language of taxonomic names and descriptions. In his time, Latin was the universal language by which European scholars could communicate. Linnaeus took classical Latin and created a new botanical form with a specialized terminology for plant structures and the flexibility for adopting Latinized words taken from other languages. Modern attempts to replace botanical Latin with English for taxonomic descriptions have so far been unsuccessful, leaving this particular Linnaean heritage intact.

#### **Origins of Taxonomic Names**

Many Linnaean names for agricultural plants have their roots in the vernacular of the ancient Greeks and Romans. For example, the genus names for grains were derived from names that often related to a particular function, activity, or trait. In other cases, common names developed out of an association with a family or person. Thus, Triticum comes from the Latin "tero" meaning "I thresh"; "Cicer," which translates to chickpea, has its origin in the Greek "kiros," referring to the Roman family "Cicero;" cereale is taken from "Ceres" the Roman goddess of agriculture in a broad sense and of cereal grain in a more narrow one. In the original Linnaean system, species names usually conveyed information about a trait so that when combined with the genus name, they produced an identifying designation for the taxa in question as these examples illustrate. Amaranthus caudatus translates as the "amaranth ending with a tail-like appendage," a reference to this species' long, tail-like inflorescence (Figure 2); Cicer arietinum as "chickpea shaped like a ram's head," a reference to the kabuli type of chickpea; Fagopyrum esculentum as "edible buckwheat" (Figure 1); Triticum monococcum as "one-grained wheat" (Figure 3); Secale cereale as "cereal rye."

For species named by Linneaus, the intertwining of scientific names with the original vernacular helps to explain their respective derivations. The origin of the term common wheat hails back to an older, no longer accepted, species name T. vulgare, which translates as "common wheat." The use and derivation of corn and the genus name, Zea, provide another example. In Europe, corn refers generally to cereal grains, or the seed itself, whereas in the Americas, it specifically denotes the species Zea mays. The English word corn has its roots in the German word for grain, "korn." The Greek word "zea," like corn, referred to grain in a general sense. Linnaeus selected Zea as a genus name for the new cereal-grain species that had arrived in Europe following the discovery of America and for which there was no European vernacular name upon which to draw. As a consequence, the English common name corn was adopted into the American English vernacular to refer to the only major New World cereal grain known in the eighteenth century.

#### The Use of Genus and Species Names

**Tables 1** and **2** list common and scientific names for grain species. The usual convention is to write both genus and species names in italics, with the genus name having a capital as the first letter, followed by the species name (known as the specific epithet) all in lower case. When a grain consists of a group of species, the plural abbreviation "spp." is used. In text, the genus name component of the binomial is spelled out the first time and abbreviated to its first letter, except when beginning a sentence, for each following use as was done above with *Amaranthus* and *Triticum*.

**Table 1** Classification of the grass family, Poaceae (Gramineae), all monocotyledonous plants: cereal grain species and their common names<sup>a</sup>

Subfamily	Tribe	Genus and species	Common name
Bambusoideae	Oryzeae	Oryza glaberrima	African rice
(Ehrhartoideae) <sup>b</sup>		Oryza sativa	Rice
		Zizania aquatica	Wild rice
		Zizania palustris	Northern wild rice
Chloroidoideae	Eragrostideae	Eleusine coracana	Finger millet, African millet, Indian millet
		Eragrostis tef	Tef, teff, teff grass
Panicoideae	Andropogoneae	Sorghum bicolor	Sorghum, grain sorghum, great millet, Kaffir-corn, durra, milo
		Zea mays	Corn, maize
		Coix lacryma-jobi	Coix, Job's tears, Adlay millet
	Paniceae	Digitaria exilis	Fonio, fonio millet, hungry-rice
		Echinochloa esculenta	Japanese barnyard millet, Japanese millet, Siberian, or marsh millet
		Panicum miliaceum	Common millet, broom millet, broomcorn millet, proso millet
		Panicum sumatrense	Blue panic, little millet, sama
		Paspalum scrobiculatum	Kodo millet
		Pennisetum glaucum	Pearl millet, cattail millet
		Setaria italica	Foxtail millet, Italian millet
Pooideae	Agrostideae (Poideae) <sup>b</sup>	Phalaris aquatica	Phalaris, Harding grass, towoomba Canary grass
	Aveneae (Poideae) <sup>b</sup>	Avena sativa	Oat, side oat
	,	Avena abyssinnica	Abyssinian oat
	Triticeae	Hordeum vulgare	Barley
		Secale cereale	Rye, cereal rye
		× <i>Triticosecale</i> sp. <sup>c</sup>	Triticale
		Triticum aestivum	Wheat, common wheat, bread wheat
		Triticum dicoccum	Emmer, farro
		Triticum durum	Durum wheat, hard wheat, macaroni wheat
		Triticum monococcum	Einkorn, small spelt, farro
		Triticum spelta	Spelt, spelt wheat, hulled wheat, dinkel wheat, farro
		Triticum turgidum	Rivet wheat, cone wheat

<sup>&</sup>lt;sup>a</sup> Following the classification system outlined in Heywood VH (1993) Flowering Plants of the World. Oxford, UK: Oxford University Press.

In cases where two genera with the same first letter are discussed together, then the second letter of the species name is included in the abbreviation, e.g., in discussions of the millets, *Pa.* for *Panicum* and *Pe.* for *Pennisetum*. Authority names (usually abbreviated, and not in italics) are not included in **Tables 1** and 2 as they are not essential for the completeness of the binomial. However, it is useful in research articles and literature reports to include the authority citation at the first mention of the species as a way of clarifying which taxonomic concept is being followed.

The authority refers to the botanist or researcher who named the species, e.g., "L." for Linnaeus in *T. monococcum* L.; "Desf." for R L Desfontaines in *T. durum* Desf. Authorities are important for synonymy, the component of taxonomy that deals with the historical naming of a species. When the name and classification of a grain species is in question

due to competing treatments, synonymy provides a way to trace its taxonomic handling and to determine which is the correct and legitimate name according to the ICBN. In dealing with synonymy, knowing the authority is critical.

#### Taxonomy – Rank and Hierarchy

In classification, taxonomy provides a system for grouping species into ranked hierarchies. All grain species are flowering plants and thus are classified together in the class Angiospermae. Members of this class reproduce sexually with the male and female organs located in flowers, which can be showy (pseudocereals and pulses) or inconspicuous (cereals). Their seeds are protected by a fruit, which can be fleshy or dried. In the case of cereals, the fruit is reduced to a thin outer tissue that is adherent to the seed. In botanical

<sup>&</sup>lt;sup>b</sup> Alternative subfamily and tribal classification assignment according to the Grass Phylogeny Working Group (GPWG).

<sup>&</sup>lt;sup>c</sup>Triticale is an intergeneric hybrid that was created by a cross between wheat and cereal rye. The × preceding its genus name, which combines *Triticum* and *Secale*, indicates its hybrid origin. The "sp." (one species) is required because no species name has been assigned.

**Table 2** Dicotyledonous grain and oilseed species and their common names<sup>a</sup>

Family	Tribe	Genus and species	Common name
Amaranthaceae	Amaranthoideae	Amaranthus caudatus	Amaranth, grain amaranth, Inca-wheat
		Amaranthus cruentas	Purple amaranth, red amaranth
		Amaranthus hypochondriacus	Prince's feather
Asteraceae (Compositae)	Cardueae	Carthamus tinctorius	Safflower
	Heliantheae	Guizotia abysinnica	Niger, niger seed
		Helianthus annus	Sunflower
Brassicaceae (Cruciferae)	Brassiceae	Brassica napus	Canola, oilseed rape, rape
		Brassica spp.	Mustard
		Crambe abysinnica	Crambe
Chenopodiaceae	Cyclobeae	Chenopodium quinoa	Quinoa
Fabaceae (Leguminosae)	Aeschynomeneae	Arachis hypogaea	Peanut, groundnut, gooper
	Cicereae	Cicer arietinum	Chickpea, garbanzo bean, gram
	Genisteae	Lupinus albus	White lupin
		Lupinus angustifolius	Blue lupin
	Phaseoleae	Glycine max	Soybean
		Phaseolus lunatus	Lima bean, butter bean
		Phaseolus vulgaris	Bean, kidney bean, pinto bean, navy bean, cannellini bean
		Vigna angularis	Adzuki-bean
		Vigna radiata	Mung bean, golden gram, green gram
		Vigna unguiculata	Black-eyed pea, cowpea
	Vicieae	Lathyrus sativus	Chickling pea, chickling vetch
		Lens esculentus	Lentil
		Pisum sativa	Garden pea, field pea
		Vicia faba	Faba bean, broad bean
Linaceae		Linum usitatissimum	Linseed, flax
Malvaceae	Hibisceae	Gossypium spp.	Cottonseed
Pedilaceae		Sesamum indicum	Sesame, sesame seed
Polygonaceae		Fagopyrum esculentum	Buckwheat, Japanese buckwheat, silverhull buckwheat

<sup>&</sup>lt;sup>a</sup> Following the classification system outlined in Heywood VH (1993) Flowering Plants of the World. Oxford, UK: Oxford University Press.

terminology, this unusual fruiting structure of the cereals is called a caryopsis.

The most useful grouping at this level of the hierarchical ranking system divides grain species into two evolutionary lines known as the monocots (subclass Monocotyledoneae) and dicots (subclass Dicotyledoneae) (Table 3). Monocot and dicot refer to the presence of one or two embryonic leaves (cotyledons) in the seed and young seedling. Other easily identifiable morphological traits that differentiate between members of these two subclasses include flower parts in threes, parallel-veined leaves, scattered vascular bundles, and fibrous adventitious root system for monocots; flower parts in fours or fives, net-veined leaves, vascular bundles located in a ring, and a primary tap root for dicots. It is noteworthy that botanists often disagree on the higher rank categories (e.g., division Anthophyta versus class Angiospermae; class Monocotyledones versus subclass Monocotyledoneae) and particularly on classification at the order level. Thus, Tables 1-3 reflect one of several possible higher-order classifications.

The taxonomic rank family is the most useful of the higher ranks. Family names end in "-aceae" except

for several older names that were in use before the ICBN standardized the suffix spelling. Thus, in grain taxonomy, two family names are occasionally encountered – Gramineae (old) and Poaceae (new); Leguminosae (old) and Fabaceae (new); Cruciferae (old) and Brassicaceae (new); Compositae (old) and Asteraceae (new). The other useful higher taxonomic ranking is tribe, a subdivision of family, which is identified by an "-eae" ending. All the cereal grains (e.g., maize, rice, sorghum, wheat) are monocots and belong to the grass family, Poaceae. On the other hand, the group of dicot grains and oil seeds comprises a diverse mixture of families. The pulses or grain legumes (e.g., beans, peas, soybeans), which are the largest group of dicot grains, are classified in the family, Fabaceae. Other families include one or just a few dicot grain or oil species, e.g., Amaranthaceae (grain amaranths), Polygonaceae (buckwheat), Asteraceae (safflower and sunflower), Malvaceae (cottonseed), and Brassicaceae (canola). Subdivisions of these families into subfamilies and/or tribes are reflections on the size of the family and the necessity for subgroupings within it.

Grain species can be subdivided into infraspecific categories known as subspecies (ssp.), variety (var.),

Table 3 Hierarchical classification<sup>a</sup> from subclass to genus for grain and oilseed species with their use-category assignments

Subclass and superorder	Order	Family	Subfamily	Tribe	Genus	Use category
Monocotyledone	eae					
Commelinidae	Poales	Poaceae	Bambusoideae (Ehrhartoideae) <sup>b</sup>	Oryzeae	Oryza	Cereal
					Zizania	Cereal
			Chloridoideae	Eragrostideae	Eleusine	Cereal
					Eragrostis	Cereal
			Panicoideae	Andropogoneae	Coix	Cereal
					Sorghum	Cereal
					Zea	Cereal
				Paniceae	Digitaria	Cereal
					Echinochloa	Cereal
					Panicum	Cereal
					Paspalum	Cereal
					Pennisetum	Cereal
				,	Setaria	Cereal
			Pooideae	Agrostideae (Poeae) <sup>b</sup>	Phalaris	Cereal
				Aveneae (Poeae) <sup>b</sup>	Avena	Cereal
				Triticeae	Hordeum	Cereal
					Secale	Cereal
					× Triticosecale	Cereal
					Triticum	Cereal
Dicotyledoneae						
Asteridae	Asterales	Asteraceae	Lactucoideae	Cardueae	Carthamus	Oil seed
			Asteroideae	Heliantheae	Guizotia	Oil seed
					Helianthus	Oil seed
	Scrophulariales	Pedaliceae			Sesamum	Oil seed
Caryphyllidae	Caryophyllales	Amaranthaceae	Amaranthoideae		Amaranthus	Pseudocereal
		Chenopodiaceae		Cyclobeae	Chenopodium	Pseudocereal
	Polygonales	Polygonaceae			Fagopyrum	Pseudocereal
Dilleniidae	Malvales	Malvaceae			Gossypium	Oil seed
Rosidae	Fabales	Fabaceae	Papilionoideae	Aeschynomeneae	Arachis	Pulse, oil seed
				Cicereae	Cicer	Pulse
				Genisteae	Lupinus	Pulse
				Phaseoleae	Glycine	Pulse, oil seed
					Phaseolus	Pulse
					Vigna	Pulse
					Linum	Oil seed
				Vicieae	Lathyrus	Pulse
					Lens	Pulse
					Pisum	Pulse
	0	Donation		Description	Vicia	Pulse
	Geraniales	Brassicaceae		Brassiceae	Brassica	Oil seed
		1 Section 1			Crambe	Oil seed
		Linaceae			Linum	Oil seed

<sup>&</sup>lt;sup>a</sup> Following the classification system outlined in Heywood VH (1993) Flowering Plants of the World. Oxford, UK: Oxford University Press.

and forma (f.). Because there is no clear agreement on subspecies and variety, the two are often used interchangeably, although it is possible to treat variety as a subrank to subspecies. When the subspecies (or variety) is the taxonomic type that defines a particular species, then the ICBN requires that this subspecies (or variety) repeats the species name — e.g., *T. aestivum* ssp. *aestivum*. In general discussions, it is not necessary to include the repetitious infraspecific form. Thus, in this example, it is

sufficient to cite the species name *T. aestivum* when referring to bread wheat. Forma is the smallest category and is infrequently encountered.

The infraspecific ranks are intended to describe variants that form distinct groups defined by genetics, morphology, ecology, and distribution. How a particular taxonomist chooses to classify infraspecific variation often appears arbitrary. For example, a recent taxonomic treatment of the wheats uses variety for the wild wheat genus *Aegilops* 

<sup>&</sup>lt;sup>b</sup> Alternative subfamily and tribal classification assignment following the Grass Phylogeny Working Group (GPWG) revision.

(e.g., A. speltoides var. ligustica) but subspecies for the domesticated genus Triticum (e.g., T. turgidum ssp. durum). Here, the infraspecific ranks should be viewed as approximately equivalent. This example also illustrates the difficulty in reflecting evolutionary relationships within the framework of taxonomic classification. The infraspecific variation in domesticated Triticum species is the product of human selection pressure, which is arguably on a different evolutionary scale than the variation in the wild species of Aegilops. Therefore, the taxonomist's choice of variety for Aegilops and subspecies for Triticum should not be construed as a necessarily accurate representation of evolutionary status.

#### **Taxonomic Disagreements**

With taxonomic classification, there is not necessarily a right or a wrong treatment of a group. Taxonomists and the researchers working with a particular group often disagree. As a consequence, different names for the same species can be found in use. For those who are not schooled in the synonymy of the group in question or unaware of the details of the controversy, conflicting taxonomic treatments are confusing. In the case of grain species, taxonomic debates usually deal with both the status of cultivated taxa (usually species status) and the relative importance of genetic evidence in defining species concepts. There is no clear separation of these two points, partly due to the historical tradition on which taxonomy has been built and the inconsistencies in how domesticated plants are viewed within an evolutionary context. The opposing species concepts evident in the handling of the wheats throughout the encyclopedia offer an excellent example of the dilemmas found within a taxonomic debate. Morphological diversity in the tetraploid and hexaploid wheats illustrates the potential for variation that developed under the direction of humans acting as evolutionary selection agents. This variation originally correlated with eco-geographical distributions and if allowed to continue in isolation over geologic time would have possibly led to new species. Wheat geneticists stress the need to portray accurate genetic relationships, circumscribing wheat species on the basis of their close genetic similarities. Thus, in the AB-, ABD-genome groups, geneticists accept only two species, each with several infraspecific forms -T. turgidum and T. aestivum. On the other side of the fence, are those who stress the importance of recognizing the variation of domesticated forms by naming many tetraploid and hexaploid wheat species (Table 1). Provided that the rules of nomenclature are followed, differing treatment concepts are acceptable. On this basis, T. spelta and T. aestivum ssp. spelta are both correct as are *T. durum* and *T. turgidum* ssp. *durum*, respectively describing the same wheats, albeit from different taxonomic perspectives.

#### **Cultivated Plant Taxonomy**

Another difficulty in infraspecific taxonomy is the confusion over variety and cultivar. In the realm of cultivated plant taxonomy, variety and cultivar have been used as equivalent terms. As in the case of subspecies and variety, there is no consensus. However, it is important to make the distinction between the botanical use of variety (*varietas*) as an infraspecific rank and the horticultural (i.e., domesticated plant) use of variety to describe a domesticated plant variant. Unfortunately, the ICNCP lends some confusion to this issue because it treats variety and cultivar as synonymous but with the qualification that a botanical variety is a taxonomic entity whereas a cultivated plant variety is not.

A domesticated variety or cultivar has a fancy name not a Latinized botanical name that is italicized. At their first mention, cultivar names are enclosed by single quotation marks. When mentioned alone without their Latin binomial, they can be preceded by cultivar or its abbreviation "cv." A good rule of thumb is to use variety within the botanical context as a term that is interchangeable with subspecies and to reserve cultivar for describing a named domesticated variant. The derivation of cultivar should help to maintain this distinction because it is formed from "cultivated variety" by combining the first five and three letters respectively. Several examples should help to clarify this usage – Zea mays "Bronze Beauty" or maize cultivar "Bronze Beauty"; Lens culinaris "Laird"; Avena sativum "Wallaroo" Arachis hypogaea ssp. fastigiata "Georgia Red"; Amaranthus cruentus "K343"; Cicer arietinum "Kranthi."

The term landrace is often encountered in discussions of crop germplasm. It refers to an indigenous grain species within a local farming system. Landraces predate modern agriculture and often are ancient forms that may have been in cultivation for hundreds or thousands of years. Landraces are usually composed of a mixed population of several forms that vary by agronomic traits such as disease resistance, maturity, yield, and cold tolerance. The mixture also may include different species or morphological forms. For example, wheat landraces collected during the late 1940s in southeastern Turkey can be composed of 10 or more different types of T. aestivum (=T. aestivum ssp. aestivum) and T. durum (=T. turgidum ssp. durum). The advantage of landraces in subsistence farming systems is the guarantee of a crop under all types of growing conditions. Thus, if the farmer faces a particularly bad disease year, the

resistant types of the landrace will survive and yield a harvestable grain. Unfortunately, a clear-cut distinction between landrace and cultivar is not always maintained. It is not uncommon for a landrace to be identified as a cultivar by one genebank and as a landrace by another. The vernacular names for landraces do not have taxonomic standing.

#### Taxonomy - In Use

As a standardized system for classification of plants, taxonomy provides a useful way for tracing the origins of grain species. By knowing the names of wild progenitors and related domesticated species, researchers can make informed decisions when selecting material for study or for breeding programs. Likewise, knowing the taxonomic characters by which species are classified enables researchers to verify the identity of research material. It is possible for genebank and research collections to hold and distribute mislabeled or misidentified wild and domesticated germplasm.

The wheats offer a practical example of the importance of taxonomy in the selection and use of research material. Because there are several conflicting taxonomic concepts for the wild and domesticated wheats, a seed request may not be filled with the species actually requested. A general, unspecified request for accessions of the diploid A-genome progenitor of tetraploid and hexaploid wheat species may be incorrectly filled with either T. boeoticum or T. monococcum. While the wild T. boeoticum and domesticated T. monococcum both have a form of the A-genome, neither was involved in the evolution of T. durum (AB genomes) and T. aestivum (ABD genomes) (Figure 3). The correct species for this request would be T. urartu. Since T. urartu and T. boeoticum are morphologically very similar to each other, a researcher, who is unfamiliar with the taxonomic characters that differentiate them, may erroneously mistake T. boeoticum for T. urartu.

#### **Taxonomy and Allergies**

Taxonomic relationships have a practical application for identifying potential allergic cross-reactions. For example, a person with a proven allergic reaction to wheat grain would be more likely to have similar problems with rye but less with the more distantly related cereal grain species such as rice or maize (Table 1). This consideration is particularly relevant to celiac disease (*see* CELIAC DISEASE), for which the prime cause is the gluten proteins found in wheat. Celiacs are also warned to avoid eating the grain of barley, rye, and triticale, closely related species in the tribe Triticeae. Although oats, which are classified in

tribe Aveneae, are considered to be less harmful for celiacs, some such individuals find that they must avoid them. On the other hand, celiacs may eat foods based on other cereal grains, which are classified in more distantly related tribes (e.g., maize, millet, rice, sorghum) as well as any of the dicot grains. Within the context of taxonomy, the possible cross-reaction with oats but not with maize, millet, rice, and sorghum is understandable. The tribes Triticeae and Aveneae are located in the same subfamily, the Pooideae. On the other hand, maize, millet, and sorghum are classified in the subfamily Panicoideae and rice in the subfamily Bambusoideae.

Attempts at prediction of allergic cross-reaction may not be valid if making comparisons with respect to different anatomical parts of the plant and different routes into the body – for example, the inhalation of pollen versus the ingestion of grain. It may thus be possible for a person, who is allergic to wheat pollen, to suffer no adverse reactions to the wheat grain. However, there is at least anecdotal evidence that individuals, who experience mild ingestion intolerance to cereal grains, may have these symptoms exacerbated in pollen season. Also, allergic cross-reactions are observed between pollens of different species, implying that sensitization by one species can confer allergy to others. Thus, a positive test reaction to pollen from one species does not necessarily mean that it is the one causing clinical symptoms, nor need it have been the one responsible for the original sensitization. The real culprit may be another, cross-reacting pollen. Here, taxonomy offers a practical tool for elucidating such situations. For the monocot grains, only the grass family Poaceae is necessary to consider. Within the very diverse group of dicot grains, pseudocereals, and oil seeds, the higher taxonomic rankings of Super Order and Order are of practical use for evaluating allergic cross-reactions. In providing only a brief introduction into the taxonomy of grain species, Table 3 illustrates the hierarchical relationships that should be considered in dealing with potential cross-reactions.

#### **Future Trends in Taxonomy**

As is evident in the above discussion, taxonomy is not an exact science but is subject to conflicting, and often changing, interpretations. When taxonomic disagreements prevail, the focus shifts away from taxonomy's real value for aiding communication among scientists. In the case of the wheats, a 50-year-old controversy over how to classify wild and domesticated taxa by evolutionary relationships has created a confusing situation with many different classifications and no consensus for names or species concepts. This controversy illustrates the possible problems that

lie ahead should the current trend continue among many leading molecular systematists to restructure traditional taxonomy into a "natural" system that accurately displays phylogenetic relationships (cf., Grass Phylogeny Working Group website below). Because concepts of plant phylogeny are changing rapidly with the sophisticated tools of molecular biology, an attempt to structure taxonomy around evolutionary concepts may lead to a situation of constant taxonomic flux. Traditionalists argue that taxonomy should continue as it now stands guided by the ICBN. Molecular systematists suggest other naming systems and other ways of classifying, which could lead to a re-invention of taxonomy. Suggestions for two systems have been proposed. In this scenario, the traditional Linnaean system would remain intact and another separate system, which would be designed to accommodate to changing concepts, would be developed for phylogenetic reconstruction.

See also: Amaranth. Barley: Genetics and Breeding. Buckwheat. Canola: Genetics and Breeding. Cereals: Evolution of Species. Maize: Genetics. Oilseeds, Overview. Rice: Genetics. Wheat: Genetics.

#### **Further Reading**

Dahlgren RMT (1997) A revised system of classification of the angiosperms. *Botanical Journal of Linnean Society* 80: 615–626.

Greuter W, McNeill J, Barrie FR, Burdet HM, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, and Hawksworth DL (eds.) (2000) International Code of Botanical Nomenclature (St Louis Code). Regnum Vegetabile 131. Königstein, Germany: Koeltz Scientific Books.

Heywood VH (1993) Flowering Plants of the World. Oxford, UK: Oxford University Press.

Judd WS, Campbell CS, Kellogg KA, and Stevens PF (1999) Plant Systematics: A Phylogenetic Approach. Sunderland, MA: Sinauer Associates.

Mabberley DJ (1997) *The Plant-Book: A Portable Dictionary of the Higher Plants*, 2nd edn. Cambridge: Cambridge University Press.

Mangelsdorf PC (1953) Wheat. Scientific American 189: 50-59.

Stuessy TF (1990) Plant Taxonomy: The Systematic Evaluation of Comparative Data. NY: Columbia University Press.

Trehane P, Brickell CD, Baum BR, Hetterscheid WLA, Leslie AC, McNeill J, Spongberg SA, and Vrugtman F (eds.) (1995) *International Code of Nomenclature for Cultivated Plants*. Regnum Vegetabile 133. Wimborne, UK: Quarterjack Publishing.

Vietmeyer ND (ed.) (1989) Lost Crops of the Incas. Washington, DC: National Academy Press.

Wiersema JH and León B (1999) World Economic Plants: A Standard Reference. Boca Raton, FL: CRC Press.

Williams JT (ed.) (1995) Cereals and Pseudocereals. London: Chapman and Hall.

#### **Relevant Websites**

http://www.bgbm.org — This online version for the most recent edition of the ICBN, which is known as the St. Louis Code, is in English or Slovak.

http://biodiversity.uno.edu — The Biodiversity and Biological Collections Server is a comprehensive source for locating many useful taxonomy and biodiversity resources.

http://www.eti.uva.nl — The Expert Center for Taxonomic Identification (ETI) is an on-line taxonomic database service supported by an international consortium. The site hosts a searchable taxonomic database for taxonomic hierarchies, species names, synonyms, and descriptions.

http://www.ars-grin.gov — The United States Department of Agriculture website for GRIN (Germplasm Resources Information Network) which is set up for taxonomic queries on holdings of economic plants and with links to other relevant websites for the taxonomy of economic plants. Economic plant listings include vernacular names and economic uses. Information on this website is drawn from "World Economic Plants: A Standard Reference."

http://gmr.landfood.unimelb.edu.au — The Multilingual Multiscript Plant Name Database has a searchable database for locating scientific and vernacular names in all applicable languages for a particular grain species.

http://www.ksu.edu — This website contains a comprehensive listing of all the historical and current taxonomic treatments of the wheats under the subject heading of *Taxonomy of the Triticeae*. http://biodiversity.soton.ac.uk — Location for the International Legume Database and Information Service (ILDIS), this website has a searchable database for species information about the grain pulses. http://www.virtualherbarium.org — The Grass Phylogeny Working Group (GPWG) is a recently established collaborative group of systematic botanists who are reexamining the phylogeny of the grass family. Information about their project and publications are posted here.

### TEFF

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#### Introduction

Teff [Eragrostis tef (Zucc.) Trotter], commonly known as "tef," is a tropical cereal. Teff cultivation and consumption as human food is mostly confined to Ethiopia. This article highlights the origin, cultivation of the teff plant, production, and storage of teff grain. Teff grain structure, anatomy, chemical composition, and physico-chemical properties of the major chemical components are assessed and described. Teff milling, processing of injera (the staple food of the majority of Ethiopians, a fermented, pancake-like, soft, spongy, sour, circular flatbread), and other food products made from teff are described. Finally, its potential, grain production limitations, and recommendations for future research to improve the teff crop and its grain utilization are enumerated.

#### **Teff Crop Description and Cultivation**

#### **Description**

Teff is a  $C_4$  self-pollinated tetraploid cereal plant with a chromosome number of 2n = 4x = 40. Teff is also an allotetraploid plant. The teff plant and panicles of some varieties are shown in Figure 1. Teff's root system is fibrous and most stems are erect, while others are bending or elbowing types. It has a panicle type of inflorescence showing different forms, from loose to compact. Its spikelets have 2-12 florets. Each floret has a lemma, palea, three stamens, mostly two ovaries (in some exceptional cases three), and feathery stigmas. In most varieties, the plant height is 50-120 cm. A single teff plant can produce up to  $50\,000$  grains.

#### Name and Origin

Teff [Eragrostis tef (Zucc.) Trotter] belongs to the family of Poaceae, subfamily Eragrostoidae, tribe Eragrosteae, and genus Eragrostis. About 300 species are known in the genus Eragrostis, of which teff is the only cultivated species. Chloridoideae is used synonymously for Eragrostoidae of teff. Vernacular names in different parts of the world are as follows:

- Tahf: Arabic,
- Tef, teff, Williams lovegrass: English,

- Xaafi, tafi, taafi: Oromo (O)/Afar/Sodo, tafe-e: Had, t'ef, teff, taf: Amarinya (A), Tigrinya (T): Ethiopian languages,
- Mil èthiopien: French, and
- Chimanganga, ndzungula (Ch), chidzanjala (Lo): Malawi.

Teff is indigenous to Ethiopia. Ethiopia is also considered the leading world center for teff genetic diversity. Records indicate that by 1997, the Ethiopian Biodiversity Institute had conserved some 3842 accessions of teff for varietal improvement study and to reduce genetic erosions. The exact details on teff domestication are unclear. However, teff is believed to have been first domesticated by the pre-Semitic inhabitants of Ethiopia and is assumed to have originated in northeastern Africa.

Even though teff is an allotetraploid plant, to date, its diploid putative ancestors are not exactly established. But, based on the morphological data and cytological evidence, the following species have been suggested as the ancestors and contributors to teff origin:

- Eragrostis. aethiopica, E. atrovirens, E. longifolia, E. macilenta, E. pilosa, and E. psedudo tef as ancestor species of teff.
- E. aethiopica, E. bicolor, E. cilianensis, E. curvula, E. pilosa, and E. mexicana as contributor species to the origin of teff.
- E. aethiopica, E. barrelieri, E. bicolor, E. cilianensis, E. heteromera, E. mexicana, E. minor, E. papposa, and E. pilosa as very closely related species to teff.
- E. aethiopica 2x, E. barrelieri 6x, E. cilianensis 2x, 4x, 6x, E. mexicana 6x, E. minor 2x, 4x, and E. pilosa 2x based on cytological evidence as closely related species to teff.

An attempted interspecific cross between teff and some wild *Eragrostis* species (*E. curvula*, *E. cilianensis* (4x), *E. pilosa* (4x), and *E. minor*) was not successful. However, it was recently reported that *E. tef* and *E. pilosa* can be crossed with fertile offspring, suggesting that *E. pilosa* or an ancestor closely related to *E. pilosa* is the most probable putative ancestor of teff.

#### **Cultivation and Production**

Teff is one of the major cereals in Ethiopia, comprising  $\sim$ 20% of cereal production. The annual production in Ethiopia is estimated to be  $\sim$ 2 × 10<sup>6</sup> t. In Idaho, USA some teff grain is produced for the health-food market

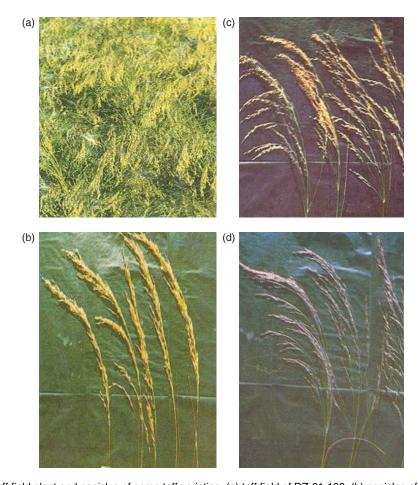
and for *injera* making. In South Africa, teff is widely grown as a fodder crop during the summer-rainfall season. However, the production of a combination of grain and fodder varieties has been introduced only recently. In Australia, India, and Kenya, it is cultivated as a forage crop. Teff can adapt to a wide range of environments, i.e., moisture stress, high rainfall, different soil types, and a wide range of altitudes from near sea level to over 3000 m. However, the best conditions are 1800–2100 m above sea level, a temperature range of 10–27°C during the cultivation period, an annual rainfall of 750–850 mm, and rainfall of 450–550 mm during the growing season.

Teff is known to have fewer disease and pest problems in the field as compared to maize, sorghum, wheat, and barley. However, the productivity is low. The average national yield in Ethiopia is 9.4 quintal per hectare (q ha<sup>-1</sup>). Lack of high-yielding cultivars, lodging, weeds, waterlogging, low moisture, and low-fertility conditions are major factors that contribute to the low grain yield. Threshing its tiny

seed is also not an easy task. The yields of improved varieties using enhanced technologies (fertilizer, weed control, appropriate harvesting, and grain threshing) are in the range of  $17-22 \, \text{q ha}^{-1}$ , and in some farms, under research conditions, the yield is as high as  $27 \, \text{q ha}^{-1}$ .

#### **Harvesting and Storage**

Teff grain is harvested when the vegetative and reproductive parts (pedicel, lemma, palea, and glumes) turn yellow or straw color (45-60 days) for very early maturing, 60-120 days for early maturing, and 120-160 days for late maturing). If harvested late, the grain loss will be significant due to shattering and the natural grain color can also fade. However, if the grain is harvested early, it may become vitreous or translucent. In Ethiopia, traditional harvesting is done manually using a sickle. The harvested panicles are gathered in batches, either on the day of harvestation or after  $\sim 1-3$  days, and temporarily stacked



**Figure 1** Typical teff field plant and panicles of some teff varieties: (a) teff field of DZ-01-196, (b) panicles of DZ-01-196 (compact), (c) panicles of DZ-Cr-37 (less compact), and (d) panicles of DZ-01-99 (loose). (Reproduced with permission from Tefera H, Ayele M, and Assefa K (1995) Improved varieties of teff [*Eragrostis tef* (Zucc.) Trotter] release of 1970–1995. *Research Bulletin*. 1. Debre Zeit Agricultural Research Center, Ethiopia.)

(in a conical shape, with the panicles towards the center), outdoors, under the shade, till it is ready for threshing.

Threshing is usually done by oxen-trampling or manually with sticks, after spreading the dried panicles on a dry, circular threshing floor. The threshing floor is prepared by smearing cow dung, cement, or other suitable materials. The grain is traditionally winnowed by wafting in the open air, with the help of a rectangular, flat piece of dried leather, called an *afarsa* or *hafarsaa* (O) ( $\sim$ 0.4 m width  $\times$  0.8 m length). Threshers or combine harvesters can also be used. However, grain loss is large because the teff grain has very small size and light mass, and can be easily blown away with the chaff.

In Ethiopia, the grain is traditionally stored in *gotera* (A, T), *gotaara* (O) (small hut-like stores), or pots or sacks. In comparison with other common cereals, teff grain is less prone to attacks by weevils and other storage pests. Thus, it can be safely stored under traditional storage conditions.

#### **Teff Grain Marketing**

In local markets of Ethiopia, several small-scale grain traders distribute teff grain from major growing areas to the urban consumers and to regions of shortage in teff grain production. At Addis Ababa, teff grain is marketed in a place called *Ehil berenda* (Markato). At present, international teff grain export is not common. But wherever there is a market, companies (government-owned, joint ventures, and private firms), such as Ethiopian Grain Trading Enterprise and Oromo Development Association, which export oil and pulses have the potential to export teff grain as well.

# Physico-Chemical Properties of Teff Grain

#### Morphology

Teff grain is hull-less (naked) and comes in a range of colors – from milky-white to almost dark brown. The most common colors are white, creamy-white, light brown, and dark brown (Figure 2). The grain is oval-shaped with size  $0.9-1.7\,\mathrm{mm}$  (length) and  $0.7-1.0\,\mathrm{mm}$  (diameter). The individual grain mass is generally  $\leq 2\,\mathrm{mg}$ ,  $\sim 0.6-0.8\%$  of the wheat grain mass.

#### **Anatomy**

**Pericarp** The outer pericarp is thin, membranous, and is equivalent to the beeswing bran of wheat. The mesocarp and endocarp present in the inner



Figure 2 Grains of different teff varieties: (a) DZ-01-196 (white), (b) DZ-Cr-37 (creamy white), (c) DZ-01-99 (light brown), and (d) South African Brown (dark brown).

surface of the pericarp are fused and appear as a single layer. As in the case of sorghum grain, this fused layer contains some starch granules.

Seedcoat (testa) Next to endocarp is the testa, which is adjacent to the aleurone layer. In some teff varieties, the testa is reported to contain tannins, and is thus presumed to be thick. However, in the varieties that we have analyzed, including brown varieties, we have not found any significant tannin levels.

Aleurone layer The aleurone layer is one cell thick and is rich in protein and lipid bodies.

Germ Like in other small-grain cereals, the germ occupies a relatively large proportion of the grain and is rich in protein and lipids.

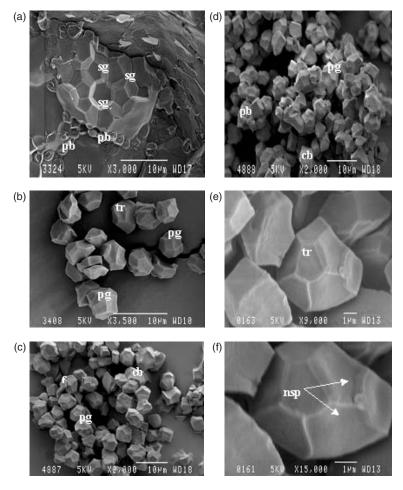
**Endosperm** The endosperm is the largest component of the grain and consists of outer and inner layers. The outer layer is vitreous and contains most of the protein

reserves of the endosperm and a few starch granules. The inner layer is mealy consisting mainly of thinwalled cells containing mostly starch granules with a few protein bodies. Teff has compound-type starch granules (Figure 3a), representing the contents of one amyloplast-like rice, oats, amaranthus, and quinoa starches. On milling, individual starch granules are released along with small groups of protein bodies. The protein bodies are individual entities in nature, spherical in shape (Figure 3a) and unlike those of wheat, they do not coalesce to form a matrix.

#### **Teff Grain Chemical Composition**

The proximate chemical composition of teff grain is shown in Table 1.

Carbohydrate Carbohydrate content of teff grain is  $\sim$ 73%, of which virtually all is starch. The teff starch properties are given in Table 2. Individual starch granules are very small (2–6  $\mu$ m in diameter)



**Figure 3** Compound (a) and individual (b-f) starch granules from different teff varieties: (a, b) South African Brown, (c) DZ-01-1681, and (d-f) DZ-01-196 (sg = starch granules, pg = polygonal, cb = cubic, Tr = tortoise-shell, pb = protein, f = fiber, and nsp = no surface pores).

(Figures 3b-3f), similar in size to rice starch granules, but larger than amaranthus and quinoa starch granules. The shape is polygonal, smooth with no surface pores (Figure 3f). A few granules are essentially cubic and at high magnification some appear as

**Table 1** The proximate composition (db) of grain teff, *Osborne* protein fractions, and food energy

Biochemical class	Compound	Range (%)	Typical value (%)
Protein (%) (N × 6.25) Carbohydrate (%)		9.38-13.3 73.0	11.0 73.0
Crude fiber (%)		1.98-3.5	3.0
Fat (%)		2.00-3.1	2.5
Ash (%)	A IIa	2.66-3.0	2.8
Osborne protein fractions	Albumins	24-39	36
(% protein recov.)	Globulins	7–34	18
	Prolamins	3–15	10
	Glutelins	28-42	40
Food energy (kJ per 100 g)		1406	1406

tortoise-shell shaped (**Figure 3e**). The composition of teff starch granules is similar to other normal native cereal starches, with 25-30% amylose. Gelatinization temperature (*Kofler* hot stage and Differential scanning calorimetry (DSC) methods) is high, similar to other tropical cereal starches. X-ray diffraction is A-type of granule crystallinity,  $\sim 37\%$ , similar to rice. Pasting temperature is similar to that of maize starch, but cooking time for peak viscosity is longer. Peak, breakdown, and setback viscosities are lower than those of maize starch. The paste clarity of teff starch is opaque. The gel texture is short and smooth.  $\alpha$ -Amylase degradation of teff starch granules is by surface erosion and endocorrosion in nature.

Because teff starch granules are very small, smooth, and of uniform size, they offer good functionality as a fat substitute, flavor and aroma carrier, similar to other small-granule starches. Teff starch has good resistance to shear breakdown, and thus it may find good application in high-shear processed foods.

Table 2 Physico-chemical properties of grain teff starch

Property	Mean and standard deviation for five teff varieties	Remark
Individual granule diameter	2-6, majority are 3-5	Individuals are from compound granules
(µm) and shape	Polygonal, smooth surface	, -
Amylose (%) (db)	$28.4 \pm 2.8$	Concanavalin A method
	$28.2\pm0.8$	lodine binding method
Ash (%) (db)	$0.16 \pm 0.04$	-
Protein (%) (N × 6.25) (db)	$0.19 \pm 0.03$	
Lipids [db]: Total (mg per g)	$8.9\pm0.7$	24% HCl hydrolysis followed n-hexane and in situ generated ethyl formate extract
Internal (mg $g^{-1}$ )	$7.8\pm0.4$	Hot (90°C) water saturated butanol extract
Phosphorus (db) (mg g <sup>-1</sup> )	$0.65 \pm 0.08$	
Kofler gelatinization temp. range (°C): $T_o-T_p-T_c$	68.0-74.0-80.0	$T_{\rm o}$ is onset, $T_{\rm p}$ is peak, and $T_{\rm c}$ is conclusion gelatinization temperatures
DSC gelatinization endotherms	63.8-65.4, 70.2-71.3,	$\Delta H$ is gelatinization enthalpy
range: $T_o$ , $T_p$ , and $T_c$ in °C, and $\Delta H$ in J g <sup>-1</sup> respectively	81.3-81.5 and 2.28-7.22	,,
Pasting properties: $T_i$ (°C),	$74.0 \pm 1.1$ , $269 \pm 13$ , $79 \pm 17$ ,	Where T <sub>i</sub> , PV, BV, Rst, and
PV (RVU), BV (RVU), Rst (RVU/min), SBV (RVU)	8.4 $\pm$ 1.8, 101 $\pm$ 11	SBV are pasting temperature, peak viscosity, breakdown viscosity, rate of shear thinning, and setback viscosity, respectively
Peak viscosity	Medium-low	
Resistance to shear	Medium-high	
Retrogradation tendency	Medium-low	
Crystallinity (%)	37	
X-ray diffraction: <i>d</i> -value (Å) and intensity (%)	5.85 (83.8), 5.16 (97.0), 4.89 (99.4), 4.41 (36.4), and 3.84 (80.2)	A type starch crystalline polymorph
Paste clarity	Opaque	
Gel texture	Short	
In vitro digestibility with porcine pancreatic α-amylase	Surface erosion and endocorrosion type	
Acid hydrolysis	Gradual surface degradation (etching)	

Table 3 Amino acid composition of the whole grain teff protein and of the Osborne protein fractions (g per 100 g protein)

Amino acid	Whole g	ırain	Album	nin	Globu	lin	Glutel	in	Prolan	nin
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Asx	5.8-7.2	6.4	10.7-14.4	12.7	10.3-14.1	12.9	12.7-14.4	13.7	3.6-20.7	9.8
Thr	2.4 - 4.4	3.6	3.3 - 4.5	4.2	0.0 - 4.2	2.6	2.0 - 4.2	3.3	2.8 - 4.1	3.5
Ser	2.8 - 5.6	4.1	3.1 - 3.9	3.6	0.1 - 2.4	1.3	0.8 - 2.2	1.6	3.4 - 4.3	3.8
Glx	18.7-24.9	21.8	3.9 - 10.5	9.0	1.5 - 9.8	6.9	6.5 - 9.5	8.7	4.8 - 26.3	20.6
Pro	5.1-11.4	8.2	6.7 - 11.7	8.3	8.0 - 16.6	10.6	8.0-11.0	8.8	6.8 - 13.5	10.0
Gly	1.7-4.1	3.1	4.1 - 8.4	7.2	1.8-5.3	3.9	3.5 - 5.4	4.5	4.3 - 6.0	4.9
Ala	5.5 - 14.7	10.1	13.1-21.7	15.4	13.3-23.7	17.2	13.5-17.9	14.6	10.5 - 19.3	12.7
Cys	0.5 - 2.5	1.8	0.4 - 0.8	0.6	0.4 - 2.1	0.9	0.4 - 1.3	0.8	0.0 - 1.3	1.3
Val	4.1 - 9.9	5.9	8.0 - 9.6	8.6	9.2 - 11.4	10.5	9.2 - 10.9	10.0	7.3 - 9.2	7.8
Met	2.0 - 4.6	3.3	0.5 - 0.8	0.7	0.4 - 1.2	0.6	0.4 - 0.7	0.6	0.7 - 2.8	1.8
lle	3.2 - 5.4	4.0	3.4 - 4.7	4.2	3.4-5.1	4.5	3.9-5.1	4.5	2.5 - 4.6	4.0
Leu	6.0 - 9.7	8.1	6.1 - 6.5	6.3	5.4 - 7.4	6.5	6.6 - 7.4	7.0	5.1 - 7.3	6.5
Tyr	1.7-4.0	3.0	0.9 - 2.5	1.3	0.9 - 1.6	1.3	0.8 - 1.7	1.3	1.3-4.8	3.4
Phe	2.7 - 5.9	5.0	2.3 - 3.5	2.9	2.1 - 3.9	3.0	2.6 - 3.9	3.2	2.5 - 6.6	5.1
His	2.1 - 3.7	2.8	2.8 - 4.2	3.4	3.6 - 5.6	4.6	4.5 - 5.1	4.8	0.7 - 3.7	1.9
Lys	1.4-4.0	3.0	3.7 - 6.6	5.6	1.4-4.2	3.0	2.5 - 3.9	3.2	0.3 - 2.7	1.3
Arg	2.9 - 6.2	4.5	5.8-7.3	6.4	8.4-13.7	10.3	8.9-10.7	9.5	1.2-5.3	2.8
Trp	1.3	1.3								

Asx is Asp + Asn and Glx is Glu + Gln.

Also, because of its slow retrogradation tendency, it could have attractive applications where starch staling is preferred to be reduced (i.e., in baked and in refrigerated foods).

Fiber The fiber content of teff grain (Table 1) is apparently higher than most other common cereals, because the grain is very small and the bran proportionally large.

Protein and amino acids Typical teff grain protein content (N  $\times$  6.25) is  $\sim$ 11%, with a normal range of 9–13%. Thus, the protein content of teff grain is similar to other common cereals. The major amino acids are glutamic acid, alanine, proline, aspartic acid, leucine, and valine (Table 3). Methionine, phenylalanine, and histidine are slightly higher than in most other cereals, but serine and glycine are lower. Lysine and arginine are essentially higher in teff than in most other cereals, except rice and oats. The balance among essential amino acids is similar to the whole edible portion of egg protein, except for its lower lysine content. The overall amino acid profile of teff can be regarded as well-balanced.

Osborne protein fractions of teff grain are shown in **Table 1**. Glutelins, albumins, and globulins are major fractions. The teff prolamin fraction is lower than in most other cereals, except in rice and oats. The major prolamins of teff are similar to the  $\alpha$ -prolamins of maize, sorghum, and *coix*. Teff is thus different from other cereals in having lower prolamins and higher albumins and globulins. Teff protein is

Table 4 Microelement composition of grain teff (db)

Microelements (mg per 100 g or μg per 100 g)	Range	Typical value
Calcium (mg)	104-223	165.2
Chloride (mg)	13	13.0
Chromium (µg)	250	250.0
Copper (mg)	0.7 - 5.3	2.6
Iron (mg)	4.7 - 19.6	5.7 <sup>a</sup> , 15.7 <sup>b</sup>
Magnesium (mg)	138-190	169.8
Manganese (mg)	1.6 - 6.4	3.8
Phosphorus (mg)	378-480	425.4
Potassium (mg)	330-570	380.0
Sodium (mg)	11.8-47.0	15.9
Zinc (mg)	2.0-6.7	4.8

<sup>&</sup>lt;sup>a</sup>Mean of iron from cleaned, acid and/or water washed samples.

essentially free of the type of gluten found in wheat. Because of this, teff grain can be used as an alternative food by consumers allergic to wheat gluten (e.g., for celiac patients). As the main protein fractions (albumins and globulins) are the most digestible types, teff protein digestibility is also presumed to be high. Amino acid compositions of the various *Osborne* protein fractions are shown in Table 3.

Ash and minerals The ash content in teff grain (Table 1) is apparently higher than in wheat, rye, maize, barley, oats, rice, and millets, in part because teff grain bran is proportionally large. In particular, calcium, copper, iron, and zinc (Table 4) content is higher compared to that in barley, wheat, and

<sup>&</sup>lt;sup>b</sup>Mean of iron from uncleaned samples.

sorghum. The iron content of traditionally harvested teff grain is especially high ( $\sim$ 15.7 mg per 100 g), in part because of grain contamination with the soil during harvest. However, when cleaned (with water and/ or dilute acid) the level ( $\sim$ 5.7 mg per 100 g) is similar to other cereals. Most teff foods such as *injera* are fermented. Destruction of phytic acid by fermentation is known to contribute to high iron availability in diets where fermented teff foods are the staple. Because of these two factors, iron deficiency disease — anemia, is rare among teff consumers in Ethiopia.

Fat and fatty acids Teff grain fat (Table 1) is lower than, for example, in maize and oats. Thus, teff is different from other small-grain cereals in having low fat even though the germ is large. As in most other cereal grains, palmitic, oleic, and linoleic acids are the major fatty acids (Table 5). Linolenic acid in teff is higher than in maize, sorghum, and wheat.

Vitamins In teff (Table 6), thiamin is typically lower, when compared to wheat, rye, barley, oats, rice, maize, millet, and sorghum. Though riboflavin content is considered to be high, it is nevertheless lower than in rye, barley, and oats. Niacin levels are similar to those in maize.

#### **Processing and Usage of Teff Grain**

#### Cleaning

Normally, the grain is cleaned by manual sifting.

#### Milling

The cleaned grain is usually dry-milled to obtain whole flour. Traditionally in Ethiopia, this was done by Wafcho (A) (T), wafcoo (O) (top and bottom hard stones). Today, milling with hand, using hard stone, has been replaced by grist mills run by electric power, and, where electric power is not available, by diesel engine or water power. The grist mill is made up of two abrasive hard-disk stones. During operation, one stone is stationary while the other is rotating. The grain, fed into the center (eye) of the upper stone, is fragmented and ground between the two stones, and flour is issued at the periphery. At present, wet-milling of teff grain for chemical component extraction like starch is not carried out.

#### Usage

Food made from teff grain is a staple diet for many Ethiopians. Teff is considered to have a better food value than the major grains, namely, wheat, barley, and maize, as it is normally used as a whole grain, i.e., the germ and bran are consumed along with the

Table 5 Fatty acid composition of grain teff fat

Fatty acids (%)	Range	Mean
Palmitic (C16:0)	14.0-16.4	15.9
Palmitoleic (C16:1)	0.1 - 0.6	0.3
Stearic (C18:0)	3.0 - 3.7	3.3
Oleic (C18:1)	23.3-24.9	24.0
Linoleic (C18:2)	41.3-46.5	44.2
Linolenic (C18:3)	6.9 - 9.9	7.9
Arachidic (C20:0)	0.6 - 0.9	0.7
Arachidonic (C20:1)	0.5-1.2	0.8
Behenic (C22:0)	0.3-1.1	0.5
Erucic (C22:1)	0.0-0.9	0.4

Table 6 Vitamin and antinutrient composition of grain teff (db)

Component	Typical value
Vitamins	
Vitamin A (RE)	8
Thiamine (mg per 100 g)	0.3
Riboflavin (mg per 100 g)	0.2
Niacin (mg per 100 g)	2.5
Vitamin C (mg per 100 g)	88
Antinutrients	
Phytate (mg per 100 g)	707
Trypsin inhibitor activity (TIU g <sup>-1</sup> )	5584

RE is retinol equivalent; TIU is trypsin inhibitor unit.

endosperm. Teff flour is used primarily for making of injera (A), caabitaa or budeena (O), and tayeta (T). The flour is also used to make sweet unleavened bread called *kitta* (A), *bixxille* (O), and *daguwalo* (T). Kitta can be consumed as bread or it can be used as an adjunct in traditional opaque beer (tella (A), farsoo (O)), or local spirit (katikalla (A), araqii (O)). Porridge (genefo (A), marqaa (O)) can also be made from teff flour. Thin, fermented teff flour batter is used to prepare soup (*muk*, (A)). Unfermented teff flour dough is also used in the preparation of traditional snacks (dabbo Kolo (A), hunkuroo (O)), where the dough is rolled into small balls and then roasted on a hot griddle. In the USA, teff has been promoted as a thickener for soups, stews, and gravies probably because teff flour paste gives the product a short and stiff texture. Teff grain flour imparts a slight molasses-like sweetness to food products, making its inclusion in porridges, pancakes, biscuits, cookies, cakes, stir-fry dishes, casseroles, soups, stews, and puddings desirable. Processing of teff grain in Ethiopia has been limited to the household level. To date, technologies for large-scale commercial processing of teff grain, for the preparation of foods like *injera* is not well advanced. However, apart from traditional usage, recent reports indicate that teff grains, along

with soybean, chickpea, and other grains, are being used in the baby-food industry.

#### Teff Grain Injera Making

In Ethiopia, *injera* is regarded as the national staple food. A flowchart of *injera*-making process is shown in **Figure 4**. The process involves fermentation and then baking of the batter.

Fermentation Flour is mixed with water and the dough is kneaded, usually by hand. Fermentation for *injera*-making involves two phases that can last a total of 24–72 h. The first phase starts spontaneously when flour is wetted, due to contaminating microorganisms. Or it can be initiated by addition of *irsho* (A) (T), *raacitii* (O) (yellowish liquid saved

from the previous batch fermentation). The initial 18–24 h are notable for vigorous gas evolution and maximum dough expansion. At about 30–33 h an acidic yellowish liquid appears on the dough surface. This phase is characterized by a decrease in gas evolution up to 31 h, an increase in liquid volume up to 48 h, and decrease in pH to below 5.8.

The first phase of fermentation results in a liquid/solid separation after  $\sim$ 24 h. The layer of liquid is then removed. About 10% of the fermenting dough is mixed with water (1:3 ratio), boiled (2–5 min), and as a result of starch gelatinization, a dough binder, called *absit*, is formed. The *absit* is cooled and added to the fermentation vat signaling the second phase of fermentation. The second phase (0.5–2 h) is characterized by a short duration of dough expansion and gas formation.

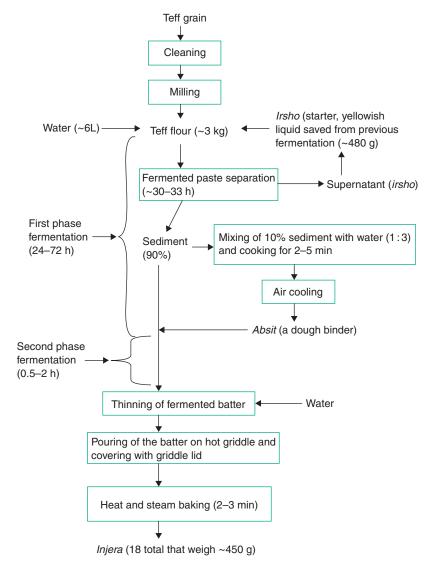


Figure 4 Flowchart of teff injera-making process.

During the first phase of fermentation, the yellowish liquid that is removed contains water-soluble nutrients (amino acids, sugars, minerals) and large number of microorganisms involved in the fermentation. This has negative nutritional consequences. Thus, *injera* baked from a batter, after  $\sim 31\,\mathrm{h}$  of fermentation without discarding the liquid, is recommended as being more nutritious.

A complex group of microorganisms is known to be involved in teff fermentation. Bacteria belonging to Enterobacteriaceae family are thought to initiate the fermentation. During the first 18 h of fermentation, the activities of these bacteria reduce the dough pH to  $\sim$ 5.8. A group of lactic acid bacteria (*Leuconostoc* mesenteroides, Streptococcus faecalis, Pediococcus cerevisiae, Lactobacillus brevis, Lactobacillus plantarum, and Lactobacillus fermentum) are involved at the later phase of fermentation (18-72 h) in reducing the dough pH from 5.8 to 3.8. During the later phase of fermentation (22–24 h), yeasts of two genera Saccharomyces and Torulopsis are reported to be involved. In the later phase ( $\sim$ 48 h), yeast belonging to the genera *Candida* and *Pichia* are the dominant types isolated from the yellowish liquid removed from dough. In addition to amylases present in the grain, the bacteria species *Bacillus* sp. A-001 involved in the fermentation has been characterized as one of amylase-producing bacteria involved in partially attacking the starch granules.

In the traditional teff fermentation for *injera* making, commercial yeast is not added externally. The source of the yeast is either from *irsho* or from the endogenous microflora of teff grain, and its flour that grows in the batter after the flour is wetted with water. Therefore, the yeast in the fermentation of teff for *injera* making can be regarded as symbiotic yeast.

Baking Injera is usually baked after  $\sim$ 24 h of fermentation. After the fermentation, the batter is diluted slightly with water and then, poured using circular motion from the outer perimeter towards the center, onto a hot-round smooth griddle called a metad (A), eelee caabitaa (O). It is then covered with a metad lid called akambalo (A), qadaada eelee caabitaa (O) to prevent steam from escaping. The griddle is traditionally made from clay. Before pouring the batter, the metad surface is swabbed with ground oilseeds, commonly rapeseed or with animal fat in a piece of cloth. This prevents the injera from sticking to the metad surface. Depending on the batter thickness, heat intensity applied, and steaming, injera can be baked in 3-6 min.

Based on the duration and nature of the fermentation involved, three common types of *injera* are prepared: (1) injera made from dough which does not contain absit, characterized by a soft, thin, fine appearance, and a sour taste without the "eyes" of *injera* (surface air cells), (2) *injera* made from partially fermented paste (12-24 h fermentation) called aflegna (A), bekuo (T), characterized by a sweet flavor, pleasant odor, and a rusty red underside, and (3) injera made from over-fermented paste called komtata injera (A), (T), qomxoxaa caabitaa (O) which tastes very sour and is regarded as less nutritious. The thick batter used for aflegna injera is also used to prepare a slightly concave, thick flatbread called cumboo (O). Cumboo is traditionally baked on the preheated surface of a small-size concave griddle, which is placed on a flat larger griddle. Depending on the batter size and heat intensity applied, the baking time for *cumboo* is between 1 and 3 h.

# Prospects, Problems, and Recommendations

Teff can be cultivated under harsh environmental conditions where most other cereals are less viable. It has relatively few pest-and disease-related problems in the field. The grain is less prone to attacks by weevils. The nutrient composition of teff grain indicates that it has good potential to be used in foods and beverages worldwide. However, the grain yield of teff is low. The mechanized farming technologies that are used for the production of other cereal grains can be problematic for teff, because the plant stems are very thin and short, and the grain is very small. Manipulation of teff plant genes through research and finding suitable technologies for maximum grain production and harvesting are required.

At present the milling of teff grain is limited to cottage-type millers. Processing of teff for different foods is usually done by traditional ways and is mostly limited to the household level. More research on large-scale milling of teff, processing for different commercial foods, and grain component extraction (e.g., starch) is needed to promote worldwide teff utilization.

See also: Amaranth. Cereals: Overview. Grain, Morphology of Internal Structure. Grain and Plants, Morphology. Grain Production and Consumption: Overview; Africa. Millet: Pearl; Minor. Nutrition: Mineral Composition. Oats. Rice: Genetics; Breeding. Sorghum: Breeding and Agronomy; Harvest, Storage, and Transport; Utilization. Appendix: Grain Composition Tables; Foods for Celiac Diets; Glossary of Grain-Industry Terms; Test Methods for Grain and Grain-Based Products.

#### **Further Reading**

- Areda A (1990) Studies on the Nutrient Composition of tef [Eragrostis tef (Zucc.) Trotter] and the Interactive Influence of Environment and Genotype. PhD thesis, University of London, London, UK.
- Areda A, Ketema S, Ingram J, and Davis RHD (1993) The iron content of tef [*Eragrostis tef* (Zucc.) Trotter]. SINET: An Ethiopian Journal of Science 16: 5–13.
- Bekele E (1995) Essential and non-essential amino acids in a free state and in the major protein fractions of teff seeds. SINET: An Ethiopian Journal of Science 18: 79–99.
- Bultosa G (2003) Physico-Chemical and Functional Properties of Grain tef [Eragrostis tef (Zucc.) Trotter] Starch. PhD thesis, University of Pretoria, Pretoria, South Africa.
- Costanza SH, DeWet JMJ, and Harlan JR (1979) Literature review and numerical taxonomy of *Eragrostis tef* (T'ef). *Economic Botany* 33: 413–424.
- Ebba T (1969) Tef (*Eragrostis tef*): the cultivation, usage and some of the known diseases and insect pests. Part I: Debre Zeit Agricultural Experiment Station. Addis Ababa University, College of Agriculture: Dire Dawa, Ethiopia.
- Ebba T (1975) Tef (*Eragrostis tef*) cultivars: morphology and classification. *Experimental Station Bulletin*, No. 66. Addis Ababa University, College of Agriculture: Dire Dawa, Ethiopia.
- Hundera F (1998) Variations of morpho-agronomic characters and grain chemical composition of released

- varieties of tef [Eragrostis tef (Zucc.) Trotter]. Journal of Genetics and Breeding 52: 307-311.
- Ketema S (1993) Tef (*Eragrostis tef*): breeding, genetic resources, agronomy, utilization and role in Ethiopian agriculture, IAR, A.A, Ethiopia.
- Ketema S (1997) Tef. Eragrostis tef (Zucc.) Trotter. Promoting the conservation and use of underutilized and neglected crops. Booklet 12. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.
- National Research Council of the USA (NRC) (1996) Lost Crops of Africa, vol. 1: Grains, pp. 214–338. Washington, DC: National Academy Press.
- Tefera H, Ayele M, and Assefa K (1995) Improved varieties of tef [*Eragrostis tef* (Zucc.) Trotter] release of 1970–1995. *Research Bulletin*. 1. Debre Zeit Agricultural Research Center, Ethiopia.
- Umeta M and Parker ML (1996) Microscopic studies of the major macro-components of seeds, dough and *injera* from tef (*Eragrostis tef*). SINET: An Ethiopian Journal of Science 19: 141–148.

#### **Relevant Websites**

http://mansfeld.ipk-gatersleben.de – Mansfeld's world database of agricultural and horticultural crops.

http://www.ars-grin.gov – USDA's GRIN database.

## **TORTILLAS**

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#### Introduction

A tortilla is a flat, round, unfermented bread produced from wheat (*Triticum aestivum* L.) flour or lime (CaO)-cooked maize (corn – *Zea mays* L.). Sorghum (*Sorghum bicolor* L.) is used alone or in mixtures with maize for tortillas in parts of Central America. Processing and characteristics of wheat flour and maize tortillas differ considerably. Both types of tortillas

originated in Mexico, where they are considered the national bread and are consumed with others foods such as beans, meat, and vegetables. Tortillas, traditionally, are homemade, prepared on a daily basis, and consumed fresh. The technology for maize tortilla production was developed by early Mesoamerican civilizations. Tortillas and "masa" products constitute the staple food for most people in Mexico and Central America. The annual per capita intake in 2001 was 85 kg in Mexico (up to 120 kg in some areas), 6 kg in USA, and 0.3 kg in Europe. The technology for flour tortillas was developed during the sixteenth century in northern Mexico.

Tortillas are increasing in popularity throughout the world. Global sales in 2002 are estimated at \$9 billion for tortillas and \$10 billion for maize

"tacos," "tostadas," tortilla chips, and other corn snacks. Mexico accounts for 42% of the world's production of tortillas, USA 36%, Central America 9%, and other countries 13%. Maize comprises 98% of the tortillas consumed in Mexico and Central America. In Mexico, 40% of the maize is used for tortillas. The tortilla industry is the fourth largest industry (0.90% of GDP) in Mexico. Wheat flour tortillas are more prevalent than maize tortillas throughout the rest of the world. Processing plants for tortillas and alkaline-cooked corn snacks are located on almost every continent.

#### **Wheat Flour Tortillas**

All wheat tortillas contain flour, water, fat, and salt. Tortillas prepared using just these ingredients are leavened by steam generated during baking, have dark toast points, and areas which are translucent (i.e., not opaque). These tortillas are common in northern Mexico and are  $\sim$ 1 mm thick, up to 60 cm in diameter, and contain up to 20% fat (on a weight of flour basis). Most flour tortillas outside of Mexico and Central America contain baking powder for leavening to "improve" flavor, opacity, texture (softness, rollability), and shelf life (1 week to 6 months). Tortillas with good appearance, uniform whiteness or opacity, large diameter, and long shelf stability are prepared using chemical leavening agents, emulsifiers, reducing agents, antifungal agents, acidulants, gums or hydrocolloids, sugar, maltodextrins, proteins, and nonactive yeast.

Flour tortillas in Mexico and Central America are usually consumed fresh on the day of production, just as "chapatis," an unleavened, flat bread in India and southern Asia, which is prepared from wheat flour, water, salt, and fat (optional). The formula and processing of flour tortillas in the USA have changed to yield a longer shelf life tortilla, due to the cultural practices of infrequent shopping, refrigerated storage of foods, and preparation and consumption over several weeks. Hot-press, die-cut, and hand-stretch methods are used to form wheat dough into thin disks in the preparation of flour tortillas by the food industry. These methods have replaced the traditional processes of preparing tortilla disks by hand. Each requires different flour specifications, dough preparation, and baking conditions, resulting in distinct tortilla characteristics.

Hot-press tortillas (Figure 1) are consumed as gourmet table tortillas, "fajitas," and soft tacos, and are prepared in restaurants for immediate consumption. Hot-press tortillas have a smooth surface, a tender bite, some elasticity, resist moisture absorption from fillings and have longer shelf stability than other flour tortillas. Hot-press tortillas are the most popular and account for >90% of the retail market.

Die-cut tortillas are mainly used in "burritos," frozen Mexican foods, and fried products, i.e., taco salad bowls, taco shells, "chimichangas," and "buñuelos." Die-cut tortillas often have dusting flour on the surface, lower moisture content, a pasty mouthfeel, and are less resistant to cracking during storage. The die-cut method greatly reduces the cost of production of tortillas.

Hand-stretch tortillas are consumed as table tortillas, burritos, and used to prepare certain fried products. Hand-stretch tortillas have dusting flour on the surface, irregular shapes, moderate elasticity, and a firmer bite than hot-press tortillas. They are more costly to produce and are decreasing in popularity.

Tortilla dough is mixed to incorporate microingredients, fat, and water into the flour and form a pliable, extensible dough (Figure 1a). The optimum dough temperature should be 32-34°C after mixing and gluten development. Dough properties are primarily modified by flour quality, levels of reducing agent, emulsifier, fat and water, and dough temperature. The dough is rested  $0-20 \,\mathrm{min}$  (Figure 1c) before being divided and rounded into dough balls (Figure 1b) for the hot-press and hand-stretch procedures. The dough balls are rested in a warm, moist environment for 2-20 min (Figure 1c) to relax the gluten network. Rested dough balls form larger diameter tortillas with more opacity and longer shelf stability. Tortilla dimensions (thickness, diameter), opacity, and shelf stability are affected by the hot-pressed dough ball's pressure, temperature, and duration of hot-pressing (Figures 1d and 1e).

In the hand-stretch procedure, the dough balls pass through two pairs of rollers, set perpendicular to each other, prior to stretching by hand on a hot griddle and baking. The dough for hand-stretch and die-cut tortillas is dusted with flour to prevent adhesion to equipment. The dough, in die-cut operations, is pumped and shaped into a sheet that is further thinned by a series of rollers and cross-rollers on a moving belt. The thin sheet of dough (0.5-2.5 mm) is cut by a circular die, which forms the tortilla shape. The scrap dough is returned to the dough pump and reprocessed.

The formed tortilla disks, regardless of the process, are baked (190–260°C for 18–50 s) in gas-fired ovens (Figure 1f) that usually have three tiers. Oven conditions vary depending upon tortilla thickness, type of conveyor (slat or wire), and method of preparation. Puffing of the tortilla occurs near the end of baking and is common in hot-press and hand-stretch tortillas. Tortillas are typically "cooled" to < 32°C on conveyors (Figure 1g) for 2-5 min prior to packaging



**Figure 1** Processing of flour tortillas using the hot-press method. Note: (a) = tortilla dough (D) in bowl with dough hook (H); (b) = dough balls; (c) = resting chamber for dough and dough balls; (d) = dough ball moving on teflon belt into the hot-press (P) behind inset area (I; arrow indicates press); (e) = pressed disk moving on teflon belt immediately after hot-pressing (arrows indicate direction of movement); (f) = tortilla at the end of the first tier of a three-tier oven; (g) = tortillas on wire conveyer; and (h) = stack of flour tortillas.

in plastic bags. Moisture and heat are lost during this operation. Improper cooling increases not only the microbiological problems but also the "stickiness" of the tortillas.

Fresh tortillas (Figure 1h) have a soft and pliable texture which changes to a firmer and less extensible texture during storage. Improvements which make it possible to retain the freshness of hot-press tortillas

throughout the storage period are naturally preferred. About 80% of flour tortillas are prepared using the hot-press method even though the die-cut method is more efficient. Hot-press flour tortillas are the preferred "bread" in the Space Program because of their versatility, functionality, long shelf stability, and lack of crumbs. Consumers prefer hot press tortillas because they retain their freshness during storage. Nontraditional fillings for breakfast, lunch, dinner, and desserts increase their versatility and convenience.

Wheat flour requirements are determined by the desired tortilla characteristics, the formula, processing conditions, and equipment. Wheat flour is usually hard wheat flour with a protein content ranging from 9.5% to 12.5%. Flours for hot-press and hand-stretch tortillas generally contain less protein and gluten strength than flours for die-cut tortillas. Tortillas that are made with flours with poor gluten strength remain pliant for only a couple of days. Hot-pressed tortillas which are made with good-quality flours are generally ones with smaller diameter, less opacity, and tend to remain more pliant during storage. Flour ideal for hot-pressed tortillas has intermediate gluten strength and levels of protein quality with small amounts of damaged starch.

Water (45-55%) of flour weight) is a reaction medium for gluten formation and chemical reactions (leavening) during mixing. Low levels (10–80 ppm) of reducing agents, such as sodium metabisulfite or cysteine, reduce dough mixing time and increase the extensibility of dough during hot-pressing, which yields larger diameter tortillas. Salt (1-2%) is added for taste and to strengthen the gluten complex. Baking powder (0.05-2.5%) causes tortillas to have a less dense, spongy structure, i.e., increased whiteness or opacity and greater specific volume. The high dough temperatures (25–40°C) cause premature leavening reactions during mixing and less leavening is available during baking. Leavening acids and bicarbonates that dissolve more slowly and react to form CO<sub>2</sub> during latter stages of baking improve tortillas opacity and thickness. Various natural and modified cellulose gums are added at 0.1-0.5% levels to improve dough machinability and decrease the stickiness of baked tortillas.

Solid or liquid fats (3-20%) of flour weight) are added to improve dough properties by weakening gluten strength. Fats also contribute to softer and more flexible tortillas by limiting amylose retrogradation during storage. Dough-strengthening emulsifiers, such as sodium stearoyl-2-lactylate, improve dough cohesiveness and integrity of the outside perimeter of hot-press tortillas. Emulsifiers, such as glycerol monostearate, limit amylose retrogradation and improve

tortilla softness. Low-fat (< 3 g fat per serving) and fatfree (<0.5 g fat per serving) flour tortillas have been developed; these have proved to be extremely popular with sales exceeding \$40 million in the USA in 2002.

Antimicrobial agents (propionates, sorbates, or mixtures thereof) limit fungal growth and extend shelf life and are used at 0.2-0.6% of flour weight. Optimum antifungal activity occurs at less than pH 5.5. Tortilla pH is lowered by acidulants (citric, fumaric, phosphoric acids). Soluble acids, however, cause the early release of CO<sub>2</sub>, hence granular fumaric acid or fat-encapsulated acids are commonly used to delay their solubility so as not to interfere with leavening reactions.

#### **Lime-Cooked Maize Products**

Three basic types of products are industrially produced from lime-cooked maize: table or soft tortillas, corn chips, and tortilla chips. Corn and tortilla chips are primarily produced and consumed in developed countries, where they have an important share of the salted snack-food market. Sales of corn and tortilla chips in the US in 2002 totaled \$5.7 billion. Modern production of tortilla chips and corn chips have evolved into specialized processes. They are quite different from the orginal tostadas made by frying stale, maize table tortillas.

#### **Table Tortillas from Maize**

Tortillas are produced using traditional and industrial processes. Maize (Figure 2a), lime, and water are three basic ingredients needed for the production of masa. In the traditional process, maize is lime-cooked in clay pots over a fire, followed by steeping for 8–16 h. The cooking liquor, called "nejayote," is discarded; then, the "nixtamal" is hand-washed and ground into a fine masa with a stone grinder. The masa is hand-molded or pressed into disks which are baked on a hot griddle or "comal." The tortilla disks are baked on each side to seal the surfaces and form steam that causes the tortilla to puff. Traditionally tortillas are produced on a daily basis. Tortillas vary in thickness from different localities in Central America and Mexico. Fresh maize tortillas have an outstanding flavor and texture, which firms rapidly into an unacceptable product. In Mexico, the bulk of tortillas are consumed fresh daily and leftover tortillas are fried into tostadas and "totopos." Tacos are soft tortillas wrapped around meat, sauces, beans, and other fruits and vegetables in Mexico. The tacos in the US, however, are deepfried tortillas in a U shape, filled with meat, sauces, etc. Other masa-based products include "tamales," "atoles," and "pozol" which is a fermented masa.



Figure 2 Processing of maize into tortillas and tortilla chips. Note: (a) = kernels of maize illuminated from behind, the light areas indicate the hard, vitreous endosperm (H) while the darker areas indicate the soft, floury endosperm (S) and germ (G); (b) = maize entering cooking water containing calcium oxide; (c) = cooked maize or nixtamal with adhering partially hydrolyzed pericarp (HP) before the washing step; (d) = separated grinding stones with nixtamal (center, n) and masa (outside edge, m) in the grooves (arrow); (e) = stone-ground masa; (f) = sheeting rollers forming disks of masa and a wire mesh belt transferring the disk to the oven (arrow indicates direction of movement); (g) = tortilla oven with a tortilla (T) on the third tier leaving the oven; (h) = tortillas cooling on a wire conveyer; (i) = stack of maize tortillas.

A popular Mexican soup, "posole," is made from the large, soft, nixtamalized maize kernels.

The industrial tortilla process begins when the maize is lime-cooked in agitated open baths, vertical cookers, or steam kettles (Figure 2b). The grain is generally mixed with three parts water and 1% lime,

based on grain weight, and cooked for 15–45 min at temperatures ranging from 85°C to 100°C. The nixtamal is then steeped for 8–16 h in the hot lime solution. After steeping, the cooked maize or nixtamal (Figure 2c) is pumped with the steep liquor or dropped by gravity to washers. The cooking

liquor is drained and the nixtamal washed with pressurized water. Most of the pericarp and lime is removed during this step. The washed, cooled nixtamal is ground using two radially-carved, volcanic or synthetic (aluminum oxide) stones (Figure 2d) or stainless-steel plates. One stone is stationary and the other rotates at 500-700 rpm. Masa particle size is directly related to the gap and pressure between the stones and the size, depth, and pattern of the grooves. During grinding, the nixtamal is disrupted into an array of particles from starch granules (10 μm) to endosperm pieces (2 mm). Some starch granules are gelatinized and dispersed by the friction of the stones to produce the glue-like material that causes cohesiveness in the masa. Only a small amount of gelatinization of the starch occurs during cooking and steeping. Grinding produces most of the gelatinized starch that holds the particles of masa together during subsequent pumping and sheeting into unbaked tortillas.

The ground particles (Figure 2e) drop into a masa feeder with augers (masa hog) which move the masa to above the sheeter head. The two rollers of the sheeter compresses the masa into a thin layer, which is die-cut into standard size disks, strips, triangles, etc., depending upon the products desired. These formed pieces of masa (Figure 2f) are transferred by mesh belts into a threetier, gas-fired oven (Figure 2g) to bake on lime-coated, metal slats or mesh belts at 280-302°C for 30-45 s. The tortillas are allowed to cool on open conveyors, counted, stacked, and packaged (Figures 2g and 2h).

Tortillas are formulated with antifungal compounds or by raising the pH to improve their shelf life when merchandized for 3-90 days. Antifungal compounds, sorbates and propionates, along with acidulants are incorporated during grinding or masa kneading. The pH must be reduced to enable the preservatives to function properly. Alternatively, tortillas are preserved by the lime that is not removed during washing. High pH is an effective preservative, if the tortilla pH is greater than 9.8. Tortilla pieces that are fried immediately do not need preservatives; however, those fried after ambient or refrigerated storage are acidified and preserved.

About 40% of the maize tortillas consumed in Mexico are prepared using dry masa flour. More than 2 million metric tons (Mt) of dry masa flour for table tortillas are produced annually in Mexico. These flours are transformed into  $\sim$ 3.4 Mt of table tortillas. Dry masa flour is produced by cooking corn with alkali, coarsely grinding the nixtamal with a modified hammermill, drying, and then, sifting. The masa is dried in large tunnels, or drying towers, in which warm airflows countercurrently over the ground particles. The particles too large for masa flour are ground by hammermilling, sifted, and the various paricle sizes are blended in the correct proportions to produce various dry masa flours for specific products with different particle size, color, pH, and water uptake. The quality and consistency of these products have improved over the years. The particle size of masa for table tortillas is finer because the tortillas hopefully will puff during baking. In the case of fried snacks, the coarser particles in dry masa flour allow water vapor to escape during frying which improves chip texture. High-quality tortillas and chips are prepared from dry masa flours. A blend of dry masa and fresh masa is sometimes used in many tortillerias. Dry masa flours for table tortillas are usually enriched with thiamin, riboflavin, niacin, folic acid, iron, and zinc.

The use of dry masa flour depends upon the processor. However, many new processors use dry masa flour because of its convenience, even though it costs 2-3 times as much as raw maize. Dry masa flour does not require experienced personnel, space, or equipment to cook, steep, wash, and grind maize, or the expense of effluent disposal. The dry masa flour is shelf stable if it contains less than 10% moisture and only requires water and mixing to form masa. Dry masa flours are mixed with 1.0-1.2 parts water for  $\sim 3-5$  min to produce a suitable dough.

Dry masa flours usually produce masa with less adhesiveness and higher viscosity, requiring more force on the rollers to form a thin sheet. Properties of rehydrated masa can be changed by modifying cooking and frying procedures or by addition of 0.1–0.7% carboxymethylcellulose. Carboxymethylcellulose makes table tortillas more chewy and improves tortilla flexibility during storage. Vital wheat gluten when added at 0.5-2% improves retention of tortilla flexibility during storage. Other hydrocolloids also increase masa softness but usually not tortilla flexibility. Product-softening emulsifiers, when used at less than 0.5%, do not usually improve table tortilla quality. Specific amylases soften masa and tortillas and improve processing and product qualities. Combinations of these additives are common in tortillas.

#### Fried/Snack Products

Frying has expanded the market for masa-based foods because the final products have excellent organoleptic properties and long shelf lives. Two popular snacks from masa are corn chips and corn tortilla chips. Corn chips are produced by directly frying extruded or sheeted masa pieces, while masa for tortilla chips is formed into triangles, strips, or circles, baked, equilibrated, and fried. Tortilla chips have less oil, a firmer texture, and a stronger maize flavor than corn chips.

Nixtamal for these snack foods is cooked and not steeped as much as the nixtamal for table tortillas. The nixtamal for fried snack-foods is ground into coarse masa because the larger particles in the sheeted pieces allow steam to escape through many small pores that develop during baking and frying. This prevents the formation of serious quality defects, such as oily appearance, pillowing, or blistering.

Corn chips are prepared by extruding masa through a die, which is cut by rotating knives before frying or by sheeting and cutting into strips. Corn chips have a more friable texture than corn tortilla chips and contain more oil than tortilla chips (Table 1). Products from white maize are fried at higher temperatures and shorter times than those from yellow maize. Beta-carotenes in yellow maize degrade into beta-ionones which are bitter and off-colored. Frying temperatures and times range from 165°C to 195°C for 20 to 90 s, depending upon maize properties. A blend of yellow and white maize is normally used for corn chips.

The moisture content of the masa or tortilla is related to the oil absorption during frying. Specifically, the higher the moisture, the higher the oil absorption. As moisture leaves the cooked product during frying, oil is absorbed into and onto the structure of the fried food. The appearance and texture of chips are also affected by masa particle size distribution, tortilla baking and equilibration, structure in the piece to be fried, maize color, and oil quality.

Corn and tortilla chips are salted and flavored immediately after frying. The hot chips are conveyed into an inclined rotating cylinder where the flavorings are applied. The most popular flavorings include nacho cheese, hot and spicy, barbecue, French onion, lemon and salt, and jalapeño. Corn and tortilla chips are packaged in moisture-proof aluminized bags, flushed with nitrogen to protect the product physically and to limit oxidative rancidity.

Specialty products include blue chips and reducedfat snacks. Blue maize tortilla chips, served in specialty restaurants, are available as organic and regular products. Blue maize has a pigmented aleurone that imparts an intense blue color. It has high levels of flavanoids and other phenolics that may have nutraceutical properties.

Reduced-fat snacks can be achieved by processing or by using a fat-replacer. Baked, low-fat tortilla chips are prepared using air impingement, infrared, and/or microwave ovens. Olestra, a nonabsorbed fat, is utilized to fry tortilla chips and reduce fat content and calories. Sales of low fat and Olestra-fried chips have decreased apparently because consumers prefer the taste and texture of full-fat products and/or dislike the perceived gastric side-effects. The trends toward

organic and natural health products are continuing with a wide variety of organic chips available.

## Chemistry of Nixtamalization (Alkaline Cooking)

Cooking and steeping maize in lime solution (pH>11), softens the pericarp, hydrates the endosperm, partially solubilizes proteins and cell walls, facilitates starch swelling, and gelatinizes only a small amount of starch. Significant dry matter is lost in the steep solution especially soluble proteins, sugars, and other components. The breakdown of the cell walls of the pericarp forms gums which are useful in table tortillas. However, for snacks the partially solubilized pericarp is removed by washing, as these pieces cause processing problems. The cooking and steeping times vary depending upon the desired products, equipment used, and the maize quality.

The quality of maize for alkaline cooking is critically important to produce high-quality products. Whole, sound, mature kernels of maize with a high proportion of hard to soft endosperm (Figure 2a) yields more masa and tortillas after nixtamalization. Uniform flat kernels without cracks and broken kernels and intermediate to hard endosperm are preferred. Pericarp removal is affected by genetics and by the environmental conditions during maturation of the maize.

The maize must be cooked uniformly to provide adequate hydration and partial solubilization so that grinding produces a nonsticky, cohesive masa that can be formed into a thin disk and baked into a desirable tortilla or fried into chips or tortilla chips. During cooking and steeping, the pericarp is converted into gums and insoluble materials. These materials are washed from nixtamal before grinding, especially when prepared into fried products; however, nixtamal for maize table tortillas is not washed as much, since the gum binds water and improves the texture of tortillas. In tortilla chips, the partially hydrolyzed pericarp causes discoloration and darkening of tortillas. However, extra washing increases drymatter losses and sewage charges since the dry-matter losses range from 5% to 10% or higher of the original maize depending on the maize quality and the processing parameters.

Table tortillas are excellent when fresh but become rigid after 4-8 h. Firming or staling of tortillas is affected by pH, extent of cooking, moisture content, and storage conditions. Alkaline tortillas retains oftness and flexibility longer than regular tortillas because starch retrogradation is inhibited by the many negative charges on the starch chains at pH>9. Many tortillas are acidified (pH 5.0-5.5) to activate the preservatives, but starch retrogradation and staling are not inhibited

at this pH. Individually or in combination, addition of carboxymethylcellulose, vital wheat gluten, amylases, and waxy maize (100% amylopectin) produces tortillas that retain flexibility longer than normal tortillas. Softness and flexibility of tortillas containing soy flour, barley flour, or beta-glucans from barley flour were retained longer than normal tortillas. The use of emulsifiers, neutral gums, shortening, and modified starches were less effective in limiting firming of tortillas.

Quality of end products depends on the nature of manufacturing practices and of raw materials. Specific types of maize hybrids are approved for use in alkaline cooking by processors. Yellow and white maize, or mixtures thereof, are commercially manufactured into alkaline products. Food maize suppliers arrange for producers to grow, harvest, store, and deliver clean maize to processors. Premiums are paid to producers to secure maize that is acceptable in terms of kernel hardness, size, cracks and brokens, pericarp removal, and levels of aflatoxins and fumonisins. The ideal grain should be clean, sound, large, uniform, brightcolored, free of cracks, and broken kernels, high test weight with intermediate-to-hard endosperm. In addition, the kernels should have a rounded crown and a shallow, unwrinkled dent, and the pericarp should be easily removed during lime-cooking. Broken and cracked kernels cause increased dry-matter losses and poor-quality (sticky) masa. The environment

affects corn quality significantly. Anything that affects the kernel during maturation affects the processing properties of the grain.

#### **Nutritional Value**

The nutrient composition of some lime-cooked maize foods is compared with white pan bread and wheat tortillas in Table 1. Tortillas, especially wheat flour tortillas, are commonly used as a substitute for pan bread by many people. Flour tortillas are higher in gross and digestible energy because their formula contains more shortening (5-15% based on flour weight). Whole meal flour tortillas have higher amounts of fiber, protein, and ash than do white flour tortillas.

Maize tortillas are the main source of energy, protein, calcium, and other important nutrients in Mexico and Central American diets. Lime-cooking considerably increases calcium and the bioavailability of niacin, and significantly decreases the amount of aflatoxins and fumonisins in contaminated maize.

The caloric densities of corn chips and tortilla chips are significantly higher than table tortillas because of the oil absorbed during frying and their low postprocessing moisture contents. Tortillas and snacks produced from enriched dry masa flour contain higher levels of B-vitamins, Fe, and Zn than counterparts produced from fresh masa. In Mexico, dry masa flours are enriched by government regulation; some of

**Table 1** Nutrient profile<sup>a</sup> (per 100 g of edible portion) of flour tortillas, lime-cooked maize products, and table bread

Nutrient	Unit	No.	Wheat flour tortilla	No.	Table maize tortilla	No.	Corn tortilla chips	No.	Corn Chips	No.	White bread
Water	g	14	$26.80 \pm 0.90$	42	44.10 ± 0.66	42	1.80 ± 0.11	100	$1.00\pm0.05$	302	36.70 ± 0.10
Protein	g	12	$8.70 \pm 0.58$	29	$5.70\pm0.13$	38	$\textbf{7.00} \pm \textbf{0.13}$	91	$6.60 \pm 0.09$	218	$\boldsymbol{8.20\pm0.05}$
Lipid (fat)	g	11	$\textbf{7.10} \pm \textbf{0.58}$	25	$2.50 \pm 0.24$	41	$\textbf{26.20} \pm \textbf{0.49}$	94	$33.40 \pm 0.28$	224	$3.60\pm0.08$
Ash	g	12	$\boldsymbol{1.80 \pm 0.15}$	29	$\boldsymbol{1.20\pm0.06}$	35	$2.20 \pm 0.14$	92	$2.20\pm0.05$	286	$\boldsymbol{1.90\pm0.02}$
Carbohy., calc	g	1	55.60	1	46.60	1	62.90	1	56.90	1	49.50
Fiber, dietary	g	1	3.3	1	5.2	1	6.5	1	4.9	1	2.3
Minerals											
Calcium, Ca	mg	20	$125\pm7.5$	53	$175\pm7.1$	38	$\textbf{154} \pm \textbf{6.0}$	126	$127\pm2.8$	250	$\textbf{108} \pm \textbf{2.6}$
Iron, Fe	mg	38	$\textbf{3.30} \pm \textbf{0.12}$	44	$\boldsymbol{1.40\pm0.07}$	36	$\boldsymbol{1.52 \pm 0.09}$	127	$\boldsymbol{1.32\pm0.03}$	256	$3.03 \pm 0.03$
Magnesium, Mg	mg	38	$26\pm1.3$	30	$65\pm1.5$	37	$88 \pm 1.4$	125	$\textbf{76} \pm \textbf{1.2}$	108	$24 \pm 0.4$
Phosphorus, P	mg	38	$\textbf{124} \pm \textbf{7.9}$	42	$\textbf{314} \pm \textbf{26.2}$	37	$\textbf{205} \pm \textbf{5.4}$	124	$\textbf{185} \pm \textbf{3.6}$	96	$94\pm1.7$
Potassium, K	mg	40	$131\pm7.3$	35	$\textbf{154} \pm \textbf{7.2}$	37	$197 \pm 6.5$	121	$142\pm2.8$	105	$119\pm2.0$
Sodium, Na	mg	40	$478 \pm 24.0$	3	$\textbf{161} \pm \textbf{15.0}$	42	$\textbf{528} \pm \textbf{21.2}$	128	$630 \pm 12.9$	130	$538 \pm 8.6$
Zinc, Zn	mg	40	$\textbf{0.71} \pm \textbf{0.08}$	33	$\boldsymbol{0.94 \pm 0.08}$	10	$\boldsymbol{1.53 \pm 0.09}$	94	$\boldsymbol{1.26\pm0.05}$	111	$\boldsymbol{0.62 \pm 0.02}$
Copper, Cu	mg	36	$\boldsymbol{0.267 \pm 0.14}$	13	$\textbf{0.154} \pm \textbf{0.015}$	31	$\boldsymbol{0.120 \pm 0.009}$	127	$\textbf{0.161} \pm \textbf{0.017}$	102	$0.126 \pm 0.003$
Manganese, Mn	mg	35	$0.462 \pm 0.011$	12	$0.402 \pm 0.011$	28	$0.382 \pm 0.010$	95	$\textbf{0.381} \pm \textbf{0.014}$	103	$0.383 \pm 0.008$
Selenium, Se	mcg	34	$\textbf{23.4} \pm \textbf{2.4}$	5	$\textbf{5.5} \pm \textbf{1.1}$	39	$6.7 \pm 0.8$	39	$6.7 \pm 0.8$	175	$\textbf{28.2} \pm \textbf{1.1}$
Vitamins											
Folate, food	mcg	10	$12\pm1.1$	11	$15\pm1.3$	1	10	1	20	1	34
Niacin	mg	14	$3.572 \pm 0.135$	33	$1.498 \pm 0.096$	35	$1.279 \pm 0.051$	92	$1.183 \pm 0.057$	198	$\boldsymbol{3.969 \pm 0.034}$
Pantothenic Acid	mg	12	$\textbf{0.582} \pm \textbf{0.061}$	12	$\boldsymbol{0.194 \pm 0.014}$	2	$\boldsymbol{0.788 \pm 0.026}$	1	0.394	25	$\boldsymbol{0.390 \pm 0.015}$
Riboflavin	mg	14	$0.293 \pm 0.069$	23	$0.073 \pm 0.008$	30	$\textbf{0.184} \pm \textbf{0.024}$	88	$\textbf{0.144} \pm \textbf{0.010}$	177	$\textbf{0.341} \pm \textbf{0.006}$
Thiamin	mg	14	$\textbf{0.531} \pm \textbf{0.025}$	31	$0.112 \pm 0.015$	29	$\boldsymbol{0.075 \pm 0.008}$	83	$\boldsymbol{0.027 \pm 0.005}$	190	$0.472 \pm 0.005$
Vitamin A	IU	1	0	1	0	12	$196\pm23$	29	$94 \pm 11$	1	0
Vitamin B-6	mg	2	$0.050 \pm -0.01$	11	$\boldsymbol{0.219 \pm 0.03}$	14	$0.286 \pm -0.01$	18	$\textbf{0.24} \pm \textbf{0.03}$	6	$0.06\pm0.01$

<sup>&</sup>lt;sup>a</sup> US Department of Agriculture, Agricultural Research Service, 2002. USDA National Nutrient Database for Standard Reference, Release 15. Nutrient Data Laboratory Webpage, http://www.nal.usda.gov/fnic/foodcomp

these flours are optionally fortified with 5% soy flour. Human nutritional studies have demonstrated that fortified and enriched flours significantly upgrade the nutritional status of low-income people, especially children. Quality protein maize (QPM), which contains 50% more lysine and tryptophan, is processed into tortillas in some areas of Mexico. The tortillas have improved protein quality but limited production of QPM restricts its application.

See also: Grain Production and Consumption: Cereal Grains in North America; South America. Maize: Quality Protein Maize; Foods from Maize. Wheat: Grading and Segregation; Dry Milling.

#### **Further Reading**

- Almeida Dominguez HD, Cepeda M, and Rooney LW (1996) Properties of commercial nixtamalized corn flours. *Cereal Foods World* 41: 624–630.
- Betran J, Bockholt AJ, and Rooney LW (2001) Blue corn. In: Hallauer A (ed.) *Specialty Corns*, 2nd edn., pp. 293–301. New York, NY: CRC Press.
- Bressani R (1990) Chemistry, technology, and nutritive value of maize tortillas. *Foods Reviews International* 62(2): 225–264.
- Dally V and Navarro L (1999) Flour tortillas: a growing sector in the US food industry. *Cereal Foods World* 44: 457–459.
- McDonough CM, Seetharaman K, Waniska RD, and Rooney LW (1996) Microstructural changes documented by traditional and environmental scanning electron microscopy in wheat flour tortillas during baking. *Journal of Food Science* 61(5): 995–999.
- McDonough CM, Gomez MH, Rooney LW, and Serna-Saldivar SO (2001) Alkaline-cooked corn products. In: Lusas E and Rooney LW (eds.) *Snack Foods Processing*, pp. 73–113. New York, NY: CRC Press.
- Rooney LW and Serna-Saldivar SO (2003) Food use of whole corn and dry milled fractions. In: White P and

- Johnson L (eds.) Corn (Zea mays L.): Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.
- Rooney LW and Suhendro EL (1999) Perspectives on nixtamalization (alkaline cooking) of maize for tortillas and snacks. *Cereal Foods World* 44: 466–470.
- Rooney LW and Suhendro EL (2001) Food quality of corn. In: Lusas E and Rooney LW (eds.) *Snack Foods Processing*, pp. 39–71. New York, NY: CRC Press.
- Serna-Saldivar SO and Rooney LW (1994) Quality protein maize processing and perspectives for industrial utilization. In: Larkins B and Mertz ET (eds.) *Proceedings of the International Symposium on Quality Protein Maize*, pp. 89–120. Sete Lagoas, MG, Brazil: EMBRAPA/CNPMS.
- Serna-Saldivar SO, Rooney LW, and Waniska RD (1988) Wheat flour tortilla production. *Cereal Foods World* 23: 855–864.
- Serna-Saldivar SO, Gomez MH, and Rooney LW (2001) Food uses of regular and specialty corns and their drymilled fractions. In: Hallauer A (ed.) *Specialty Corns*, 2nd edn., pp. 303–337. Boca Ratone, FL: CRC Press.
- Snack Food Association (2003) Snack Food Association State of the Industry Report. *Snack World*, June. 20–45.
- Waniska RD (1999) Perspectives on flour tortillas. *Cereal Foods World* 44: 471–473.
- Waniska RD (2001) Evaluation methods and quality control for snacks. In: Lusas E and Rooney LW (eds.) *Snack Foods Processing*, pp. 607–629. Lancaster, PA: Technomic Publishing Co.

#### **Relevant Websites**

http://www.nal.usda.gov — US Department of Agriculture, Agricultural Research Service (2002) website. The Nutrient Data Laboratory Webpage provides information on the USDA National Nutrient Database for Standard Reference, Release 15.

## **TRITICALE**

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#### Introduction

Triticale is the first man-made cereal grain crop species resulting from the hybridization of wheat (*Triticum*) with rve (*Secale*), the name of which combines the

scientific names of the two genera involved. This synthetic amphiploid is obtained by chromosomal doubling after artificial crossing to produce fertile hybrids. It is a small-seeded cereal grain that is used for both human consumption and livestock feed. As a hybrid species, it combines many of the better qualities of both of its parents. Triticale possesses wheat's properties for food production and rye's adaptive properties, and under certain conditions can

out-yield both parents. This promising crop species is grown on more than 3 million hectares (Mha) worldwide. Furthermore, triticale is an important germplasm source for wheat improvement, providing a vehicle to transfer desirable rye characteristics to wheat.

#### **Origin and Types**

The Scottish scientist Alexander Stephen Wilson produced the first triticale in 1876. Triticale was initially developed to combine the positive traits of both parent types: the vigor and winter hardiness as well as the higher protein content of rye combined with the higher-quality gluten and baking properties of wheat. However, initial progress was limited by the fact that resulting hybrid progeny was sterile. In the 1930s, the discovery and use of the chemical colchicine, a natural chemical extracted from the autumn crocus plant to create chromosome "doubling," overcame this sterility problem. In 1938, Arne Müntzing from Sweden applied colchicine to wheat/rye hybrids, obtaining fertile plants. Once a fertile hybrid was established, it became possible to utilize modern plant-breeding methodologies. Early varieties were primitive and had numerous agronomic disadvantages such as low grain yield, poor seed set, shriveled grain, excessive height, low germination, and late maturity. Triticale improvement commenced in the 1960s to create new and better combinations between wheat and rye, triticale and triticale, and triticale and wheat. Most notable were breeding programs at the International Center for Maize and Wheat Improvement (CIMMYT) in Mexico and the University of California at Davis (UCD) for spring triticales and programs in Poland and the University of Manitoba, Canada for winter varieties.

There are two main types of cultivated triticales: octoploid types produced from the hybridization of bread wheat, Triticum aestivum L., with rye, Secale cereale L., and hexaploid types using durum wheat, T. turgidum L., followed by chromosome doubling of the hybrid plant (Figure 1). Octoploid triticales (2n = 56) contain the A, B, and D genomes of bread wheat and the R genome of rye, while the hexaploid triticales (2n = 42) contain the A and B genomes of durum wheat and the R genome of rye. However, most triticale cultivars are hexaploids. There is a third type of triticale (2n = 28) produced from the hybridization of diploid wheat, T. monococcum (2n = 14) with rye, but is not considered to be important economically. Although triticale is a cross between wheat and rye, it is self-pollinating (similar to wheat) rather than cross-pollinating (like rye). Most agronomically desirable triticales that breed true have

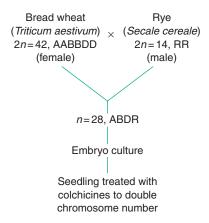


Figure 1 Flow diagram for octoploid triticale development showing chromosome number and genome identifications. The hexaploid type (2n = 42) is produced similarly, though using durum wheat (T. turgidum, 2n = 28, AABB) as the female parent. (Adapted with permission from Qualset CO (2002). Triticale. In: McGraw Hill Encyclopedia of Science and Technology, 9th edn. The McGraw-Hill Companies, Inc.)

resulted from several cycles of improvement, and are primarily from the durum-rye crosses with some common wheat parentage occasionally involved.

In general, triticales can be divided into three groups

- 1. "Primary triticales" are the initial product of the wheat × rye hybridization followed by doubling of chromosome numbers to produce the hexaploid or octoploid types.
- 2. "Secondary triticales" are produced by intercrossing primary triticales or by crossing a primary triticale with wheat.
- 3. "Substituted secondary triticales" are hexaploid with A, B, or D genomes of wheat substituted for one or more R genome chromosomes of rye.

Primary triticales are often found to be fragile, poor producers, and genetically unstable. They are used as breeding stock to produce the more stable and agronomically favorable secondary and substituted secondary triticales. Secondary triticales can be either hexaploid or octoploid and often contain complete genomes of wheat and rye, whereas substituted triticales never have complete rye genomes (Table 1). One advantage of the secondary hexaploid triticale is increased genomic diversity, resulting from the insertion of portions of the D genome from the hexaploid wheats. Spike type is often used as a visual morphological marker to distinguish types. Octoploid triticale spikes appear similar to wheat spikes, whereas hexaploid triticales have more distinctive spike types (Figure 2), and are classified as Beagle (for complete triticales) and Armadillo (for substituted triticales).

Table 1 Development and examples of primary, secondary, and substituted triticales

Primary triticale <sup>a</sup>			
Common wheat X  Triticum aestivum L.  AABBDD (2n = 42)	Rye <i>Secale cereale</i> L. RR (2 <i>n</i> = 14)		Octoploid triticale AABBDDRR (2n = 56)
Durum wheat $X$ $T. turgidum L.$ AABB $(2n = 28)$	Rye <i>Secale cereale</i> L. RR (2 <i>n</i> = 14)		Hexaploid triticale AABBRR $(2n = 42)$
Einkorn wheat X T. monococcum L.	Rye <i>Secale cereale</i> L.	<b>&gt;</b>	Tetraploid triticale AARR $(2n = 28)$
Secondary triticale <sup>b</sup>			
Triticale X AABBDDRR AABBDDRR AABBRR	Triticale AABBDD AABBRR AABBRR		AABBDDRR AABBDDRR or AABBRR AABBRR
Triticale X AABBDDRR or AABBRR AABBDDRR or AABBRR AABBDDRR or AABBRR	Wheat AABBDD AABB AA		
Substituted triticale <sup>c</sup> $A_7A_7B_7B_7D_2D_2R_3R_3$ $A_6A_6B_6B_6D_6D_6R_3R_3$ $A_7A_7B_6B_6D_7D_7R_1R_1$			

<sup>&</sup>lt;sup>a</sup>Colchicine treatment is given to hybrid plants to double chromosome number.

Adapted from Qualset CO, EA Rupert, and JD Prato (1973) Triticale in California: review of current research and appraisal as a new cereal crop. In: Yang SP (ed.) Proceedings of the International Triticale Symposium. Lubbock, TX: International Center for Arid and Semi-Arid Land Studies.



Figure 2 Spike types representing substituted (left, Armadillo) and complete (right, Beagle) forms of triticale.

#### **Adaptation and Production**

Triticale is grown using cultural practices similar to wheat and rye. However under some conditions, earlier planting can result in better yields. It works well planted alone, as a companion crop for establishing alfalfa and for interseeding into established alfalfa, and as a double crop with corn and other summer annuals. There are both spring and winter growth habits depending on the parents used in the cross, with environmental requirements similar to other winter and spring sown cereal grains. Drought tolerance is

<sup>&</sup>lt;sup>b</sup> Products of these hybrids have variable chromosome constitutions.

<sup>&</sup>lt;sup>c</sup> Examples with 2n = 42. Subscripts indicate the number of chromosomes present from each genome.

**Table 2** Desirable characteristics of wheat, rye, and triticale<sup>a</sup>

Wheat	Rye	Triticale
High-yield potential Large, filled grain High harvest index Tillering efficiency Short straw Sprouting resistance High-energy grain	Many grains per ear High biomass Low-temperature growth Winter hardiness Drought tolerance Disease resistance Grain high in lysine	High yield High-quality straw High feed value Disease resistant Stress tolerant Winter hardiness High lysine content

<sup>&</sup>lt;sup>a</sup>Modified from Semundo Limited (1994) Triticale The Hybrid Evolution. Cambridge: Semundo Limited.

the primary advantage that spring triticales have over other spring cereal crops. Winter triticale provides a high-yielding early maturing alternative to spring triticale for short-season areas. The University of Manitoba began the first intensive program in North America in 1953, working mostly with durum wheat-rye crosses. Since then, triticale has been the subject of modern plant breeding efforts for and has resulted in excellent gains in yield and quality. Triticale most closely resembles its wheat parent but exhibits more vigorous growth characteristics. As a hybrid species, it contains many of the better traits from each parent (Table 2). Triticale can combine the bread-making qualities of wheat with much of rye's adaptive properties such as disease resistances, drought tolerance, and adaptability to harsh soil conditions. As a result, varieties have been produced with a wide adaptive range as well as site-specific adaptation. Triticale does well in regions where wheat performs poorly, such as cold and infertile soils, extremely sandy soils, soils with high levels of boron, salty soils, acidic soils, manganese-deficient soils, and dry soils. One particular concern, however, is the presence of ergot infection (caused by the fungus Claviceps purpurea) in some areas.

The first commercial triticale cultivars were released in 1969. Today triticale is becoming a crop in its own right and is grown on over 3 Mha worldwide and in at least 27 countries (Table 3). This crop contributes more than 10 Mt year<sup>-1</sup> to global cereal production. Since its introduction, the area harvested has increased over 7 times and amount harvested has increased over 18 times (Figure 3).

Although it is grown throughout the globe, the countries that produce the most triticale are China, Poland, and Germany. There is also significant production in Canada and United States.

#### Usage

Use of triticale for human consumption has not yet become widespread. Although triticale flour and

Table 3 World triticale production, 2002

Country	Growth type <sup>a</sup>	Area (ha)	% World
China	S+W	500 000	16.1
Poland	W	920 523	29.7
Germany	W	560 466	18.1
France	S + W	269 000	8.7
Australia	S	264 000	8.5
Hungary	W	132 000	4.3
Belarus	W	94 200	3.0
Czech Republic	W	53 093	1.7
Canada	S + W	47 282	1.5
Denmark	W	37 657	1.2
Austria	W	37 621	1.2
Sweden	W	30 740	1.0
Spain	S	29 900	1.0
Portugal	S	25 000	8.0
Lithuania	W	20 000	0.6
Slovakia	W	18 372	0.6
Latvia	W	15 500	0.5
United Kingdom	W	14 000	0.5
Switzerland	W	13 500	0.4
Belgium	W	12 000	0.4
United States	S + W	8979	0.3
Estonia	W	6847	0.2
Netherlands	W	4618	0.1
Luxembourg	W	4000	0.1
Norway	W	1000	0.03
Tunisia	S	1000	0.03
Mexico	S	850	0.03
Algeria	S	3	0.0001
World		3 122 151	100

<sup>&</sup>lt;sup>a</sup>S: spring type; W: winter type.

Sources: United Nations FAOSTATS, Statistics Canada, and United States Census of Agriculture.

products are available commercially (namely in specialty markets such as health food stores), this availability is limited. It comes in several forms including whole berry, flakes, and flour. Whole triticale can be cooked and used in a variety of dishes. Quality evaluations have shown triticale grain inferior to wheat for milling and baking, making large-scale commercial baking not feasible. Triticale flour is low in gluten, and bread made from it alone is heavy. For that reason, it is usually combined half-and-half with wheat flour. If mixed with wheat or rye flour, triticale flour can be used to make a number of breads and pastries. In developing countries, triticale flour is often mixed with wheat flour during wheat shortages. It is of course important that the crop is not infected with ergot, as this is highly toxic to humans.

Most triticale production is used for animal feed. It offers better amino acid balance, lysine content, and higher protein, particularly important for swine and poultry. However, triticale has lower energy content than other grains, and feeding of triticale must be supplemented with other grains. It can also be used as forage, silage, or hav for ruminants, offering high

Figure 3 World production (Mt) and area (ha) trends since the 1970s. (Source: United Nations FAOSTAT.)

digestibility and out-yielding traditional crops in dry soils. Care should also be taken to insure that the crop is not infected with ergot.

Alternative uses of triticale include use as a cover crop to prevent soil erosion and land reclamation. Triticale has also been used in limited amounts as raw material in bioethanol production. Ethanol plants pay a premium for triticale over barley, since it has more starch and no hull, making alcohol production more efficient.

#### **Genetic Resources**

As a synthesized species, triticale has no wild ancestors and there are no existing landrace varieties. In addition, the actual wheat and rye parents used in triticale synthesis are often either unknown or no longer available. It is therefore often not possible to resynthesize unique triticale genotypes through hybridization. Genetic resources for the development and enhancement of triticale include existing triticales, wheat and rye, and the ancestral species of both wheat and rye. Itself, triticale exists as a genetic resource for the improvement of wheat, providing a vehicle to transfer desirable characteristics from rye.

In order to insure continued improvement in triticale, it is important to maintain a comprehensive genetic resource collection. CIMMYT has established a world gene bank for triticale and has over 15 000 accessions. The North American triticale genetic resource collection was evaluated at the UCD and showed a great deal of variation in both qualitative and quantitative traits. The collection is now maintained at the USDA Small Grains Collection in Aberdeen, ID, and at CIMMYT in Mexico.

#### **Future Prospects**

Triticale production has increased tremendously since the 1970s and genetic improvements have been vast. It can only be expected that improvements will continue, especially with the tools provided by biotechnology. *In vitro* regeneration of plants will allow for successful genetic transformation. Genomic maps for wheat and rye have been completed and will provide invaluable assistance for marker-aided selection.

Although used primarily for animal culture, it holds promise on a number of additional levels. Perhaps one of triticale's greatest potential is as a vehicle for gene exchange for wheat improvement, extending wheat's gene pool. It, however, still holds promise to be a leading food crop in some areas of the world. Continual improvements are being made to increase triticale's grain quality for commercial production. There is a great deal of potential for triticale products in the specialty markets, especially in the west where a healthier and more varied diet is becoming increasingly popular and commercialized. Triticale also has potential for increasing global food production in developing countries. It grows in many areas unsuitable for wheat production and can out-yield wheat in certain areas. Already used in many of these countries to some degree, increased production for food would likely find a market, especially in areas where wheat shortages are prevalent. Triticale may also play an ecological role in the future, both for soil reclamation and biogas usage.

See also: Animal Feed. Cereals: Protein Chemistry. Consumer Trends in Consumption. Nutrition: Effects of Food Processing. Rye. Taxonomic Classification of Grain Species. Wheat: Genetics; Breeding.

#### **Further Reading**

- Darvey NL, Naeem H, and Gustafson JP (1991) Triticale: production and utilization. In: Kulp K and Ponte JG, Jr (eds.) Handbook of Cereal Science and Technology. New York: Marcel Dekker.
- Forsberg RA (ed.) (1985) Triticale CSSA Special Publication Number 9. Madison, WI: Crop Science Society of America.
- Furman BJ, Qualset CO, Skovmand B, Heaton JH, Corke H, and Wesenberg DM (1997) Characterization and analysis of North American triticale genetic resources. *Crop Science* 37: 1951–1959.
- Guedes-Pinto H, Darvey N, and Carnde VP (eds.) (1996) Triticale: Today and Tomorrow. Boston, MA: Kluwer Academic.
- Gupta PK and Priyadarshan PM (1982) Triticale: present status and future prospects. Advances in Genetics 21: 255 - 345.
- International Board for Plant Genetic Resources (1985) Descriptors for rye and triticale. IBPGR Secretariat, Rome.
- Lorenz K (1974) The history, development, and utilization of Triticale. Critical Reviews in Food Technology 5(2): 175 - 280.

- MacIntyre R and Campbell M (eds.) (1973) Triticale Proceedings of an International Symposium. Ottawa, Canada: International Development Research Center.
- Muntzing A (1979) Triticale Results and Problems. Berlin, Germany: Verlag Parl Parey.
- National Research Council (1989) Triticale: A Promising Addition to the World's Cereal Grains. Washington, DC: National Academy Press.
- Qualset CO (2002) Triticale. In: McGraw Hill Encyclopedia of Science and Technology, 9th edn. The McGraw-Hill Companies, Inc.
- Qualset CO, Rupert EA, and Prato JD (1973) Triticale in California: review of current research and appraisal as a new cereal crop. In: Yang SP (ed.) Proceedings of the International Triticale Symposium. Lubbock, Texas: International Center for Arid and Semi-Arid Land Studies.
- Semundo Limited (1994) Triticale The Hybrid Evolution. Cambridge: Semundo Limited.
- Tsen CC (ed.) (1974) Triticale: First Man-Made Cereal. St. Paul, MN: American Association of Cereal Chemists.
- Yang SP (ed.) (1973) Proceedings of the International Triticale Symposium. Lubbock, TX: International Center for Arid and Semi-Arid Land Studies.

# V

## VARIETY IDENTIFICATION OF CEREAL GRAINS

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#### Introduction

The need to identify varieties and species of grains arises because they may be significantly different in their genetic traits with respect to grain-quality attributes and according to their agronomic potential, e.g., resistance to pathogens. Therefore, it is important that there should be adequate means available to permit distinction to be made between these various genotypes (genetically different forms). This article summarizes the range of methods in use and under development for routine identification of cereal varieties.

The need for variety identification arises especially at the stages of sowing and harvest. The farmer needs to insure that the seed being planted is of the correct genotype, and the grain buyer needs assurance at harvest that the quality type of the grain is appropriate for the planned utilization.

Variety identification is especially needed for wheat, because of the wide range of quality types available. The examples in **Table 1** include two hard-wheat varieties suited to conventional bread production

(Marquis and Gabo) and a soft wheat (Rosella), whose weaker dough properties make it suitable for making cookies and cakes. The varieties Marquis and Gabo are very old wheats, now superseded by varieties with better resistance to rust and superior yield potential. Furthermore, these two wheats themselves are examples of families of wheats adapted to very different growth environments, namely, spring sowing after the winter of the Canadian prairies (Marquis) versus the moderate climate of the wheat belt in northern New South Wales, Australia. These three examples, all of the same genus and species, must in turn be distinguished from durum wheat, which is a different species, suited specifically to the manufacture of pasta products.

#### **Historical Background**

Variety identification often involves the need to make subtle distinctions among plants or grain samples of similar appearance. The need for such distinctions has arisen since the early twentieth century as a result of the efforts of plant breeders to produce genotypes suited for specific niches of agronomy, climate, and quality type. At the turn of the twentieth century, there was poor knowledge about distinctions between genotypes within grain species. Seed for sowing may have contained a range of genotypes, due to poor

Table 1 Examples of different species and varieties of grain

Common name	Genus and species	Variety (date of release)	Characteristics
Bread wheat	Triticum aestivum	Marquis (1911): at least two biotypes	A hard red spring wheat of good bread-making quality adapted to the Canadian prairies
Bread wheat	Triticum aestivum	Gabo (1945)	A good baking white-grained wheat adapted to the Australian prime-hard region
Bread wheat	Triticum aestivum	Rosella (1986)	An Australian soft white wheat suited to biscuit manufacture
Durum wheat	Triticum turgidum	Tamaroi(1998)	A very hard grain, suited for pasta manufacture
Malting barley	Hordeum vulgare	Clipper (1968)	A two-row barley, grown in Australia for malting purposes
Feed barley	Hordeum vulgare	Cape (from South Africa, about 1900)	A very old six-row variety, not suitable for malting, once grown in Australia for animal feed
Maize	Zea mays	StarLink	Maize, genetically modified to provide herbicide resistance

**Figure 1** Pedigree chart of two malting barley varieties, illustrating their descent from a cross between Proctor and Prior A, which in turn came from the earlier cross (Prior  $\times$  Kwan) and selection from Archer.

attention to the need to select at least for uniformity of plant and grain appearance. Mixtures of seed types also arose as a result of neglect in failing to keep different types separate.

Early attempts to produce improved varieties involved the selection of superior plants from an otherwise varied crop, to permit the separate propagation of genotypes that showed, for example, well-filled heads or resistance to pathogen attack. Improvement by selection is illustrated in Figure 1, by the selection of the malting barley Prior (released in Australia in about 1900) from the traditional English barley Archer. Individual farmers often practiced this approach, passing on their improved variety to neighbors and selling the seed on a wider scale. The introduction of crosspollination, a century or so ago, greatly increased the genetic diversity from which to select improved genotypes.

#### **Varieties and Other Taxonomic Levels**

Cross-breeding is illustrated in Figure 1 by the production of two Australian malting barleys, Clipper and Cutter, released respectively in 1968 and 1975. They are "sister lines," being separate selections from the same cross, involving an old English variety Proctor (named in 1952) and Prior A, from the cross between the varieties Kwan and Prior. The task of distinguishing between these sister lines would be expected to be more difficult than making a distinction between either of them and, for example, the unrelated variety Cape (Table 1). Even more evident, of course, is the difference between any of these barleys and representatives of a distinct species or genus, e.g., wheat (genus *Triticum*) or maize (genus *Zea*: Table 1).

The practice of finally selecting a variety for registration often involves taking several plants from an advanced line many generations from the original cross. The combinations of genes in these lines are thus "fixed" (homozygous, of stable genotype), but they may differ from one another in subtle ways, although they are uniform in appearance as plants. Subsequent analysis of individual grains by sensitive

methods of variety identification may provide a distinction between these component genotypes (termed "biotypes"), although all are authentic parts of the same variety. For example, gel electrophoresis of the grain proteins of the wheat Marquis (Table 1) has shown that there are distinct biotypes; one of these, Marquis K has been suggested as a standard sample for electrophoretic identification.

The term "cultivar" has been used to indicate a homogeneous population of plants of common pedigree which breeds true. The word is loosely used as being equivalent to the term "variety," but, in theory, "cultivar" refers mainly to plants having a single phenotype, whereas the term "variety" is slightly less restrictive. Nevertheless, common usage continues with the term "variety," and it is adopted throughout this article.

Recent years have seen a further breakthrough in breeding technologies, involving the use of genetic modification (GM) to introduce genes from species unrelated to the target genotype. An example is the maize variety, StarLink (Table 1). The need for "identity preservation" of GM varieties in some regions adds to the need for variety identification, so that such grain is received and transported separately from conventionally bred varieties. However, identity preservation is not restricted to GM varieties; it is a standard practice in many grain-producing countries to insure that grain of premium quality is not mixed with that of inferior or different quality type.

#### **Plant Breeders' Rights Requirements**

Another important reason for satisfactory methods of variety identification is the need to implement plant breeders' rights (see Variety Registration and Breeders' Rights), the system for breeding organizations to be remunerated financially for the use of their varieties, in a process similar to that of copyright laws. These systems require that a new variety must be registered, defining its identity, with evidence that it is distinct from other varieties of the respective species, that it is uniform with respect to relevant characteristics, and that it is stable for those characteristics from one generation to another. This process should also provide the means of identification, permitting this variety subsequently to be identified at the time of sale of seed or delivery of harvested grain.

However, it has become difficult to provide methods that are sufficiently discriminatory for this purpose, especially for closely related varieties (such as sister lines – Figure 1) without the need for excessive cost, labor input, or resources in expertise or equipment. Methods used for registration under PBR may not be readily deployable in "field" situations.

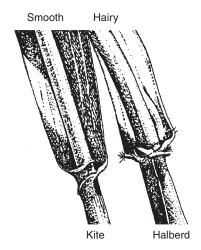
The range of methods devised for distinguishing between varieties of cereal grains differs according to their discriminating ability and the degree of difficulty in implementation.

#### Grain and Plant Morphology for Identification

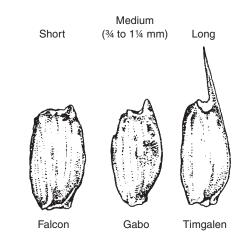
Traditionally, distinction has involved visual inspection of plants or grain, probably aided by reference manuals listing systematic descriptions relevant to the species under examination (*see* Grain and Plants, Morphology). This approach satisfies the requirements of speed and minimal resources, but it is subjective and its capability for discrimination is generally poor. Visual examination can be implemented in any situation "on the spot," requiring no more than good lighting, and possibly a hand lens for magnifying small details. Experience is obviously essential, even when reference manuals are available, often involving a lifelong vocation.

The specific characteristics that provide distinction differ from one species of grain to another. Ideally, these distinguishing markers are genetically determined, without modification by variations in growth conditions. This ideal situation is often not possible. Nevertheless, such markers are available to a limited extent. For example, color differences are an obvious basis for distinction, such as blue aleurone coloration in barley grain, and red/white grain in wheat. Other useful characteristics are illustrated in Figures 2 and 3 for wheat plants. The auricles of a young wheat plant (3–6-leaf stage) may be either smooth or hairy, the auricle being the claw-like appendage that clasps the stem of the wheat plant at the base of the leaf (Figure 2). Genetic variation is also seen in the shape of the glumes – the outer husk that surrounds the grain in the mature wheat head (Figure 3). Particularly evident is the length of the "beak" (a spike projecting from the creased edge of the glume at the end of the glume opposite to the point of attachment to the rachis). This characteristic is especially useful since glumes are often found in grain samples, so that its value is not necessarily confined to plant identification.

In recent years, attempts have been made to obtain objective identification of varietal differences in morphological characteristics using image analysis, taking advantage of the speed and power of computing to analyze the image of grain profiles from a video camera. The technology appears to require further development for it to permit sufficient discrimination for routine variety identification, but image analysis is well suited to provide distinction between grains of different quality types or distinct species.



**Figure 2** Smooth and hairy auricles of young wheat plants. (Reproduced with permission from Ferns GK, Fitzimmons RW, Martin RH, Simmonds DH, and Wrigley CW (1975) *Australian Wheat Varieties: Identification According to Growth, Head and Grain Characteristics.* Melbourne: CSIRO.)



**Figure 3** Variation in beak length for glumes (husk) of wheat. (Reproduced with permission from Ferns GK, Fitzimmons RW, Martin RH, Simmonds DH, and Wrigley CW (1975) *Australian Wheat Varieties: Identification According to Growth, Head and Grain Characteristics.* Melbourne: CSIRO.)

# **Established Laboratory Methods for Variety Identification**

To overcome the subjective nature of visual examination, recourse has been made to a range of laboratorybased methods to achieve objective and definite identification.

#### The Phenol Test

A simple test, for example, for wheat varieties is the phenol test. This involves soaking grains in water for several hours, and spreading them on filter paper that is wetted with a 1% solution of phenol in water. After a few hours at room temperature (20–25 $^{\circ}$ C), there is color development (Figure 4) according to the genotype of the variety, shown as degrees of brown from colorless through to black. Generally, four degrees of coloration are distinguished – no change, light, medium, and dark - these are gaged by the inclusion of grains of known phenol reaction. The test thus does not have great discriminating power, but it has the advantages of low cost and ease of performance, especially for large numbers of grains. This latter advantage is considerable in relation to assessing statistical significance for a sample containing a mixture of varieties that differ in phenol reaction. The genes responsible for the phenol reaction have been mapped to specific chromosomes of wheat, namely, chromosomes 2A and 2D (see Wheat: Genetics), based on studies of the variety Chinese Spring. The phenol test can also be applied to the glumes of wheat as a further indication of variety, provided head samples are available, or if the grain sample contains husk material. The results of the phenol test are not affected by variations in the growing conditions of the wheat grain, except in the case of severe deficiency of copper,



**Figure 4** Color changes in wheat grains due to application of the phenol test. Most of the grains on the disk of filter paper have given a light-brown reaction. Grains of a different variety have turned dark brown.

as the enzymes responsible for the phenol reaction require copper as a cofactor.

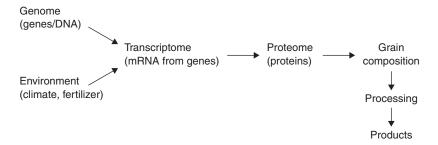
#### **Testing at the DNA and Protein Levels**

The most commonly used laboratory tests for variety involve analysis at the gene (DNA) or protein levels. New technologies at the genome level offer the possibility of conducting varietal identification by DNA analysis efficiently and economically. Analysis at the genome level excludes the possibility of interference from fluctuations in the growth conditions, because it occurs at the start of the sequence of events shown in Figure 5. These events extend from the genes through protein synthesis to the formation of all components of the grain. This composition largely determines processing characteristics and final product quality.

The second major possibility for variety identification — the analysis of protein composition — provides the risk that the influence of growth environment may interfere with the attempt to identify genotype. However, analyses of the storage proteins of many cereal grains have demonstrated that the influence of growth conditions is not so great as to cause major difficulties in the interpretation of the results. Major advantages of choosing protein composition as the basis for study are that proteins are more abundant, compared to DNA, and that proteins are easier to extract for analysis by traditional methods. Several technologies of protein fractionation have been applied to variety identification. They differ in their complexity, cost (in capital and consumables), and distinguishing ability.

#### **Gel Electrophoresis**

Analysis of grain-protein composition by polyacrylamide gel electrophoresis (PAGE) has been accepted for some decades as the routine method of identifying cereal varieties in the laboratory. There are standard methods for the conduct of electrophoretic identification adopted by several scientific bodies, including the International Association for Cereal Science and Technology (ICC), the International Seed Testing



**Figure 5** The sequence of events from genes, through protein synthesis to the final determination of product quality. Analysis of varietal identity is generally directed at the DNA or protein levels.

Association, and the Royal Australian Chemical Institute.

Extraction of grain proteins For electrophoretic identification, proteins are extracted from the milled grain (or a single crushed grain), using a solvent that selectively dissolves the relevant class of proteins (e.g., wheat gliadins). The proteins in solution are separated from the insoluble material by centrifugation. A small amount (a few microliters) of the protein solution is applied to the top of a slab of gel material, held between parallel plates, and under the electrolyte buffer that serves to conduct the electric current (DC) that is next applied (Figure 6). The protein molecules migrate into the gel according to the strength of attraction caused by their electric charge, with a retarding effect proportional to the size of the protein molecules.

At the end of the electrophoresis run, the gel material is removed and is stained to visualize the protein zones inside the gel. Figure 6 shows protein extract being loaded on to the top of a precast gel in the electrophoresis equipment. Figure 7 shows the results from one slab gel, on which several protein samples have been run in parallel. The result for each sample is represented by a series of horizontal lines ("bands") down the gel, each band representing a different protein. Some protein bands (in the same positions) are common to all the varieties (as shown in Figure 7), but there are other protein bands that are the basis of distinction between the varieties.

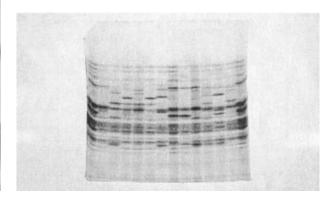
The gel medium In early versions of electrophoretic identification, a starch gel was used as the electrophoresis medium. This was subsequently changed to polyacrylamide gel, involving the polymerization of acrylamide (plus cross-linker) in the laboratory, but

**Figure 6** Electrophoresis equipment in use for variety identification. Grain-protein extract is being loaded on to the top of a precast gel, prior to switching on a voltage (direct current), which will attract the protein molecules down into the gel for separation into their various fractions.

also using precast commercially available gels, provided ready to use, in disposable cassettes. The standard method of the Royal Australian Chemical Institute specifies the use of polyacrylamide gels in which the concentration of polyacrylamide increases from the top (where the sample is applied) to the bottom, thereby creating decreasing pore sizes to sort the proteins according to their size, and to cause sharpening of the protein zones as they pass through the gel medium under the attraction of the electric field.

Different gel ranges are specified for the various cereal grains, and also different extractants and run times, as tabulated in Table 2. These conditions are designed to provide electrophoretic profiles for the prolamin class of proteins from these cereal grains, known as gliadins for wheat, secalins for rye, and hordeins for barley. (see Cereals: Protein Chemistry. Protein Chemistry of Dicotyledonous Grains and Wheat: Grain Proteins and Flour Quality.) In the case of wheat, for example, the gliadins represent ~40% of the protein content of the grain. A further 40% consists of the glutenin proteins, which are not extracted under the conditions of Table 2, due to their large size.

SDS gel electrophoresis The polypeptides of wheat glutenin can be included in the electrophoretic profile by using an alternative system of extraction and electrophoresis. This involves the inclusion of a reducing agent to break disulfide bonds that hold the polypeptides of glutenin, together with the inclusion of the detergent sodium dodecyl sulfate (SDS) in both extraction and gel systems. In this way, many more protein zones may be displayed, thereby increasing the chances of showing differences in protein composition, and thus providing distinction between varieties.



**Figure 7** The gel patterns resulting from electrophoretic identification of a series of wheat varieties on the basis of their gliadin bands, separated by cathodic gel electrophoresis in a precast gel (3–15% polyacrylamide) at pH 3. The gel measures  $\sim$ 7 cm wide and 7 cm high, and is 1 mm thick.

**Table 2** Variations in the conditions of the acidic gradient polyacrylamide gel electrophoresis (PAGE) method, suiting it for various cereals<sup>a</sup>

	Wheat, rye, triticale	Barley	Oats	Rice
Extracting solution Extracting solution volume mg <sup>-1</sup> grain Gradient range of polyacrylamide gel Electrophoresis time at 200 V	6% urea	6% urea + 1% ME	15% urea	18% urea + 1% ME
	6 μl	4 μl	6 μl	4 µl
	3–13%	3-27%	3–27%	3–27%
	150 min	120 min	140 min	90 min

<sup>&</sup>lt;sup>a</sup> Reproduced with permission from Westcott R and Ross D (1995) Official Testing Methods of the Cereal Chemistry Division of the Royal Australian Chemical Institute. Melbourne, Australia: Royal Australian Chemical Institute.

Table 3 Relative efficiencies of four protein-based methods of variety identification

Steps of analysis	Pre-cast PAGE gels	RP-HPLC	CE	MS
Prepare gel or column	10 min	10 min	2 min	0
Extract sample	20 min	20 min	20 min	20 min
Analyze sample	10-90 min	30 min	10 min	>1 min
Reveal protein profile	20 min or overnight; and	Instant	Instant	Instant
Interpret data	10 min	Instant	Instant	Instant
Equipment cost	Low	High	High	Very high
Consumables cost	Medium	Medium	Medium	Low
Labor cost	Moderate	Low	Low	Low

PAGE, polyacrylamide gel electrophoresis; RP-HPLC, reversed-phase high-performance liquid chromatography; CE, capillary electrophoresis; MS, mass spectrometry.

# Towards Better Methods for Variety Identification

The shortcomings of established methods of identification have prompted research into better laboratory-based methods, with the aims of providing good distinction and more efficient handling of samples. The following methods, recently developed, offer these advantages (Table 3).

# Capillary Electrophoresis (CE) and Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

CE and RP-HPLC offer alternatives to gel electrophoresis as a means of achieving efficient fractionation of the grain proteins for variety identification. The bases of fractionation by PAGE, by CE, and by RP-HPLC appear to differ from one another, so the use of any of the three holds promise of providing distinctions between varieties. Standard procedures have been developed for the use of CE and RP-HPLC, detailed in the "Official Testing Methods Handbook" of the Royal Australian Chemical Institute.

Figure 8, for example, shows CE profiles for gliadins from grain of wheat varieties. The respective gliadin proteins have been separated according to their charge properties during passage through a CE column. As the protein zones exit the column, they are detected by their absorption of ultraviolet light, so that the

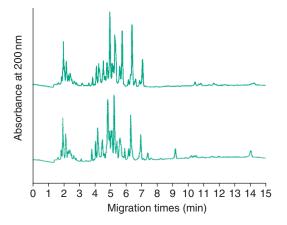
separate fractions appear as a series of peaks, the overall profile providing a "fingerprint" characteristic for the individual variety.

The system of detection is similar in the case of RP-HPLC, but the separation medium is different (a chromatography column is used) and fractionation is based on a different characteristic of the proteins, namely, their hydrophobicity, the respective protein components being retarded to varying degrees as they pass through the column.

Both CE and RP-HPLC are much faster than conventional gel electrophoresis. Rapid identification of wheat varieties is possible in less than 1 h using either CE or RP-HPLC to determine gliadin composition. Furthermore, automatic loading facilities permit analyses to proceed unattended, for example, overnight. They offer the additional advantage of providing the results as electronic data for automatic processing of the results. In this way, the profile of unknown sample can be matched against the stored profiles of authentic samples, allowing the best match to be obtained as the identity of the unknown sample.

# **Mass Spectrometry (MS)**

Mass spectrometry is another alternative developed recently for rapid analysis of identity based on protein composition. The main demonstration of its capability is again based on the analysis of gliadin proteins



**Figure 8** Capillary electrophoresis profiles for gliadin proteins, extracted from grain of wheat varieties Halberd (top) and Cranbrook. (Reproduced with permission from Wrigley CW and Bekes F (2002) Grain-protein composition as a document of wheat-quality type: new approaches to varietal identification. In: Ng PKW and Wrigley CW (eds.) *Wheat Quality Elucidation: The Bushuk Legacy*, pp. 65–86. St. Paul, MN: American Association of Cereal Chemists.)

from wheat grain. In this case, an accurate analysis is provided of the molecular mass of all the gliadin molecules, the resulting profiles being characteristic of the varieties analyzed. In these experiments, growth environment had little impact on the MS spectra. Analysis took only 2 min per sample manually, or 15 s per sample if automated. However, the equipment is expensive. The role for this approach to variety identification may in the future involve a central laboratory, with robotics for sample handling, and large numbers of samples to justify the high costs of the equipment. Nevertheless, the future also holds the prospect of smaller and cheaper MS equipment that may permit the more general use of this approach to variety identification.

#### **Immunoassav**

Aspects of protein composition can also be determined by using the specificity and speed of antibodies to indicate the presence (or absence) of proteins that are markers of specific varieties. The main possibility for this type of analysis is the use of a series of antibodies that would provide color reactions to indicate if the respective marker proteins are present in the extract of grain proteins. In practice, this system can be applied efficiently in the laboratory by using multi-well microtiter trays, with automatic multichannel pipettes and an automatic enzyme-linked immuno-sorbent assay reader. Alternatively, it may be adapted to on-the-spot identification with an extension of the immunochromatography test card, similar to that used in medical diagnostics.

# **Scenarios for Identification**

These various methods of protein fractionation differ in their potential for distinction between varieties, and in their suitability for routine use, based on considerations such as speed, expertise required, and cost. For example, PAGE has the advantage of low cost for the equipment needed, compared to the much more expensive capital costs for the other alternatives described above. However, PAGE is labor-intensive, requiring a degree of skill, and it does not provide the possibility of immediate computer-based interpretation of the results. Relative suitability depends on the situation in which identification is needed. These situations are summarized in Table 4, namely:

- the need for rapid identification, when delivered grain must be assessed within a few minutes, with minimal facilities;
- the regional laboratory, with modest equipment and modest expertise, where questionable samples may be sent for a prompt result to be provided, possibly overnight; and
- a major centralized laboratory, with sophisticated equipment and trained staff, where the emphasis is on the efficient analysis of large numbers of samples. This third scenario suits the approach of having farmers' samples taken at the time of grain delivery, for subsequent analysis to verify the declaration of variety made at delivery.

# Philosophy of Analysis

#### Clarification of the Aim of Identification

A proper appreciation of the aim of identification is needed in designing the best approach to the task, namely, "What is the question?"

- In general, the question to be addressed is one of verification of identity, that is, varietal identity has been specified for the grain sample to be analyzed, and testing requires a "yes/no" answer.
- The more difficult question follows from a "no" answer, namely, "What variety is it?"
- An intermediate possibility is the question "Is this sample variety A or variety B?"

The precision of the answers to any of these questions depends on the discriminating power of the test(s) applied. A reference list of varieties and their characteristics is essential to providing the information needed to make decisions about the type and degree of testing necessary to answer these questions. A test system that discriminates a large set of varieties into only two or three groups (e.g., the phenol test) cannot alone provide a precise answer to any of the

Table 4 The three major situations in which variety identification is needed and relative suitability of methods of identification

Examples of these situations	Situations			
	On-the-spot Grain receival at mill or grain elevator (silo)	Regional lab Backup at mill Breeders' or seed lab Export terminal	Central lab Contract lab for large numbers of post harvest samples	
Requirements in the respective situations	Speed	Overnight results	Efficiency for large numbers of samples	
Visual examination	++			
Image analysis	$+^a$	+	+	
Phenol test		+		
Rapid immunoassay	+ <sup>a</sup>	+		
Immunoassay in lab			++	
Gel electrophoresis		++	+	
RP-HPLC		++	+	
Capillary electrophoresis	$+^{b}$	++	+	
Mass spectrometry			++	

<sup>&</sup>lt;sup>a</sup>Distinguishing ability yet to be established.

Table 5 Statistical interpretation of results from the identification of individual grains sampled from a total consignment of grain

Distinct identity found for given %	Confidence limits <sup>a</sup> (%) depending on total number of grains identified				
of grains identified	10 grains	20 grains	100 grains	500 grains	
0	0-31	0-17	0-4	0-1	
5	_	0-25	2–11	3-7	
10	0-45	1-32	5-17	7-13	
20	3-56	6-44	13-29	17-23	
50	19-81	27-73	40-60	46-54	

<sup>&</sup>lt;sup>a</sup>Limits at 95% confidence level.

above questions. The strategy for efficiently answering the third question requires the results from testing the two varieties to be available, such as would be provided in a reference list. The adequacy of an answer to the second question depends on how comprehensive is the size of the catalog of test results, as well as the discrimination of the tests being applied.

# **Grain Sampling and Statistics**

A basic requirement of satisfactory analysis of variety is that the sample taken for analysis is representative of the whole load of grain. This is relatively simple if it can be assumed that the load is uniform or homogeneous. Otherwise, the taking of a sample for analysis must follow a procedure designed to insure that the subsample reflects the composition of the whole consignment.

An additional consideration relates to interpreting the analysis of many individual grains. Obviously, the results for only one or a few individual grains do not indicate the identity of the whole load if it cannot be assumed to be uniform. Analysis of a milled sample provides a much better indication of identity if only one analysis is to be undertaken initially. In the case of a heterogeneous consignment, it may be necessary to perform grain-by-grain analysis — a tedious and expensive task — and the results must be interpreted for statistical significance.

Table 5 illustrates the problem. If analysis of 10 grains individually showed that they all had the same identity, it could not thus be assumed that the whole sample was uniform; the table shows that this result would indicate a 95% probability that there could be between 0% and 31% of a different variety present. These confidence limits become much narrower with the analysis of more grains. Likewise, if two grains (20%) in 10 were found to be of a different variety, the confidence limits for that different variety in the whole load would be 3–56%.

<sup>&</sup>lt;sup>b</sup>Small-scale capillary electrophoresis equipment promising, but yet to be proven suitable.

RP-HPLC, reversed-phase high-performance liquid chromatography.

Adapted from Wrigley CW and Bekes F (2002) Grain-protein composition as a document of wheat-quality type: New approaches to varietal identification. In: Ng PKW and Wrigley CW (eds.) Wheat Quality Elucidation: The Bushuk Legacy, pp. 65–86. St. Paul, MN: American Association of Cereal Chemists.

# **Future Prospects**

Further developments already at the research stage promise better capabilities for the automatic analysis of large numbers of samples, using DNA or antibody probes, with microarray technologies. In this way, reactions to many such probes are determined simultaneously, thereby greatly increasing the possibility of obtaining distinctions. The remaining tedium of handling large numbers of samples is likely to be overcome by the use of robotics in a central laboratory where such expense would be justified.

See also: Barley: Genetics and Breeding. Cereals: Grain-Quality Attributes. Genetically Modified Grains and the Consumer. Grain and Plants, Morphology. Maize: Genetics. Variety Registration and Breeders' Rights. Wheat: Genetics.

# **Further Reading**

Agrawal RL (1997) *Identifying Characteristics of Crop Varieties*. Enfield, NH: Science Publishers.

Cooke RJ (1992) Electrophoresis Handbook: variety identification *Handbook of Variety Testing*, pp. 1–50. Zurich, Switzerland: International Seed Testing Association.

Ferns GK, Fitzimmons RW, Martin RH, Simmonds DH, and Wrigley CW (1975) Australian Wheat Varieties: Identification According to Growth, Head and Grain Characteristics. Melbourne: CSIRO.

Hervey-Murray CG (1980) The Identification of Cereal Varieties. UK: Cambridge University Press.

Kruger RP and Bietz JA (1994) High Performance Liquid Chromatography of Cereal and Legume Proteins. St. Paul, MN: American Association of Cereal Chemists.

National Institute of Agricultural Botany (regular issues) Botanical Descriptions of Cereal Varieties. Cambridge: National Institute of Agricultural Botany.

Westcott R and Ross D (1995) Official Testing Methods of the Cereal Chemistry Division of the Royal Australian Chemical Institute. Melbourne, Australia: Royal Australian Chemical Institute.

Wrigley CW (ed.) (1995) Identification of Food-Grain Varieties. St. Paul, MN: American Association of Cereal Chemists. Wrigley CW and Bekes F (2002) Grain protein composition as a document of wheat-quality type; new approaches to varietal identification. In: Ng PKW and Wrigley CW (eds.) Wheat Quality Elucidation: The Bushuk Legacy, pp. 65–86. St. Paul, MN: American Association of Cereal Chemists.

Wrigley CW, Autran JC, and Bushuk W (1982) Identification of cereal varieties by gel electrophoresis of the grain proteins. In: Pomeranz Y (ed.) *Advances in Cereal Science and Technology* vol. 5, pp. 211–259. St. Paul, MN: American Association of Cereal Chemists.

# **Relevant Websites**

http://www.icc.or.at/6/0.htm — This website gives details of the Study Group no. 6 on methods of variety identification of the International Association for Cereal Science and Technology (ICC), based in Austria.

http://www.seedtest.org — The International Seed Testing Association (ISTA), whose Secretariat is based in Switzerland, exists to develop, adopt, and publish standard procedures for sampling and testing seeds, and to promote uniform application of these procedures for the evaluation of seeds moving in international trade. The ISTA also promotes research related to seeds, including variety certification.

http://www.niab.com — Originally the National Institute of Agricultural Botany, NIAB is situated in Cambridge, UK. The company is involved in variety evaluation, plant variety rights, seed certification, and seed technology.

http://wheat.pw.usda.gov/index.shtml — GrainGenes is a compilation of molecular and phenotypic information on wheat and other crops. The project is supported by the US Department of Agriculture Plant Genome Research Program and by the community of scientists who are providing the information.

http://www.acas.on.net — Website of the Australian Crop Accreditation System (ACAS), a governmental authority for the registration of crop varieties. ACAS advises on the choice of suitable varieties.

# **VARIETY REGISTRATION AND BREEDERS' RIGHTS**

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# Introduction

It is well known that there are different species of grain crops, with varying end uses. However, within each grain crop there also exists a large number of different types, which might be farmers' selections, landraces, or cultivated varieties (cultivars) actively produced by plant breeders. Such varieties may differ significantly in their genetic potential. These differences relate both to their agronomic potential (e.g., yield, maturity, or disease resistance) and to their suitability for end use, hence affecting the market value of the grain. Thus, for instance, varieties of wheat suitable for bread making may attract a premium in the marketplace, both nationally and internationally. Because there are these differences between varieties of any grain species, it is necessary to have a system of market regulation and consumer protection, usually achieved by naming and registering each variety. Furthermore, there must be a recognition that this range of beneficial characteristics has been "built in" by plant breeders, at considerable cost to the breeding organization.

Accordingly, most grain-growing countries have an official system for the registration of new varieties. In addition, it has been considered appropriate internationally that the efforts of the breeder should be rewarded by the payment of royalties on the seed of their varieties sold. These payments are thus generally paid by the farmer, as the primary beneficiary of the breeding effort. To establish such payment systems, legislation has been enacted in most grain-producing countries under the title of Plant Breeders' Rights (PBR) or Plant Variety Rights (PVR). These are intellectual property protection schemes, operating in a manner somewhat analogous to that of copyright or patenting systems.

# **Historical Background**

When farming, as opposed to the hunter-gatherer life style, became established, the wise farmers set aside part of their crop as seed for the next season. This permitted early attempts to produce improved "varieties" to be undertaken, simply by the selection of superior plants from an otherwise varied crop. In turn, such selection permitted the separate propagation of parts of the crop that showed, for example, well-filled heads or resistance to pathogen attack. An example of this process is the selection of the malting barley Prior (released in Australia in about 1900) from the traditional English barley, Archer (Variety Identification of Cereal Grains). Individual farmers often practiced this approach, and, as they wished to improve their planting stock, they would pass on their improved variety to neighbors or sell the seed on a wider scale. This led, in turn, to the establishment of a trade in seed, both locally and, in time, internationally. It was usual for the selling agent to promote the seed for sale, using a name that was designed to enhance its attractiveness to the buyer. However, in these early days, there was little regard for the authenticity of these names. Systematic plant breeding by selection was established seriously by the end of the eighteenth century when the plants being grown by farmers were the result of several thousands of years of partly conscious, partly unconscious selection. However, the potential for improvement based solely on selection was very limited.

It was not until early in the twentieth century, with the rediscovery of Mendel's laws of heredity, that deliberate cross-pollination followed by selection contributed to the establishment of plant breeding on a much more scientific basis. The essence of plant breeding is the creation of genetic variation within a species and the subsequent selection from within that variation of plants with suitable combinations of desirable traits that can be inherited in a stable fashion. The plant breeder's final selections of superior plants will form the basis of one or more plant varieties. The introduction of cross-pollination greatly increased the genetic diversity from which to select improved lines or genotypes. This mechanism is illustrated (Variety Identification of Cereal Grains) by the production of the Australian malting barleys, Clipper and Cutter, released, respectively, in 1968 and 1975. These are "sister lines," being separate selections from the same cross, involving an old English variety Proctor (bred in 1952) and Prior A, a line arising from the cross between the varieties Kwan and Prior.

The breakthrough of cross-pollination a century ago also moved the scene for producing better varieties from the individual farmer or interested amateur to organized science — either government sponsored or in private industry. The success of a breeder depends, to

a large extent, on the number of potential new varieties that can be screened within a breeding program, and on the ability to identify the traits of interest. Scale is thus very important and plant breeders will use all available technologies, both to create genetic variation and to select from within that variation. Recent years have seen breakthroughs in breeding technologies, involving the use of genetic markers, which permit the selection and identification of desirable traits at the DNA level, reducing both time and costs. Furthermore, genetic modification (GM) is now possible, permitting the introduction of genes from species unrelated to the target genotype, much more rapidly than conventional techniques.

It is thus clear that the breeding of a plant variety takes place over many years, and requires significant investment, for example, in land, specialized equipment, and skilled scientific manpower. Not all breeders or all varieties are successful and, even where successful, changes in market requirements may reduce the returns on investment. However, there is undoubtedly a benefit to society and to countries in the continued production of new and improved varieties, not least in terms of securing the food supply. Hence, it has been generally recognized that there are good reasons to encourage investment and risk taking in this field, and one way of encouraging such investment is through variety registration and PBR schemes.

# PBR - Background and the Role of UPOV

PBR schemes are a mechanism for intellectual property protection, applicable to newly developed varieties of all agricultural and horticultural species, including all of the major grain crops. PBR systems are attracting an increased interest and significance worldwide due to developments within the World Trade Organization (WTO). As part of being a signatory to the WTO, countries agree to abide by the conditions of trade related intellectual property systems (TRIPSs), and a condition of TRIPS is that an "effective system" for the protection of new plant varieties exists. WTO members are thus establishing (or have already established) systems for PBR. Most of them are adopting PBR based on the principles of the International Union for the Protection of New Varieties of Plants (known as UPOV, from the French acronym – see www.upov.int for more information).

UPOV has its headquarters in Geneva and has over 50 members. One of the primary functions of UPOV is to coordinate the legal and technical aspects of PBR systems worldwide, and to this end, there is a UPOV

Convention (latest version 1991), setting out the principles for PBR. The cornerstone of the UPOV PBR system is that in order to qualify for protection, a newly bred plant variety has to be shown to be new, distinct from others "of common knowledge" and sufficiently uniform and stable in the characteristics used to demonstrate distinctness. An important activity of UPOV is to produce guidelines for conducting this distinctness (D), uniformity (U), and stability (S) testing in a wide range of crops – there are now UPOV guidelines for more than 250 different species, with others in development. The guidelines contain lists of characteristics that can be used for distinctness, uniformity, and stability (DUS) testing of a given species, as well as instructions for how to record these characteristics and "example varieties" which demonstrate the various states (or "UPOV notes") of a characteristic (see Table 1 – extract from current wheat guideline). The characteristics currently used for DUS testing are almost exclusively morphological features of the seed, seedling, or developing plant. For instance, in barley there are 29 characteristics in the current UPOV guideline, whereas in wheat there are 26 in total. These include features such as plant length, growth habit, time of ear emergence, color of ears, and width of straw. While some of these are clearly measurable in absolute terms (e.g., length), others exist in only a few discrete states (ears are either white or colored) and others again are relative (frequency of plants with recurved flag leaves – absent/very low to high). In most cases in grain crops, the characteristics are recorded on a 1-9 scale (e.g., plant length is recorded as very short (note 1) to very long (note 9)). In addition, countries can use extra characteristics, which they have found to be useful for DUS purposes in their own environments. It must be remembered that the guidelines are not mandatory - they are lists of characteristics that have been shown to be useful for distinctness testing and variety description. Some of the characteristics are asterisked, to indicate that they should always be recorded and included in the variety description (see Table 2).

In principle, DUS testing requires that each new (or candidate) variety received for PBR is grown in replicated plots and all of the UPOV characteristics (plus any agreed additional ones) are recorded at the appropriate growing stage. These data are used to compile a description of the candidate variety, which is then compared with the descriptions of all of the varieties in a reference collection. This comparison is in theory made with all varieties of common knowledge. However, in practice this is not really possible, and most testing authorities only make comparisons with a "working reference collection," which might typically consist of current varieties grown in a particular

Table 1 Extract from UPOV guideline for wheat DUS testing

	Characteristics Caractères Merkmale	Stage Stade Stadium	English	Francais	Deutsch	Example varieties Exemples Beispielssorten
1.	Coleptile: anthocyanin coloration	09-11 VS	absent or very weak	nulle ou trés faible	fehlend oder sehr gering	Herzog; Delos
			weak	faible	gering	Niklas; Baldus
	Coléoptile: pigmentation anthocyanique		medium	moyenne	mittel	Andros; Planet
	antinocyanique		strong	forte	stark	Obelisk; Briscard
	Keimscheide: Anthocyanfärbung		very strong	trés forte	sehr stark	Albatros; -
2.	Plant: growth habit	25–29 VG	erect	dressé	aufrecht	Castan; -
			semi-erect	demi-dressé	halbaufrecht	Frandoc; Remus
	Plante: port au tallage		intermediate	demi-dressé à demi-étalé	mittel	Obelisk; Troll
			semi-prostrate	demi-etalé	halbliegend	Boss; -
	Pfalanze: Wuchsform		prostrate	étalé	liegend	Beaver; -
3.	Flag leaf: anthocyanin coloration of auricles	49–51 VG	absent or very weak	nulle ou trés faible	fehlend oder sehr gering	Soissons; Prinqual
			weak	faible	gering	Niklas; Troll
	Dernière feuille: pigmentation anthocyanique des oreillettes		medium	moyenne	mittel	Cardigoc; -
	anthooyanique des oremetes		strong	forte	stark	Cargo; Sunnan
	Oberstes Blatt: Anthocyanfärbung der Auricula		very strong	trés forte	sehr stark	Recital; Dollar
4.	Plant: frequency of plants with recurved flag leaves	47–51 VG	absent or very low	nulle ou trés faible	fehlend oder sehr gering	Apollo
			low	faible	gering	Recital; Axona
	Plante: fréquence de plantes avec la dernière feuille retombante		medium	moyenne	mittel	Obelisk; Filou
	retorribante		high	forte	stark	Frandoc; Prinqual
	Pflanze: Häufigkeit van Planzen mit gebogenen obersten Blattern		very high	trés forte	sehr stark	Capitole; -

country or region along with others that are available commercially or are of interest for other reasons (e.g., they display a range of expression of the UPOV characteristics). If the new variety is found to be D and sufficiently U and S on the basis of this examination, it can be granted PBR, and the description produced

then legally defines the variety, along with an acceptable variety name (or denomination). It is important to note that UPOV does not prescribe how the DUS examination is carried out, and there are many different approaches to the award of PBR that have been adopted by UPOV members (see below).

Table 2 Example of a variety description

Char.	no.	Character	State
UPOV	UK		
01	01	Coleoptile – anthocyanin coloration*	Medium to strong
02	02	Plant – growth habit	Intermediate
04	06	Plant – freq. of plants with recurved flag leaves	Absent
05	08	Time of ear emergence* (first spkt visible - 50% ears)	Late
03	09	Flag leaf – anthocyanin coloration of auricles	Absent/very weak
06	14	Flag leaf – glaucosity of sheath*	Very strong
08	16	Culm – glaucosity of neck	Very strong
07	17	Ear – glaucosity*	Very strong
09	21	Plant – length (stem, ears, awns, and scurs)*	Short to medium
10	22	Straw – pith in cross-section*	Thin
16	25	Ear – color*	White
14	26	Awns or scurs – presence*	Scurs present
15	28	Awns or scurs at tip of ear - length*	Very short to short
11	30	Ear – shape in profile*	Tapering
13	31	Ear – length (excluding awns or scurs)	Medium
12	33	Ear density*	Dense
20	41	Lower glume – beak length	Long
21	42	Lower glume – beak shape	Slight/moderately curved
18	45	Lower glume – shoulder width	Medium
19	46	Lower glume – shoulder shape	Elevated with 2nd point
22	52	Lower glume – extent of internal hairs	Group $1+$ to 2 (Jonard $1-2$ )
23	55	Lowest lemma – beak shape	Strongly curved
17	68	Apical rachis segment – hairiness convex surface	Weak to medium
24	69	Grain – color*	Red
25	79	Grain — coloration with phenol	Medium
26	80	Seasonal type	Winter type
27	81	SDS-PAGE electrophoresis – glutenin locus Glu-A1	No band
28	82	SDS-PAGE electrophoresis – glutenin locus Glu-B1	Bands 6+8
29	83	SDS-PAGE electrophoresis – glutenin locus Glu-D1	Bands 2 + 12

As an intellectual property protection system, PBR schemes have to be adopted into the national legislation of countries. The UPOV Convention sets out the scope of the protection, including the requirement that the holder's prior authorization is necessary for commercial production, marketing, sale, and marketing of propagating material of a protected variety. The breeder's right extends not only to the protected variety itself but also to varieties whose production requires the repeated use of the variety (e.g., F1 hybrids), and – in the 1991 Convention – to varieties that are "essentially derived" from the protected variety. This is a mechanism designed to cope with the GM of existing varieties, e.g., by the insertion of a single new gene, but also encompasses other aspects of so-called cosmetic breeding. Under PBR systems, protection is granted for a limited time, after which varieties pass into the public domain. In contrast to patents, PBR extends only to the actual variety (as defined in the Convention) and not to any single trait or characteristic of a variety in isolation. Moreover, authorization of the holder of the right is not required for the use of a protected variety for "research" purposes, including the breeding of new varieties. The Convention also

allows for a farmer's subsequent use of harvested seed of protected varieties, under national legislation.

It is important to note that DUS testing does not examine the merit or value of new varieties, and the characteristics used do not usually have any particular agronomic significance. This is primarily because of the requirement to compile a description of the variety, which becomes the "definition" of the variety and has to be maintained while PBR is in force. Performance characters such as yield or quality are not reliable indicators of identity, as they are subject to environmental interactions and are thus difficult to describe with sufficient precision. Uniformity and stability assessments of such characteristics would also be difficult to undertake. In many countries, the question of agronomic value is assessed in a different process, usually known as variety registration.

# Variety Registration and PBR Schemes in the European Union

The member states of the European Union (EU) operate a very comprehensive system of DUS testing and PBR of agricultural crops. This arises from the

existence of a Seeds Directive (72/180/EEC), which requires that before a new variety can be marketed, it has to be included on the "national list (NL)" of a member state, or on the "common catalog" (essentially a compilation of NLs). To be included on an NL, a new variety has to be shown to be D, U, and S, and also to have sufficient value for cultivation and use (known as VCU). The VCU of a variety is satisfactory if "...its qualities... offer a clear improvement...." This improvement must be in "cultivation, or as regards the uses which can be made of the crops or the products derived therefrom," and the qualities of varieties are "...taken as a whole...." The DUS criteria are also used for awarding PBR, at the breeder's request. 72/180/EEC also specifies the way in which the DUS and VCU testing are to be carried out. In general, breeders submit their varieties to a national testing PVR office, and a series of "official" trials and tests are required, usually over a 2 year period, using prescribed protocols. Following compilation and collation of the data, including statistical scrutiny/analysis if appropriate, decisions on the DUS and VCU status of candidate varieties are made by a national board or committee. There are certain differences between member states as to who actually carries out the NL testing. In some countries (e.g., Germany), the testing is all carried out by government scientists, whereas in the UK, much of the technical work is subcontracted to private organizations, with the administration of the system carried out by government departments. Generally though, all EU member states conduct DUS tests for the award of PBR, based on the principles and methods of the UPOV guidelines. A complete variety description is produced (e.g., Table 2) and evidence of the distinctness of the variety from its most similar existing variety is required. A sample of the seed of the variety must be kept by the testing office, as the official reference sample.

In addition, there exists the Community Plant Variety Office (CPVO), a supra-national organization that grants protection to breeders in the form of EU-wide PBR. The CPVO does not conduct any tests or trials for itself, but subcontracts the technical work to EU member states. At the moment, the majority of CPVO applications are for ornamental species, with breeders of the major grain crops still on the whole opting for national protection, primarily because of the VCU requirement.

Some countries within the EU operate schemes for the collection of royalties payable to plant breeders on the farm-saved seed of registered and protected varieties. This follows the introduction, in 1994, of EC regulations, allowing plant breeders "... to collect remuneration that was 'sensibly lower' than the certified seed royalty rate, for protected seed that was produced and then saved by the farmer and used on his own farm.... "For example, in the UK the British Society of Plant Breeders (BSPB) has the legislative authority to collect this remuneration on the farm-saved seed of certain protected varieties that had been granted European and/or UK rights after 1994. This applies to varieties of cereals, field peas and beans, oilseed rape, linseed, and triticale. There is only one remuneration rate for farm-saved seed per species, but each variety has its own individual royalty rate for certified seed. Such payments represent an important source of income for breeders, and additional return on their original investments.

Although it may appear unduly complex to have variety testing being conducted in 15 different countries for the same purpose, in practice the system has worked well and has delivered a series of improved, high-quality varieties to European farmers since the early 1960s. In turn, these varieties have enabled grain production in Europe to increase, without a major rise in the land under cultivation (i.e., efficiency and productivity have increased). The effects of enlargement of the EU, and the increasing number of countries in the UPOV system, along with the need to contain the costs of the system, may change this situation in the future (see below).

# **PBR Schemes in North America**

By contrast to the situation in the EU, PBR systems in the USA have no official trials system. In addition, various different types of protection are available to US plant breeders. The Plant Variety Protection Act (PVPA) provides for PBR under the UPOV system (the USA is a member of UPOV), although there are no trials and no inspection of new varieties. Rather, breeders submit a description of their variety, using UPOV guidelines where appropriate, and a comparison of this description and those of other varieties of the species in question is made by the PVPA office, using a database of existing descriptions. However, no sample of the new variety is required or kept by the office. The Plant Patent Act (PPA) is limited only to asexually reproducing species and hence excludes the major grain crops. However, utility patents are available for inventors (breeders) of new varieties, a decision which has been confirmed recently by a decision in the Supreme Court (I.E.M AG Supply Inc v. Pioneer Hi-Bred International Inc, 2001). The subject matter for such patents is defined in Section 1010 of title 35 of the US Code. They require a demonstration of novelty, nonobviousness, and utility, and the applicant also has to fulfill the other requirements of US Patent Law, such as the need for a substantive description — requirements which are arguably more stringent than those required under UPOV-type PBR systems. A deposit of seed of the variety is also required, which becomes publicly available. In return, the protection offered by a utility patent is more restrictive than a more traditional PBR award. For example, the variety cannot be used for breeding without the consent of the patent holder, as this would represent product development under patent law.

Canada has had a PVPA since 1990 and operates a breeder testing system, in accordance with UPOV principles. Although there are no independent official trials, applicants are required to grow their new varieties in comparison to closely similar types, and these trials can be inspected by government officials. The results of the descriptions of new varieties submitted by breeders can also be checked in the field by officials, or their advisors. Breeders have to supply an official sample of newly registered varieties, which is kept in a gene bank. Primary registration required by the Canadian Grain Act involves consideration of functional quality and kernel visual distinguishability. However, the latter requirement is in the process of change, with an added emphasis on distinguishability according to laboratory methods of identification.

# **PBR Schemes in Other Countries**

There are many other countries with PBR systems, and a range of options for operating these systems has been followed. New Zealand and South Africa have systems in place which are broadly along the lines of the Canadian model (i.e., breeder based), but can require more substantial tests, including the possibility of official tests if these are deemed necessary by the authorities and the production of a variety description by the officials rather than by the applicant. Japan has a range of systems in place, depending on the species – rice, for instance, is tested in an official series of tests by the National Center for Seeds and Seedlings, and/or by local government research institutes. Officials establish the final DUS report and prepare the variety description. There is also a VCU system for major crops, including rice and the other grain species. In Australia, a breeder-based system operates, and the applicant has to demonstrate that a new variety is D, U, and S, by carrying out comparative trials, supervised by the examination office. The variety descriptions (produced by the applicants, but checked by officials) are published, allowing for a period of public consultation and consideration before PBR is awarded. Royalties are collected for protected cereal varieties when the grain is delivered after harvest, as

distinct from other systems, in which royalties are payable when the seed is sold.

Of the many countries that have established PBR systems since the advent of the WTO agreements, most seem likely to choose a primarily breeder-based approach, largely for cost considerations. Clearly though, there is considerable scope, even under the UPOV system, as to how DUS testing is conducted. Other countries such as India, although signatories to WTO, have yet to decide the details of their systems. The relevant act establishing PBR has passed through the Indian Parliament, but India is not yet a member of UPOV and may indeed never be. The principles of DUS testing are well established in the country, and India may decide to develop a "sui generis" system.

# Advantages and Difficulties with Present PBR Systems

PBR schemes are now becoming very widespread, and the advantages that they bring to countries in terms of rewarding plant breeding effort and increasing the supply of high-quality varieties to the marketplace seem well established. For example, Brazil has reported that since the advent of PBR (1997), there have been increases in the number of varieties released, in the range of crops being bred, in private investment in breeding, and in productivity. It has been established that in the UK, PBR has expanded the genetic diversity of the major grain crops being grown, primarily by increasing the number of independent breeding companies releasing these varieties. However, there are some difficulties with the current PBR systems, both philosophical and technical.

For instance, the concept of the "ownership" of plant genetic resources is one which raises fundamental issues in some minds and fears that multinational seed companies might obtain a monopoly over food supply. It is also argued by some that the intellectual property protection systems that operate successfully in industrialized countries might not be suitable models for developing countries where agriculture is still a high-volume occupation. Again, the existence of PBR and "owned" germplasm might inhibit public-sector breeding, which is still very important in large areas of the world, and stifle investment in minor crops.

On the technical side, the expanding membership of UPOV and the number of countries operating PBR has a profound effect on the size of the reference collections that are used to make comparisons of new varieties with those of common knowledge. It is now impossible for any one country truly to compare new varieties with all those known to exist, especially

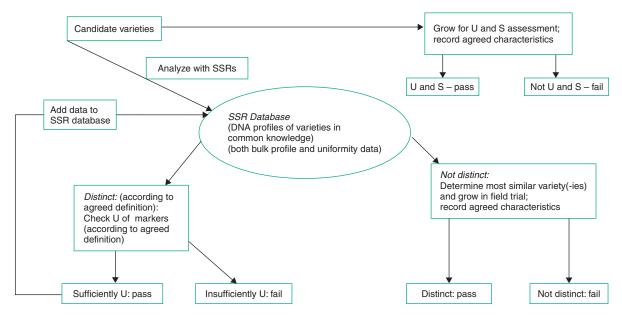


Figure 1 Possible future model for DNA-based DUS testing.

in the major grain crops. UPOV is also being forced to examine the concept and usefulness of the example varieties given in the guidelines (see Table 1), which now need to be available and to express the appropriate characteristic state on a worldwide basis. It is likely that regional example varieties and/or the use of digital photographs will be used in the future. For these reasons and also to constrain costs, new approaches to DUS testing utilizing modern technology are being seriously examined.

# **Future Prospects**

The primarily UPOV-based PBR systems have served plant breeding and agriculture more generally, very well since the early 1950s. They provide effective protection, without imposing too much cost on either breeders or the responsible testing authorities. However, science does not stand still, and the advent of molecular markers and their use in breeding, along with the expansion of UPOV, offer new opportunities to devise more cost-effective, rapid, objective DUS systems and increase the quality and scope of protection of PBR. UPOV is taking this issue seriously, and has established a Biochemical and Molecular Techniques Group (BMT) as a forum to bring together scientists, breeders, and variety testers to discuss how best to make progress. One possible scheme for using DNA microsatellites (simple sequence repeats (SSRs)) in DUS testing is illustrated in Figure 1. Other possibilities also exist, but the fundamental point is to retain at least some aspects of morphology in testing, using molecular markers to reduce the number of comparisons that need to be made in the field and to make the publication of harmonized variety descriptions more possible and more useful. The same molecular description of a variety will be obtained wherever the analysis is carried out, which clearly is not necessarily the case for multigenic, complex, continuous morphological characteristics. In the longer term, as more becomes known about the molecular basis of genes and gene function, it is conceivable that PBR systems could be based more on the assessment of the functional diversity of varieties, including quality, disease resistance, and even yield, with these characteristics predicted from laboratory analyses rather than field trials. Thus, there is the prospect that a quantum shift occurs in the way that PBR schemes are operated around the world.

See also: Barley: Genetics and Breeding. Canola: Genetics and Breeding. Lupin: Breeding. Maize: Breeding. Rice: Breeding. Sorghum: Breeding and Agronomy. Soybean: Germplasm, Breeding, and Genetics. Variety Identification of Cereal Grains. Wheat: Breeding.

# **Further Reading**

Kelly AF and George RAT (eds.) (1998) Encyclopedia of Seed Production of World Crops. Chichester: Wiley.

Mauria S (2000) DUS testing of crop varieties — a synthesis on the subject for new PVP-opting countries. *Plant Varieties and Seeds* 13: 69–90.

Wrigley CW (ed.) (1995) *Identification of Food-Grain Varieties*. St. Paul, MN: American Association Cereal Chemists.

# **Relevant Websites**

There is a large amount of material on the internet relating to PBR, from the point of view of both the technical requirements and the impact on society/ agriculture - selected sites are given below:

http://www.inspection.gc.ca details of the \_ Canadian inspection and registration system and the variety registration information relevant to Canada are available on this website.

http://www.newcrops.ug.edu.au, www.csu.edu.au, www.southcentre.org, www.journal.law.mcgill.ca - discussion on the effects of PBR on plant breeding, genetic diversity, and other issues.

http://www.ipaccess.gov.au, www.defra.gov.uk, www.inspection.gc.ca, www.gov.za - governments increasingly are making their arrangements for PBR testing available on the web.

http://www.upov.int - International Union for the Protection of New Varieties of Plants (UPOV) website – has information on UPOV generally, plus the convention, general introduction to the conduct of DUS Testing, and UPOV Guidelines for various crops.

http://www.worldseed.org - The International Seed Federation also has information on PBR and the position of breeders.

http://www.greenpeace.org, www.grain.org, www. twnside.org - views expressing opposition to the concept of PBR and patents on plant varieties and the potential impact of PBR on developing countries can be found at these websites and the links therein.

# W

# WHEAT

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**Grain Proteins and Flour Quality** 

# **Genetics**

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# Introduction

Common or bread wheat (*Triticum aestivum* L.) is an allohexaploid with 21 pairs (2n = 42) of chromosomes comprising three similar genomes (AA, BB, and DD) each of seven pairs. The species does not occur as a wild form; it evolved in agriculture  $\sim 9000$  years ago. Durum or macaroni wheat and related cultivated and wild emmers (*T. turgidum* L.) are allotetraploids (2n = 28) possessing genomes, AA and BB, in common with hexaploid wheat. Einkorn (*T. monococcum* L.) is a diploid (2n = 14) that shares genome AA with both polyploid species. Within this species, there are both cultivated and wild forms.

Common wheat products are major components of food for about one-half of the world's population. This significance in agriculture has driven detailed study of the genetics of wheat and its relatives, a number of which have contributed genes for wheat improvement. A secondary, but important, reason for studying wheat genetics is that it is the best-understood genetic model of polyploidy, and study of the species has contributed to knowledge on chromosome pairing and to an understanding of how highly duplicated plant genomes are orchestrated to function within a single organism.

Common wheat has often been described as "soft" wheat to contrast it with the "hard" durum and emmer types. However, within common wheat, there is a bimodal distribution of grain textures also described as "hard" and "soft." The main gene controlling softness is located in the D genome so the trait does not occur in the tetraploid and diploid wheat groups.

# Taxonomy of Wheat and Its Relatives

The allocation of wheat taxa to species and subspecies has changed periodically. The nomenclature of van Slageren is currently the most widely accepted. Although the closely related genus *Aegilops* shares the D and B genomes with wheat, *Triticum* and *Aegilops* have been treated as separate genera.

# The Genomic Structure of Wheat

Common wheat has the genome groups AABBDD, durum and emmer, AABB, and einkorn, AA. The tetraploid cultivated form of T. timopheevii (Zhuk.) Zhuk. (AAGG) and hexaploid T. zhukovsky Men. & Er. are relatively unimportant. However, the wild AAGG form of T. timopheevii (ssp. Ameniacum (Jukubz.) van Slageren) may be more widespread than wild emmer which is largely restricted to Israel, Jordan, and nearby areas. It is agreed that the A genome of common wheat is derived from a close relative of *T. monococcum* known as *T. urartu* Tum. ex Gand. There has been an ongoing controversy regarding the diploid source of the B genome, but the current consensus of opinion is that it was Aegilops speltoides Tausch or a close relative within the Sitopsis section of the Aegilops genus. The D genome came from Ae. squarrosa Coss. A number of Aegilops species also possess a D genome and therefore hybrids with them show a degree of chromosome pairing and occasionally, a low level of fertility, which can be exploited in wheat improvement. Among the relatives of wheat, there are a number with distinct genomes that have functional, but not chromosomal, homology with those of wheat.

# The Cytogenetic Structure of Wheat

Cytologically, common wheat behaves as a diploid species. At meiotic metaphase I, the chromosomes form 21 bivalents that regularly disjoin to produce gametes with n=21. This diploid-like behavior is genetically enforced, but mutants (ph mutants) that disrupt regular chromosome pairing have been isolated. Meiosis in these mutants is more typical of autopolyploidy permitting chromosome pairing between related or homeologous chromosomes.

# Wheat Aneuploidy and Breeding Behavior of Monosomics

Despite regular chromosome pairing, chromosomes of all species occasionally fail to pair. Unpaired chromosomes behave abnormally in meiosis, resulting in gametes with missing or additional chromosomes. In most diploid animal and plant species, the zygotes (aneuploids) formed from such gametes are lethal or sublethal. In wheat, however, with its genomic triplication, aneuploidy in the form of either chromosome loss or addition can be tolerated. Between 1930 and 1970, several different series of aneuploids were isolated in common wheat cultivar (cv.) Chinese Spring by E. R. Sears at the University of Missouri.

Table 1 Some aneuploid sets of Chinese Spring wheat

Aneuploid name	Chromosome no.	Meiotic pairing (metaphase 1)
Monosomic	41	20" + 1'
Nullisomic	40	20"
Trisomic	43	20" + 1"'
Tetrasomic	44	20" + 1""
Ditelosomic	40 + 2 telocentrics	20" + tt"
Double ditelosomic	40 + 4 telocentrics	$20^{\prime\prime}+tt^{\prime\prime}+tt^{\prime\prime}$
Nullisomic-tetrasomic	42	20" - (1") + 1""

<sup>&#</sup>x27; = univalent, " = bivalent, "' = trivalent, "" = tetravalent or quadrivalent.

These lines formed the basis of modern wheat genetics and some of them continue to have a role in the current era of molecular genetics. Some of the more common wheat aneuploids are described in Table 1.

When a monosomic wheat plant undergoes meiosis, the 20 bivalents separate in the normal reductional manner, whereas the unpaired univalent may reach one of the dyads, may divide mitotically in first division with the products reaching each dyad, or may lag to be excluded from both nuclei. At second meiotic division, the undivided chromosome, when present, divides normally with the other chromosomes, but if already divided, it may lag to be excluded from the nuclei of the resulting tetrads. The outcome of the various events over all monosomic chromosomes is that  $\sim 25\%$  of resulting gametes have n=21 and  $\sim 75\%$  have n-1=20.

On selfing or crossing to a monosomic as female, this is the realized proportion of functioning eggs. However, through the pollen, the gametes with n=21 have a competitive advantage and contribute to pollination  $\sim 96\%$  of the time (Figure 1). The results of self-pollination are that  $\sim 24\%$  of zygotes are normal euploids, 73% monosomic, and 3% nullisomic. Individuals nullisomic for most chromosomes are morphologically weaker than monosomic and disomic sibs, and often sterile or partially sterile.

# **Gene Location by Monosomic Analysis**

The unique breeding behavior of wheat monosomics allows dominant genes to be located to particular chromosomes. If Chinese Spring carries a dominant or co-dominant allele conferring a particular phenotype, that phenotype will be missing in the nullisomic for the chromosome that carries the gene (Figure 1). Another way to locate the same dominant gene (AA) in Chinese Spring is to cross the monosomics as female parents with a cultivar or line that carries

	Male	e transmission
Female	21 chromosomes	20 chromosomes
Transmission	96% (81–99) [A]	4% (1–19)
21 chromosomes	Disomic	Monosomic
25% (14–39) A	25% (11–39) <i>AA</i>	1% (0.1–7) A–
20 chromosomes 75% (61–86) -	Monosomic 72% (49–85) – A	Nullisomic 3% (0.6–16)

**Figure 1** The breeding behavior of a typical monosomic plant in Chinese Spring wheat with gene *A* located in the monosome. The percentage frequencies are mean realized values (and ranges). Frequencies in hybrid generations and other cultivars may exceed these values. For zygotic genotypes (boxes), the allele from the female gamete is written first.

the contrasting recessive allele (aa). In the "critical" cross, the monosomic individuals (-a) will have the recessive phenotype whereas in all 20 other crosses the monosomic hybrids will have the dominant phenotype (Aa).

The more usual situation is that the dominant gene is present in the nonmonosomic parent. In this case, hybrids can be generated, as above, to produce 20 sets of monosomics aA (female gamete written first) and a critical cross with -A; all have the dominant phenotype A. On self-pollination, the Aa individuals segregate normally, 3 A (1 AA + 2 Aa): 1 aa phenotypes, whereas in the critical line most of the plants (disomics + monosomics, see Figure 1) will have the A phenotype and a few may show the aa phenotype, but are actually nullisomic (–) and often characteristically weaker. Rather than demonstrate a cytological association between chromosome number and phenotype, researchers often have used a significant statistical deviation from the hypothesized segregation ratio to reach conclusions regarding gene location. Sometimes this led to failure of location, or incorrect location, due to incorrect hypotheses or to instances where nullisomic frequencies were higher than expected with the consequence that statistical deviations were not detected. Thus, the statistical test should be a guide to chromosome location and the prediction should be validated by chromosome counts or by further genetic tests.

The location of genes by monosomic F2 analysis was largely restricted to instances of single dominant gene inheritance, however other breeding strategies and appropriate cytogenetical models were designed to locate genes involved in more complex inheritance patterns. For traits controlled by two or more genes and for quantitatively inherited traits, the method of chromosome substitution was more appropriate. Chromosome substitution involves the production of a series of backcross-derived lines whereby monosomy is used to maintain the integrity of one

chromosome of a donor parent while the remaining 20 chromosomes are converted to the genotype of the recipient parent by repeated backcrossing to the relevant monosomic line. After the last backcross (usually 6-10), a monosomic plant is self-pollinated, and a disomic is selected as the fixed substitution line. The 21 substitution lines thus generated can be assessed for the traits of interest and significant deviations from the phenotype of the recurrent parent are attributed to genes on the substituted chromosome originating from the donor parent. A large number of genes were located by monosomic and chromosome substitution methods.

# **Telocentric Chromosomes**

Mala Avananciasian

During meiosis, unpaired chromosomes occasionally undergo misdivision, breaking at or near the centromere to produce either telocentric or isochromosomes. Most, but not all, monotelosomic (potentially 42) and ditelosomic plants in wheat are sufficiently normal and fertile to be maintained as distinct genetic stocks. Because telocentric chromosomes can be identified cytologically, they have special uses in gene localization to chromosome arms, in checking the identities of monosomic chromosomes by testcrossing, and for within chromosome recombination and mapping. Telocentric mapping exploits the use of the telosome as a centromere marker. Analysis of the progeny of a monotelodisomic (20'' + 1t'') heterozygote (Aa) allows determination of the genetic distance between a gene and the centromere (telocentric mapping, Figure 2).

In instances where a particular ditelocentric stock cannot be maintained due to sterility, a source of the telosome can be maintained either as a double ditelosomic stock (20'' + tt'' + tt'') or as a monoteloditelosomic stock (20'' + tt'' + tt'). For example, an individual with 2BS, and not 2BL, can be generated using a CS DT2BS MT2BL  $(20'' + tt_{SS}'' + t_{L}')$  as a female parent.

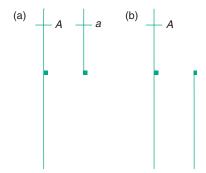


Figure 2 Method of telocentric mapping: When a plant of genotype AA is crossed to the alternative ditelocentric lines for the relevant chromosome, pairing can be as shown in (a) or (b). Genetic recombination in testcrossed or self-pollinated progenies of (a) can be estimated from the proportions of individuals with the contrasting phenotypes conferred by the A/a locus, and chromosome types. Genetic recombination for A/a and the centromere is not possible in (b); in this case, all progeny with phenotype A have an entire chromosome.

#### Nullisomic-Tetrasomic Lines

A very significant discovery by Sears was the realization that nullisomy for one chromosome can be compensated by trisomy or tetrasomy of the related or homeologous chromosomes from the other two genomes. The compensating combinations permitted identification of the seven sets of three homeologs numbered 1 to 7. Nonhomeologous nullisomic tetrasomic combinations fail to show complementation and are phenotypically similar to nullisomics. Nullisomic-tetrasomic lines, in conjunction with ditelocentrics were essential in determining chromosome locations of the restriction fragment length polymorphism (RFLP) and microsatellite markers used to develop the skeletal chromosome maps of wheat. Most nullisomic-tetrasomic stocks are acceptably stable and fertile, but chromosome numbers should be confirmed in cycles of seed regeneration. The most significant exceptions are the nullisomic 4B combinations, which are sterile due to the absence of an essential gene for male fertility.

Although wheat chromosomes are comparatively large, they cannot be identified in general chromosome staining. As for many other species, however, they can be stained by C-banding procedures to produce unique banding patterns that enable the individual chromosomes to be identified by skilled technicians. C-banding was essential to the isolation and identification of a large number of deletion stocks that are enabling the physical mapping of many genes in wheat.

# **Aneuploidy in Tetraploid Wheat**

Tetraploid wheat is much less tolerant of chromosome loss than common wheat, and monosomics are very difficult to maintain. The most stable and useful aneuploids developed for cytogenetic analysis at the tetraploid level were the durum cv. Langdon nullisomic-D genome substitution series produced in North Dakota. In these lines, the individual A and B genome chromosomes (except 4B) were replaced by D genome homeologues of common wheat cv. Chinese Spring (2n = 28 = 14''). When such plants are crossed with euploid durum the chromosome pairing in the hybrid is 13'' + 1' + 1'.

Since 1990s, there has been a significant decline in the use of cytogenetic procedures for gene identification and location in wheat. The established cytogenetic knowledge and stocks developed since the 1940s, provided the framework for generating the current genetic reference maps used for more detailed molecular mapping aimed at gene cloning and isolation.

# Interspecific Crosses and Gene Transfer

Common wheat can be crossed with all Triticum species and with many of its relatives in the Triticeae. The amount of chromosome pairing and fertility of such hybrids determines the methods used to attempt to introgress genetic material and useful agronomic traits from the related species to wheat. The most common and useful crosses for hexaploid wheat improvement have been those with close relatives such as tetraploid wheats and Ae. squarrosa, but there are some very successfully exploited introgressions from species of more distantly related genera such as Thinopyrum.

Once an accession with a trait of interest is identified in a related species, the first step is to produce a hybrid and to determine if such hybrids are fertile when self-pollinated or when backcrossed, particularly as female parents, with wheat. Bridging crosses and/or embryo rescue may be useful in achieving the fertility needed to undertake the transfer. Hybrids with at least some chromosome pairing are often sufficiently fertile to enable the transfer process through normal genetic recombination. Hybrids with little or no chromosome pairing sometimes form chromosomally doubled amphiploids, or partial amphiploids, through generation of restitution nuclei, or may undergo chromosome doubling in response to treatment with colchicine. Both backcrossing and amphiploids have been used to transfer genes from diploid wheat (via AAB, AABD or AAAABB bridging crosses) and Ae. squarrosa (via ABDD or AABBDD hybrids).

These transfers take advantage of normal homologous chromosome pairing.

Hybrids of amphiploids and partial amphiploids with wheat show normal chromosome pairing of the common wheat chromosomes with the additional "alien" chromosomes occurring as unpaired univalents. Backcrossing and selection for a trait or chromosome of interest results in recovery of single monosomic addition lines  $(2n = 43, 21''_W + 1'_A)$ . Such alien chromosomes have the ability to substitute for their wheat homeologs to produce 42 chromosome derivatives  $(20''_W + 1'_A)$  which approach normal wheat in vigor and fertility.

Progenies of monosomic addition lines, or preferably hybrids of alien substitution lines and wheat  $(20'' + 1'_{\rm W} + 1'_{\rm A})$ , can be used to select for translocation events that involve the alien chromosome and, most frequently, the unpaired wheat homeolog. Such translocations may occur spontaneously and often involve entire arm-centric fusions following misdivision, or may be induced by genetic procedures or by radiation with X-rays or fast neutrons. Through the use of further hybrids with some genotypes of Ae. speltoides or related species, deletion of chromosome 5B or mutants with disrupted control of strict bivalent pairing (ph mutants), homeologous chromosomes can be induced to pair and recombine with each other, including the homeologous alien chromosomes. Potential recombinants can be selected from progeny populations that appear to segregate in Mendelian ratios in contrast to the markedly reduced transmission rates of unpaired alien chromosomes. Candidate recombinants can be confirmed by meiotic chromosome pairing, C-band analyses, or by in situ hybridization using genomic DNA from the donor species as a dispersed probe. Obviously, small homeologous chromosome exchanges are most likely to be successfully exploited in agriculture because they should have minimal disruptive effects on inheritance and reduced likelihood of carrying undesirable genetic material. However, there are exceptions and the most widely exploited alien genetic transfer in wheat involves the replacement of the entire short arm of chromosome 1B with the short arm of 1R from cereal rye. The added rye segment carries genes for disease resistance and higher yield and better adaptation under certain conditions, but can have detrimental effects on bread-making quality.

This process of alien gene transfer, chromosome engineering, is not different in concept to that of genetic engineering where cloned genes at the DNA level are inserted into a chosen genotype. Transformation, however, is independent of sexual compatibility and can be carried out with genes isolated from another genotype of the recipient species or genes isolated from any source.

# **Wheat Genetics**

Despite the known evolution of tetraploid and hexaploid wheat and the presence of common traits in these species and in the known ancestors and related species, not all traits in polyploid wheat are controlled by triplicated and duplicated genes. Some genes apparently have been mutated or lost entirely from the genome. The genes controlling some traits are clearly triplicated at corresponding or orthologous sites in each genome (for example, the R gene series in group 3 chromosomes controlling red kernel color) whereas other traits seem to be affected by single genes of major effect (e.g., the squarehead or nonspelt gene Q on chromosome 5A) or by multiple genes located on nonhomeologous chromosomes (e.g., the strong awn inhibitors on chromosomes 4A, 5A, and 6B). Triplicated traits such as red kernel color may be controlled by one, two, or three dominant genes depending upon the particular cultivar, or the kernel may be white. In contrast, the presence of ligules (compared with nonliguled variants) in common wheat is determined by genes in chromosomes 2B and 2D, whereas in tetraploid wheat, only the gene in 2B is present. Because the ancestral A genome species, T. monococcum and T. urartu, and all related diploid species are liguled, it seems the orthologous gene in chromosome 2A was lost (null allele) in polyploid wheat. Using this knowledge, alien chromosomes of homeologous group 2 can be selectively added to wheat using a liguleless wheat stock as the recurrent parent.

Despite the powerful techniques of genetic analysis that became available with aneuploidy, the genetic map of the large wheat genome continued to be sparsely populated. This was alleviated initially by the study of isozyme variation. More than 25 proteins, and often multiple forms of related enzymes, such as the esterases, were identified and characterized by electrophoresis. Many but not all allozymes were determined by orthologous gene sets. Individual electrophoretic bands were located to chromosomes and chromosome arms using nullisomic-tetrasomic and ditelocentric lines, and allelic variation at some loci enabled genetic mapping. However, allozyme analysis did not provide the high level of variation and number of loci that was being sought. Moreover, each protein had to be extracted, separated, and stained by individual techniques, and the overall process was expensive and tedious.

Opportunities for more detailed genetic analysis of wheat became available firstly with discovery and use of restriction enzymes and RFLP analysis, and later, with techniques for studying DNA polymorphism based on the polymerase chain reaction (PCR) protocol. As with allozymes, electrophoretic bands were related to chromosome arms, but again the levels of allelic variation determined with RFLPs was relatively low in wheat compared with barley and maize. RFLPs were usually triplicated in orthologous sets. Despite the relatively low levels of polymorphism, many RFLPs were mapped and the information obtained contributed to a backbone of reference points on the wheat genetic map.

More recently, PCR-based microsatellite or simple sequence repeat (SSR) techniques provided an expanding number of chromosomally unique polymorphisms that can be readily mapped. Large "banks" of these have been developed for use by restricted "consortium" groups, or are available in the public domain. Microsatellites are the current tools of choice being applied to the rapidly expanding application of marker-assisted (marker-linked) selection in wheat improvement.

With increasingly detailed genetic maps, it became apparent that expressed genes are not evenly distributed throughout the large genome. Comparative physical and genetic maps showed a clear clustering of mapped genes to the terminal regions of chromosomes. For example, chromosome 1BS has a visible restriction about two-thirds of its length from the centromere. Most, if not all of the mapped genes in 1BS are located in the satellite demarcated by the restriction.

# **A Wheat Gene Catalog**

Since 1968, wheat researchers have maintained a wheat gene catalog that ensures that genes are named in accordance with accepted rules. The catalog provides relevant information for each named gene, including attributes such as dominance, or origin if from an alien source, synonymous names, chromosome and arm locations, available genetic stocks in increasing order of complexity, and for genes determining morphological, quality, and disease traits, proximity to molecular markers. Catalog supplements are published annually and complete revisions occur at five-yearly intervals.

# **Genetics of Quality Traits in Wheat**

Wheat is an important foodstuff for mankind because of its unique quality characteristics and the fact that large quantities of grain can be produced, harvested, stored, and transported in an efficient way. Its ability to produce a wide range of products is determined by the starch characteristics of the endosperm and the proteins that constitute  $\sim 6-15\%$  of the milled products. The main storage proteins, the glutenins and

gliadins, determine dough strength, extensibility, and elasticity. The glutenin genes are classified as high-molecular-weight (HMW) glutenin genes located in the long arms of group 1 chromosomes and the low-molecular-weight (LMW) group located in the short arms. Up to five orthologous groups of gliadin genes occur in chromosomes of homeologous groups 1 and 6. Relative amounts of protein in wheat grain are largely environmentally influenced, but there are also genetic components for which group 5 chromosomes were particularly implicated.

Grain softness in common wheat, conferred by a dominant gene on chromosome 5DS, determines the milling characteristics and hence its suitability for the manufacture of specific products. Mutations in an orthologous series of waxy proteins (GBSS) in chromosome 4AL (translocated from 7BS), 7AS, or 7DS can, in various combinations, enhance the suitability of flour for noodle production.

# The Genetics of Adaptation

Wheat cultivars used throughout the world were chosen pragmatically by farmers to suit the agronomic and climatic constraints of the environments in which they were grown. The genes that determine the growth and flowering characteristics include those responsible for response to vernalization, response to daylength, and earliness per se. In common wheat, there are up to five orthologous groups affecting vernalization response, one orthologous group (chromosome group 2) affecting daylength response and several diversely located genes for earliness per se. Nil or reduced vernalization response and reduced daylength response are conferred by dominant alleles.

Reduced plant height and increased straw strength were strongly selected by breeders in order to achieve higher yields through higher harvest index and reduced lodging. While the gibberellin-insensitive *Rht-B1b* and *Rht-D1b* alleles in chromosomes 4BS and 4DS were the choice of the Green Revolution, these alleles limit expression of coleoptile length, often resulting in low seedling emergence attributes for planting in dry environments. Consequently, attempts are being made to exploit alternative alleles at these loci, or various gibberellin-sensitive *Rht* genes in order to improve seedling vigor.

# The Genetics of Stress Resistance

There are reports of genetic variation for tolerance to drought, freezing (leaf tissue), frost (spikes and flowers), heat, salt, certain herbicides, and to both mineral deficiencies and toxicities. In some instances, major genes were documented, in others such as drought and heat tolerance, variability was largely quantitative.

Biotic stress resistances were found to have both qualitative and quantitative genetic bases. For diseases caused by highly specialized, pathogenically variable obligate pathogens such as the rusts, powdery mildew, and some smut pathogen species, as well as the Hessian fly pest, resistances were characterized in terms of several to many genes, each of large effect. For less specialized pathogens (and pests) such as those causing root rots and *Fusarium* head scab, individual gene effects were not so obvious, but there has been significant progress in genetic analysis using quantitative trait loci (QTL) mapping.

A significant recent development for the important leaf rust and stripe rust diseases has been increased emphasis on analyzing and exploiting durable adult plant resistances (APR). The genetics of durable APR have indicated oligogenetic additive combinations of genes, which individually confer relatively low levels of resistance. These findings offer the breeder the choice of selection for high levels of resistance in the disease nursery, predicting that agriculturally acceptable phenotypes will have gene combinations. To achieve this major genes should be avoided in crossing programs, or nullified in segregating populations by using appropriate virulent pathotypes for disease screening. Alternatively, specific molecular markers, if available, enable such genes to be combined in a planned way.

# **Concluding Remarks**

The methods of genetic analysis of wheat have evolved since 1990s, from a strong effort on cytogenetics to an almost exclusive emphasis on molecular approaches. Cytogenetic stocks and knowledge enabled the construction of basic molecular framework maps to which the emerging molecular mapping data were referenced. With the rapidly increasing marker density of the genetic map, and current methods of discovering DNA polymorphisms, there is an expanding effort on gene characterization by heterologous homologies and gene isolation in wheat, as well as the increased application of marker technology and transformation in both wheat and durum improvement.

See also: Cereals: Overview; Grain Diseases; Grain-Quality Attributes. Genetically Modified Grains and the Consumer. Noodles: Asian Wheat Flour Noodles.

# **Further Reading**

Borner A and Worland AJ (eds.) (1996) Selected papers from the EWAC Conference: "Cereal Aneuploids for Genetical Analysis and Molecular Techniques" held in Gatersleben, Germany, July 1994. Euphytica 89(1).

Feldman M (2001) Origin of cultivated wheat. In: Bonjean AP and Angus WJ (eds.) *The World Wheat Book. A History of Wheat Breeding*, pp. 3–53. Paris: Lavoisier.

Heyne EG (ed.) (1987) Wheat and Wheat Improvement, 2nd edn. Madison, Wisconsin: American Society of Agronomy.

Johnson R (1984) A critical analysis of durable resistance. Annual Review of Phytopathology 22: 309–330.

Kimber G and Feldman M (1987) Wild Wheats, An Introduction. Special Report 353, 142pp. College of Agriculture, University of Missouri, Columbia.

Lupton FGH (ed.) (1996) Wheat Breeding. London: Chapman and Hall.

McIntosh RA, Hart GE, Devos KM, Gale MD, and Rogers WJ (1998) Catalogue of gene symbols for wheat. In: Slinkard AE (ed.) *Proceedings of the 9th International Wheat Genetics Symposium*. University of Saskatchewan, Saskatoon, Canada: Extension Press.

Morris R and Sears ER (1967) The cytogenetics of wheat and rust relatives. In: Quisenberry KS and Rietz LP (eds.) Wheat and Wheat Improvement, pp. 19–87. Madison, Wisconsin: American Society of Agronomy.

Sears ER (1954) The Aneuploids of Common Wheat. Missouri Agricultural Experimental Station Research Bulletin, 572, 59pp.

Sears ER (1976) Genetic control of chromosome pairing in wheat. *Annual Review of Genetics* 10: 31–37.

Van Slageren MW (1994) Wild Wheats: a Monograph of Aegilops L. and Amblyopyrum (Jaub and Spach) Eig. (Poaceae). Wagengen Agricultural University Papers 94-7, 512pp.

Worland AJ and Snape JW (2001) Genetic bases of worldwide wheat varietal improvement. In: Bonjean AP and Angus WJ (eds.) *The World Wheat Book. A History of Wheat Breeding*, pp. 53–94. Paris: Lavoisier.

# **Relevant Websites**

http://wheat.pw.usda.gov — Graingenes wheat website. Displays a wide range of material relevant to wheat genetics including the wheat gene catalogue and annual supplements.

http://www.shigen.nig.ac.jp — National Institute of Genetics, Japan. Information on wheat genetics and wheat genetic resources, including genetic maps and the database for the wheat gene catalog.

http://www.ksu.edu — Wheat Genetics Resources Center, Kansas State University. Genetic stocks of wheat and related species.

# **Breeding**

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Wheat breeding is the exploitation and fixation of genetic variation by selection and evaluation for the benefit of mankind

# **Origin of Wheat**

There are two known main species of wheat: common bread wheat (Triticum aestivum L.) and pasta or durum wheat (Triticum turgidum L. subsp. turgidum conv. durum). These occur as natural interspecific hybrids.

The wheat progenitors (e.g., Triticum monococcum, Aegilops speltoides, and Aegilops tauschii) are diploid grasses, having seven pairs of chromosomes (2n = 14). The natural chromosome complement or genome of each grass is unique, which supplies the grass its species attributes.

Through many natural hybridization events, possibly over thousands of years in the West Asian region, a range of natural polyploids developed. The best known of these are durum wheat and common bread wheat.

Durum wheat is a tetraploid (4n = 28). It was derived from the natural hybridization of two diploid grasses, Triticum monococcum (A genome) and Aegilops speltoides (B genome), giving it a genomic constitution of AABB.

Common bread wheat is a hexaploid (6n = 42), a result of a natural hybridization event between Triticum dicoccoides (AABB) and Triticum tauschii (DD). Bread wheat has a genomic constitution of AABBDD.

While durum and bread wheats are tetraploids and hexaploids, respectively, at mitosis and meiosis they both behave as diploids, forming 14 and 21 pairs of chromosomes, respectively. This is because wheat has a gene that confers pairing of the chromosomes at these times in a homologous fashion, rather than across the genomes. The recognition of a naturally occurring line deficient in the pairing homologous gene allows wheat to pair across the genomes at both meiosis and mitosis. This means that genetic recombination can occur across the genomes, rather than between chromosomes within a genome. This ability to have wheat pair either within or across its genomes facilitates the movement of genes between wheat and its near and distant relatives, making wheat relatives a very valuable source of genetic variation for pest and disease resistance, grain quality, etc.

# The Earliest Plant Breeders

The earliest wheat breeders were undoubtedly man's forebears, the hunter-gatherers. Most primitive wheats contain a gene for brittle rachis, where the main branch of the flower fragments at each spikelet when mature. Harvesting would entail having to pick up each spikelet from the ground. A naturally occurring variant keeps the head intact. The ease with which this type could be harvested would have resulted in its quick selection as the preferred type by early hunter—gatherers.

# Working at the Phenotype Level

Just like the hunter-gatherer, today's wheat breeder has to work with the phenotype, or what we see when we look at the plant. The phenotype is influenced by both the genetic constitution of the plant, as well as the environment in which it is grown, plus the manifestation of the interaction between the genotype and its environment, or the genotype-environment (G–E) interaction. This can be expressed as follows:

$$\begin{aligned} phenotype &= genotype + environment \\ &+ G - E \ interaction \end{aligned}$$

As long as the breeder has to work with the phenotype, the design and implementation of all plant breeding is based around this simple but fundamental equation.

It is clear from this statement that all three factors can influence the phenotype. Critically, for breeding to be effective, an understanding of how all components interact on the phenotype is fundamental to success for any target region.

Phenotypes in which the environmental and G–E components prevail will, by definition, alter in their expression when the environment changes. This will result in differing performance of a genotype from location to location and from year to year, resulting in the absence of genetic progress.

On the other hand, phenotypes where the genotype component prevails and where the influence of the environment and G-E components are minimized will result in a consistent performance across locations and years. Under this scenario, genetic advance is made, resulting in the identification of the genetically superior wheats that get released as new varieties.

# Understanding the Environment and the Setting of Breeding Objectives

A thorough knowledge of the environment is fundamental, as it is the environment that determines breeding objectives. In its entirety, the environment consists of the soils, climate, and the biological environment in which the plant has to live. The breeder also has other environmental dimensions to consider such as the financial and physical resources available to the breeding program, access to support scientific disciplines and enabling technologies as well as the grain quality demanded by the major markets to which grain may be sold.

# The Environment

# Soils

The soil provides the plant with its base for growing, as well as its water and nutrients. Soil structure impacts on water penetration and holding ability. Highclay-content soils generally have superior waterholding capacity compared with sandy-clay loams or deep sands. Factors such as pH, with its effect on plant growth, nutrient availability, and effect on factors toxic to plant growth such as free aluminum at low pH, high levels of available boron in ex-marine soils, unavailability of essential nutrients such as zinc at high pH, unavailability of minor nutrients such as copper under waterlogging conditions, etc., all can have dramatic impacts on plant growth and, if not understood, can reduce genetic gain by appearing to be uncontrollable G-Einteractions. However, when properly understood, the production environment can be managed using agronomic approaches. In combination with genetic variability that exists for most of the above aspects, these factors can be ameliorated.

#### Climate

The amount of rainfall the wheat crop receives and the timing of the rainfall events over the growing season impact on plant growth via the obvious extremes of waterlogging or drought. Rainfall and soil fertility are two of the major factors determining grain yield and quality. Even transient periods of both events can have dramatic impacts on plant growth and thus on grain yield and quality.

Temperature has a regulating influence on plant growth. Extremes such as high temperatures cause heat stress and drought, with downsides on yield and quality. Cold temperatures during winter slowdown growth, or lead to soil freezing, and the need for breeders to incorporate winter hardiness in most of the major winter wheat-growing areas of the world.

Transient periods of low temperature to below 0°C at critical stages of plant growth, such as from terminal spikelet formation through to flowering, can cause severe yield losses as a result of frost damage. In most areas of the world, the practice is to breed for frost avoidance, by delaying flowering to a time of more acceptable frost risk. This commonly means that flowering is delayed, so that grain filling has to take place under conditions of increasing temperature and decreasing availability of water.

Wind increases transpiration from plants, so in dry areas this places crops under risk of increased moisture stress (drought at its severest). In combination with high temperatures during and after flowering, the grain-fill period experiences transient periods of moisture stresses. Breeders need to select and evaluate their materials under similar conditions to which they will be grown in order to produce varieties that can tolerate such conditions. Wind also demands that ripe crops can stand and ripen without lodging and or shedding their grain prior to harvest.

# **Biological Component**

The interaction between soils and climate provides the conditions for organisms, both favorable and destructive to plant growth, to prosper.

Favorable soil conditions like vesicular arbuscular mycorrhizae (VAM) are important for healthy plant growth. The availability of moisture and nutrients is affected by what happens in the rhizosphere. This can also result in conditions where fungi, bacteria, viruses, and nematodes pathogenic to wheat plants can prosper.

A wide range of fungi can infect wheat roots and their crowns, causing major yield losses. These include a range of Fusarium species, common root rot, Rhizoctonia root rot, a number of viruses, and plant nematodes. It is the environmental conditions in conjunction with a susceptible host plant that predisposes the plant to attack. An example is snow mold, where it is the amount and period of snow cover that determines the extent and severity of disease development.

Above the ground, it is again the interaction of all the climatic components, in conjunction with susceptible host plants, that determines which plant diseases and pests will prosper, and the range is vast.

The major foliar fungal pathogens are the rusts (stem, leaf, and stripe), the septorias (tritici and nodorum blotch), powdery mildew, and yellow (tan) spot. Each of these has unique sets of environmental conditions for development, mostly governed by the length of the dew period on the leaves for spores to germinate, and then in the cases of the septorias and yellow spot rain drops for splash dispersal of spore to adjacent plants and new leaves of existing plants.

A range of viruses can also cause yield loss. Some require vectors for transfer between plants, such as barley yellow dwarf virus that is dispersed by aphids and wheat streak mosaic virus that is spread by the wheat curl mite.

A variety of insect pests attack wheat plants causing yield and quality losses. Examples are: wheat stem saw fly, which lays its larvae into wheat stems and the metamorphosis of the larvae weakens the stem, leading to straw breakage and lodging; cereal leaf beetle, which eats the epidermal tissues from leaves, thereby reducing photosynthetic area and causing yield loss; the cinch bug and some aphids, which feed on developing grains, releasing proteolytic enzymes into the grain and causing deterioration of the gluten proteins responsible for dough quality.

This brief overview of the major pests and diseases of wheat is intended to highlight that they are many and varied, and they cause major losses in both yield and quality. As a result, most wheat breeding programs direct the major share of their resources to breeding for resistance. In order to effectively prioritize which resistances should be addressed by a breeding solution, breeders must have a comprehensive understanding of the environment of their target breeding area.

For the full range of pests and diseases of wheat, biological variability exists. For the breeder, this means that one source of germplasm rarely is effective against all races, strains, or pathotypes of the pest or disease. Thus, effective breeding requires ongoing access to resistant germplasm and reliable screening tests.

# **Grain Quality**

Major areas of world wheat production are often located at considerable distances from where the grain will be consumed, resulting in the sophisticated grain handling and transport systems as part of grain trading. Increasingly, grain is traded on the basis of rigid quality specifications of dryness, cleanliness, and protein content. More discriminating markets have even tighter specifications relating to dough properties and end-product quality. It is this diversity of requirements that breeders must satisfy when setting quality objectives for their breeding programs.

The environment of the target breeding and production region influences the quality of wheat that can be grown in any area. The production environment has a major impact on grain protein content, one of the major specifications upon which grain is traded

internationally. For example, dryer regions tend to be lower yielding and favor the production of higher-protein, better-quality wheat.

To set effective market-based quality objectives the breeder needs a reliable supply of market intelligence that has been interpreted into what is achievable by breeding. In most of the large wheat producing and exporting countries, breeding programs obtain this information from grain traders, processors such as millers and bakers, and/or grower-supported marketing and research agencies.

# Assembling and Creating Genetic Variability

Genetic variability is the cornerstone of wheat breeding. Variability can be sourced from bread wheat or its near and distant relatives. Traits can be introgressed into bread wheat from these sources by using conventional hybridization techniques. In interspecific or intergeneric hybridizations the use of chromosome doubling may be required to get a balanced chromosome complement for successful cell division of the embryo and the resultant plant.

With the advent of molecular biology, traditional interspecific, intergeneric, and plant type barriers to gene transfer have been removed. By isolating the gene for a particular trait and inserting it into a new host by a range of techniques (e.g., biolistic bombardment or Agrobacterium transfer), the gene becomes part of the host DNA, replicating itself at cell division and expressing itself in plant development. The development of such genetic engineering techniques considerably enhances the range of genes available to the modern plant breeder.

Traditionally, breeders have gone to other breeding programs or to major germplasm collections to source their genetic variability. Utilizing genetic variability is always done in a targeted manner, with the breeder seeking new genes for an agronomic, disease resistance, quality- or yield-related trait. Transfer of the new gene is usually by conventional hybridization, followed by cycles of selection until the new gene is fixed in its new background. To facilitate transfer of new genes, some knowledge of the inheritance of the trait and the ability to select for it in its new background are essential.

# Role of Germplasm Collections for New Trait Discovery

Large collections of wheat are kept in international collections (link to USDA Beltsville, Australian Winter Cereals Collection, Vavilov Collection, CIMMYT,

etc.) and are major sources of variability utilized by breeders. In some instances passbook data is held on entries in these collections. However, often when a new disease or pest problem arises, the breeder has to systematically screen the collection for sources of resistance. As this is done, data are fed back to the collections, thereby creating some passbook data for future use. However, in the case of a new disease or change in an existing disease, the breeder usually has to go back to the collection and commence the screening process all over again, as it is only with virulence in a pathogen that new resistance in a host plant can be identified. Until we understand the genetic basis of what actually confers resistance at the DNA level, this cyclic process of screening germplasm collections will continue as new problems emerge.

# **Hybridization Systems**

Wheat is a self-pollinating crop and so lends itself to hybridization by a range of methods. Breeders design their crossing programs so that the resulting populations produced will segregate for all the traits desired in the new varieties. Breeders choose one of the parents to become the female of the cross, and affect this by removing the anthers from all florets of the ear. Hybridization is completed a few hours to a few days later by transferring pollen from the plant designated as the male. This is done using a range of methods, with the transfer being completed using tweezers to shake the pollen from individual anthers onto the receptive stigma, or by cutting back florets of the designated male plant so that anthers are exposed. The dry air causes them to dehisce, and the male plant is then shaken or twirled over the stigma, thereby effecting pollination.

Other means for hybridizing wheat include the use of cytoplasmic male sterility-nuclear fertility restoration mechanisms or the use of chemical hybridization agents. Both of these systems still rely on making one of the parents the nominal female, while pollen is transferred from another parent chosen as the male of the cross. Both of these mechanisms, while useful for crossing, are better deployed for making hybrid wheat where the advantages of heterosis (hybrid vigor) can be captured.

# **Crossing Strategies**

Choice of crossing strategy depends on the range of traits available in the parents and those desired in the progeny of a cross. If all the desired attributes can be obtained from just using two parents, A and B, then the straight cross A/B strategy can be deployed. If one of the parents (A) has more desirable traits than the

other, then to increase the frequency of the desired genes from A a further cross to that parent can be made, making for an A/B//A crossing strategy.

When all the desired traits cannot be assembled from a straight crossing strategy and three parents are needed (A, B, and C) to get all of them in the progeny then a three-way crossing system can be used, such as A/B//C.

When a variety has most of the traits desired, but has had a resistance breakdown or can be enhanced by the addition of a new resistance or trait, then the new gene(s) can be rapidly introgressed using the backcross crossing strategy. If the new resistance gene is dominantly inherited then all that is wanted from this donor parent is that gene, so the original variety becomes the recurrent parent and repeated cycles of crossing between the recurrent parent and the F1 generation can be made until the desired percent of the recurrent parent is recovered. The straight cross F1 contains 50% of genes from each parent. The first backcross to the recurrent parent increases the frequency of recurrent parent genes to 75%. Subsequent backcrosses increase the gene frequency to 87%, 93%, 96%, 98% for the second to fifth backcrosses, respectively, and so on.

When deploying the backcross breeding strategy to recessively inherited genes, it is necessary to allow the first cross to go to the F2 generation and then screen for the presence of the new gene or trait, prior to making the next backcross. At each backcross cycle it is necessary to go to the F2 and conduct screening for the new trait prior to crossing to the recurrent parent.

# Selection and Evaluation Systems and **Breeding Methods**

# **Factors Influencing the Sequence of Trait Selection and Choice of Breeding Method**

Many factors influence the sequence in which traits are selected in a breeding program. These include the economic importance of the trait, its heritability, the availability of simple and effective selection methods, cost of the selection process and the extent of resources available to the breeding program. As a result, traits with high heritability and essential components of any new variety, such as most agronomic attributes such as flowering time, straw strength, and lodging resistance and many disease resistances are selected on a single plant level commencing in the F2 generation and usually fixed early in the breeding cycle (by F3 or F4 generation). More difficult to select traits like grain yield and quality are selected later in the breeding cycle (F4 onwards)

and this is done on a progeny basis in plots rather than a single plant basis.

There are many different ways in which breeders can manage material flow in breeding programs. Choice of method depends on many factors, including cost of variety development, which makes the shortening of the breeding cycle a high priority, resources available to the breeding program and the availability of effective selection methodologies for the target traits.

Pedigree method This is the method of choice where disease resistances are the priority traits that must be incorporated into new varieties. The pedigree method allows for simply inherited, often single-gene traits to be selected and fixed through repeated cycles (2–4) of selection until homozygosity is achieved. To shorten varietal development, this method is often combined with the use of two or more field-grown generations per year (Figure 1).

Modified pedigree In this process the basic pedigree cycle is followed, but with the addition of a round of yield evaluation and or quality testing as early as possible, F3 or F4, in order to remove low-yielding and poor-quality families from the breeding program.

Single-seed descent This rapid method allows for up to four generations per year using growth chambers and glasshouse conditions (Figure 1). A population of 200 or more seeds is planted and only single tiller plants produced by manipulating the watering and nutrition regime to speed up the growth of plants. At harvest, one seed from each plant is taken and planted again for the next generation. This process is repeated until plants are advanced to between the F4 and F6 generations. Seed is then increased and planted in the field in observation rows or yield plots. The advantages of single-seed descent (SSD) are speed combined with cycles of recombination, giving the possibility of desirable recombinants being produced.

Doubled haploid or dihaploid development In this method, F1 seeds are produced by conventional crossing techniques using the two parents that between them have all the traits desired in the new variety (Figure 1). Doubled haploids can be produced from microspore culture, but the wheat—maize method is the most widely used system. In the wheat—maize system, F1 seed is made by hybridization, the seeds planted and their flowers emasculated and pollinated with maize pollen. A haploid (a plant with single chromosome of each of the 21 bread wheat pairs) starts to develop. This is saved using an embryo rescue

technique and allowed to grow into a haploid plantlet, which is then treated with a chromosomedoubling agent such as colchicine, nitrous oxide, or caffeine to produce a diploid plant. Seeds from these plants are instantly homozygous for all genes, thus imparting the major advantage of this breeding system, namely, that it takes material to true breeding status in less than one year, thus considerably shortening the breeding cycle.

# Early, Mid-, and Late Generation Selection

In the early generations, the focus is on selection of traits of highest heritability. These are usually agronomic attributes and disease resistances. In the midand later generations, irrespective of what breeding method a breeder chooses to use, selection is about validating performance of breeding lines, demonstrating their merit for potential release as new varieties. This usually involves the use of replicated, multi-site, multi-year yield and quality evaluations to gather the data needed to support the decision to bulk up seed for release. Most breeding programs consider that three years of such evaluations are needed to demonstrate the reliability of any potential variety.

# **Emerging and Enabling Technologies**

One of the most exciting emerging technologies is the use of marker-assisted selection (MAS). The value of MAS is that it shifts the basis of selection from the phenotype level to the gene level. The requirement for it to work effectively is that the markers are either closely linked to, or reside within the gene to be selected.

MAS provides a more accurate and reliable method of selection as it is not influenced by the effect of the environment. Hence, it is extremely attractive to breeders for traits that are difficult and time consuming to screen for, and are highly modulated in their expression by the environment.

However, one of the major limitations to the deployment of MAS in bread wheat has been a lack of polymorphism that has often expressed itself by a lack of robustness of a marker across a range of breeding populations. MAS is being deployed widely and successfully in a range of other broad acre crops, so while the benefits to wheat breeding are recognized, the major benefits are yet to be realized.

Use of the genetically modified organism approach is in its infancy in wheat breeding. These technologies remove species and genus barriers to gene transfer, so they considerably expand the range of genes available

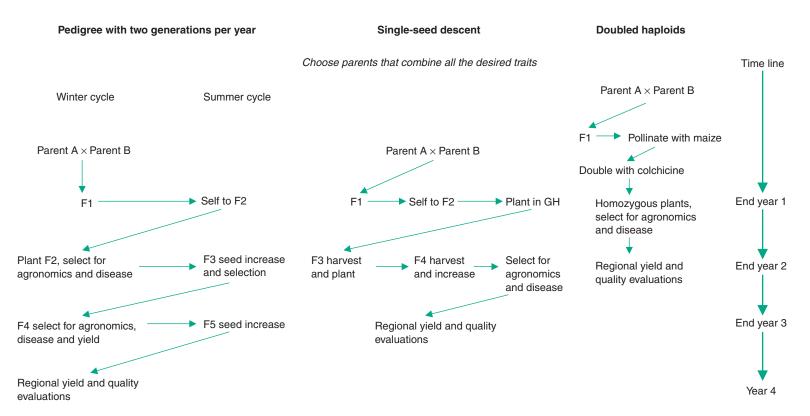


Figure 1 Schematic of three breeding methods.

to the wheat breeder. Currently the processes of inserting the genes into wheat breeding material means that the position the gene inserts itself into the host DNA is random, so success rate of gene expression is low.

As gene-splicing techniques improve, the success rate can be expected to improve. While the success rate of current methods remains low, GMO's offer breeders new genes, but these will need to be transferred into adapted backgrounds for release as new varieties using conventional breeding methods such as hybridization and selection. Backcrossing is the preferred method for rapidly transferring GMO traits into adapted wheat backgrounds.

Varietal fingerprinting is another useful breeding aid where both the DNA complement of a recurrent parent and a DNA-based marker for the gene to be introgressed in a backcross breeding program can be tracked at the DNA level. The use of these combined strategies will shorten the breeding cycle by earlier recovery of sufficient contribution of the recurrent parent DNA at an earlier stage in the backcross process. This technology will also be increasingly used to identify breeding programs for varietal identification at the point of sale for integrity of marketing and trading grain and for intellectual property issues associated with the protection of plant breeders' rights.

# Wheat Breeding as Part of Supply Chain Management

Modern wheat breeding is just one part of a sophisticated supply chain, whereby all parts of the chain are important in adding value to a major food source. All members in the supply chain can have a major impact on the overall product as it moves through the chain, so the more these components can be integrated the more efficient the process, and the less downside on the quality of the end product. This demands a good information flow up and down the supply chain. With the wheat breeder sitting at the end of the supply chain most distant from the consumer, the effectiveness of information flow and the quality of that information are critical for effective wheat breeding.

See also: Cereals: Overview; Grain Diseases. Genome Mapping. Genomics. Variety Registration Breeders' Rights. Wheat: Genetics; Agronomy.

# **Further Reading**

Allard RW (1960) Principles of Plant Breeding. New York:

Bonjean AP and Angus WJ (eds.) (2001) The World Wheat Book: A History of Wheat Breeding. Paris: Lavoisier.

Heyne EG (ed.) (1997) Wheat and Wheat Improvement, 2nd edn. Madison, WI: American Society of Agronomy. Lupton FGH (ed.) (1996) Wheat Breeding. London: Chapman and Hall.

O'Brien L and Blakeney AB (eds.) (1998) An Introduction to the Australian Grains Industry. North Melbourne, Australia: Royal Australian Chemical Institute.

Wood DR (ed.) (1983) Crop Breeding. Madison Wisonsin: American Society of Agronomy, Crop Science Society of America.

Yamazaki WT and Greenwood CT (eds.) (1981) Soft Wheat: Production, Breeding, Milling and Uses. St. Paul, Minnesota: American Association of Cereal Chemists.

# **Relevant Websites**

http://www.awb.com.au - Australian Wheat Board. http://www.inspection.gc.ca - Canadian Food Inspection Agency for plant products for variety registration and PBR.

http://www.cps-scp.ca - The Canadian Phytopathological Society.

http://www.cwb.ca — Canadian Wheat Board.

http://www.csl.gov.uk - Central Science Laboratory, UK.

http://www.cdl.umn.edu – Cereal Disease Lab (Rust Lab - USDA - ARS).

http://www.cimmyt.org - CIMMYT.

http://www.meteo.ec.gc.ca - Environment Canada weather forecast by location worldwide.

http://www.fao.org - Food and Agriculture Organization of the United Nations.

http://res2.agr.ca - Inventory of Canadian Agricultural Research.

http://wheat.pw.usda.gov - Graingenes.

http://www.grdc.com.au - Grain Research and Development Corporation, Australia.

http://pgrc3.agr.gc.ca — Plant Gene Resources Canada. http://www.ars-grin.gov - National Plant Germplasm System, USDA – ARS.

http://www.sas.com — Statistical Analysis Systems. http://www.ssc.ca - The Statistical Society of Canada.

http://www3.sympatico.ca — The Statistical Society of Canada, Professional Development Committee.

http://www.un.org - United Nations.

http://www.usda.gov - US Dept of Agriculture.

http://www.scabusa.org - US Wheat and Barley Scab initiative.

http://www.uswheat.org — US Wheat Associates. http://www.grainscanada.gc.ca - Canadian Grain Commission.

http://www.agronomycanada.com - Canadian Society of Agronomy.

http://www.defra.gov.uk - Home Grown Cereal Authority, UK.

# Agronomy

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# Introduction

Wheat is the world's most popular crop. It is grown over a large area and under a wide range of conditions and provides more nutrition to humans than any other species. Over 580 million tons (Mt) is produced annually on 220 million hectares (Mha), an average of 2.66 t ha<sup>-1</sup> (Table 1). Ripe grain is harvested and a new crop is planted somewhere in the world during every month of the year. Wheat is also the oldest of crops, one that is grown in over 120 countries with many different technologies and traditions.

Production of wheat has increased ~100% during recent decades, the maximum increase seen in any food crop. Improved agronomic practices and new varieties account equally for over 80% of the gain and increased area for less than 20%. The extreme conditions for growing wheat, the many classes that are produced, and the numerous cultural traditions create highly diverse agronomic practices. This article describes wheat in relation to its agronomy, major factors that affect its productivity, and the different agronomic practices for its production.

# Wheat in Relation to Its Agronomy

# **The Wheat Plant**

Wheat is a member of the grass family (Gramineae) that produces an edible one-seeded caryopsis called a berry, grain, or kernel. The seed normally germinates within 1 week after planting and the first true leaf, the coleoptile, emerges from the soil. Each plant produces

Table 1 Area, production, and yield of wheat by major exporters, major importers, and other countries during 2000-01<sup>a</sup>

	Countries			
	Major exporters	Major importers	Other	World
Area (Mha)	69.69	91.08	58.78	219.55
Production (Mt)	232.34	209.90	141.57	583.82
Yield (t ha <sup>-1</sup> )	3.33	2.30	2.41	2.66

<sup>&</sup>lt;sup>a</sup> Data from Production Estimates and Crop Assessment Division, Foreign Agricultural Service, US Department of Agriculture.

five to seven leaves from a growing point, or apical meristem, which then differentiates from a vegetative to a reproductive structure to form a spike that bears the grain. Tillers develop in the axils of leaves, giving each plant three to a dozen or more spikes depending on conditions, a trait that makes wheat highly adaptable to different environments.

Two root systems form on wheat: seminal roots from the embryo of the seed and adventitious roots from the coleoptile and tillers. The adventitious system is usually more extensive and may spread to several meters below the plant to absorb moisture and nutrients from the soil.

Grain growth begins with anthesis or flowering, when pollen is released from the three stamens in each floret and deposited on the stigma of the ovary. Wheat is 95-98% self-pollinated and little exchange of pollen with other plants, or out-crossing, occurs. Growth of the grain (maturation) proceeds in a sigmoid fashion, with a lag period for an increase in cell number, a linear period for an increase in cell mass, and a plateau as the grain approaches maximum weight (physiological maturity). The grain then ripens as the moisture content decreases from 35% to 40% down to 14%. Most varieties also have an after-ripening period of 1-4 weeks, during which the grain loses dormancy and becomes capable of germinating.

Yield components of wheat − the constituents that determine the harvest - include the plant density (number per area), tillers per plant, spikelets per spike, kernels per spikelet, and the mass per kernel. Agronomic practices strive to optimize, not maximize, each component because of the compensation among them. For instance, high plant density reduces tillering, and excessive tillers may decrease the number of kernels in each spike.

#### **Classes of Wheat**

Two of the many types of wheat that are grown throughout the world, common wheat and durum, are most prominent. Common wheat (Triticum aestivum L.) is hexaploid (6N = 42) and occupies  $\sim$ 90% of the world wheat cultivation area. It includes classes with spring and winter growth habit, hard and soft endosperm, and red and white pericarp (seedcoat).

Spring and winter growth habit do not denote the season that the crop is grown but instead indicate whether the plant requires cold to change from vegetative to reproductive growth, a process called vernalization. Spring wheat has no vernalization requirement, whereas winter wheat needs 1-6 weeks of  $\sim 5^{\circ}$ C to induce reproductive growth. Wheat also

requires long or lengthening days of  $\sim$ 14 h to initiate flowering, except for varieties that are bred to eliminate daylength sensitivity and are said to be dayneutral. Varieties with a third growth habit, known as facultative wheat, do not require vernalization but have strong daylength sensitivity.

Hard wheat is used primarily for raised bread and buns, but is also satisfactory for steamed bread; flatbreads such as "chapatis," "tortillas," and "pieta;" and for Asian noodles. Soft wheat is used for cakes, pastries, flat breads, and crackers. White wheat is preferred by many consumers, particularly for cereals and whole-wheat products.

Durum (*T. turgidum* L. var. *durum*) is a tetraploid (4N = 28) and accounts for  $\sim 8\%$  of world wheat production. It is more resistant to drought and heat than common wheat and is mostly grown in regions with considerable environmental stress. Nearly all commercial varieties have a spring growth habit. The grain of durum is extremely hard and is used mostly for pasta (macaroni and spaghetti), "couscous," and "burghul" but is also used for bread in some localities.

Club wheat (*T. aestivum* L. var. compactum) is a hexaploid that has spring or winter growth habit and red or white grain and is used for the same products as common wheat. Other specialty wheats include einkorn (T. monococcum L.), a diploid (2N = 14) and an ancient crop that is mostly grown in harsh environments in several European countries; emmer (T. turgidum L. var. dicoccum), a tetraploid used for bread and porridge; and spelt (T. aestivum L. var. spelt), an ancient hexaploid that is used for bread, "pilaf," and hot cereals. (See Cereals: Evolution of Species and Wheat: Genetics for the relationships among species of wheat and Triticale for a description of this durum × rye hybrid.)

Wheat has numerous uses other than as food. Considerable winter wheat is grazed for forage by livestock, and the straw left after harvest is used for bedding and feeding livestock, composting mushrooms, and making bricks and particle board. Much of the grain is fed to livestock in some countries, as are damaged or surplus grain and by-products from milling in other countries. Starch from grain has many industrial applications, including paper, adhesives, plastics, and replacement for milk and egg white in foods.

# Wheat in Relation to Its Environment

Wheat is a cool-season species that does best in temperate regions with ample sunshine and moderate moisture. Few of the major production areas – the maritime region of Western Europe and high elevations in several parts of the world being exceptions – are highly favorable for wheat. Other crops that are more productive or more valuable are usually grown in amenable regions and wheat, being well adapted to stress conditions, is often the crop of choice in marginal areas.

# **Soil Factors**

Poor soil conditions – low fertility, salinity, toxicities, waterlogging, and erosion – are major factors in the unfavorable environment for wheat. Nitrogen is the critical component of the protein that determines the food quality of wheat and is often the limiting soil nutrient for yield. Phosphorus is usually limiting after nitrogen and is particularly essential for grain growth. Potassium is especially needed for vegetative growth. Sulfur may be deficient in soils that are coarse and low in organic matter. Magnesium is not deficient for production of wheat in most areas, but marginal levels in plants may cause grass tetany in pasturing livestock. Iron may be deficient when the soil pH or manganese content is high. Copper deficiency occurs on soils that have high organic matter content, and zinc may be inadequate in soils that are eroded or low in organic matter. Responses to chlorine fertilizers have been reported in some regions, but the effect is from amelioration of foliar diseases and not a plant deficiency of the nutrient.

Salinity from accumulation of various salts in the soil has rendered parts of Asia, Australia, and elsewhere unsuitable for crops. The problem is most severe where irrigation water is high in salts or the water table is near the soil surface. Direct toxicity to wheat occurs when certain elements are highly available in the soil. Aluminum and sometimes manganese become toxic as a result of low soil pH, often from long-time application of nitrogen fertilizers. Other toxicities have been reported from excessive chlorine, boron, arsenic, molybdenum, and nickel. Hypoxia, or inadequate oxygen for plant growth, occurs where soil is waterlogged because of poor drainage, a high water table, or a shallow hard pan.

Drought is a perennial problem in many regions and an episodic problem in most other regions for wheat. Inadequate water is the major limiting factor for wheat worldwide, affecting production on an estimated 70% of the area in developing countries and at least as much in developed countries. Wheat has higher water use efficiency than most crops but still requires 1 mm per  $10-20 \text{ kg grain ha}^{-1}$  or 1-3 mmday<sup>-1</sup> during early stages and 7-8 mm day<sup>-1</sup> at anthesis.

# **Cold Temperatures**

Cold injures plants directly or by causing them to heave from the soil, smother under ice, or desiccate, processes known collectively as winterkilling. Winter wheat hardens to cold during autumn, and the hardiest varieties can tolerate direct exposure down to  $-18^{\circ}$ C. However, farmers may not plant the hardiest varieties because they often have lower yields than less hardy varieties; they may plant the crop too late for it to harden, or a snow cover may be missing to insulate the plants.

Winter wheat and spring wheat are often subject to late spring freeze injury. The plants lose tolerance to freezing as they develop and are easily injured by cold fronts or radiation freezing. Late spring freeze injury is particularly damaging during anthesis, when anthere may be destroyed by only  $-1^{\circ}$ C and the plants, being mostly self-pollinated, are sterilized. In far northern areas, late summer or early autumn freezes before wheat matures diminish the yield and quality of the grain.

# **High Temperature**

High temperature during maturation affects wheat in many regions. The optimum temperature for grain growth is 15°C; higher temperatures accelerate maturation, reduce the yield, and alter the gliadin proteins that determine grain quality. High temperature is frequently accompanied by dry winds known as "Gan Zhe Feng" in China, "Sukhovei" in Russia, "Siroccu" in North Africa, and "Khamsin" in the Middle East.

# Wind and Lodging

Wind causes soil to blow, particularly during spring when the soil surface is dry and the vegetative cover is sparse. Blowing erodes the soil and abrades the plants, causing them to desiccate. Wind also removes soil from around the roots of plants or deposits soil on top of them. Later in the season, wind causes grainladen plants to lodge or fall over, especially when the stand is dense or growth has been stimulated by nitrogen fertilizer. Lodging reduces yield and complicates harvest.

# Hail

Hail is a frequent hazard to wheat. Damage depends greatly on the plant developmental stage. Leaves are most labile at early stages, floral structures are susceptible before anthesis, and the grain shatters or dislodges from the spike at late stages.

# **Preharvest Sprouting**

Preharvest sprouting of the grain occurs when persistently wet, humid conditions coincide with ripening.

Varieties differ somewhat, but most white-grained wheats are much more susceptible than red wheats. Sprouting is most damaging to the quality of wheat for bread because of the high α-amylase enzyme activity that occurs; it is less detrimental to cakes and other products.

# **Biotic Factors**

Weeds are common problems, often seriously reducing the yield and sometimes the quality of the grain. Most species compete with wheat for light, moisture, nutrients, and space. Others are allelopathic, producing chemicals that inhibit growth of the wheat plant. They also interfere with harvest and increase the moisture content of the grain, causing heating and molds. Some weed species cause off-tastes in the grain, which lower its value. Winter annual weeds, which germinate during autumn and complete their growth cycle the following year, are most detrimental to winter wheat and spring wheat planted during autumn. Summer annual weeds that germinate during spring and mature in summer mostly affect springseeded wheat but can also occur in sparse stands of winter wheat. Perennial weeds, which live indefinitely, are problems in both winter and spring wheat. (See Plants: Diseases and Pests and Cereals: Grain Diseases for other important biotic factors in wheat agronomy.)

# **Agronomic Practices for Wheat**

# **Varieties and Seeds**

More than 80% of the wheat area in developing countries and ~100% in developed countries is planted to modern varieties. Few true hybrids are grown because the seed is costly and the yield advantage is often small.

Varieties must be adapted to the local environment, resist indigenous biotic and abiotic stresses, and produce quality grain, in addition to yielding well. Most varieties are semidwarf, 60–80 cm tall, in contrast to traditional varieties that grow up to 120 cm in height. Semidwarf varieties do not produce more biomass, but they allocate more of it to the grain. They also respond to high plant density and high levels of nitrogen fertilizer by producing more grain instead of lodging.

Improved varieties are developed primarily by public agencies, such as government ministries and universities, in most countries except in western Europe and South Africa, where they are mostly supplied by private companies (see Wheat: Breeding). Once farmers receive a new variety, they usually save grain from each harvest for seed to plant the

next crop and only purchase commercial seed when a new, superior variety becomes available. Whatever the source, wheat seed should have a minimum germination of 85-90%, a minimum volume weight of  $72 \text{ kg hl}^{-1}$ , and be free of weed seeds and diseases.

# **Rotations and Tillage**

Numerous rotations are used for wheat. Fallow, or leaving the land unplanted during alternate seasons, is common where annual precipitation is less than 400 mm. Some rotations include fallow with 2 years of wheat or wheat followed by another crop for two harvests every 3 years. Fallow benefits yield of the succeeding crop by increasing soil moisture and nitrogen and, in some cases, reducing pests.

Continuous wheat, i.e., growing wheat every year, is favored in some areas by the climate, tradition, or government programs. However, the practice often promotes buildup of diseases and weeds that eventually reduces wheat yields. Mixed farming systems that alternate wheat with other crops such as soybean, maize, cotton, and rapeseed alleviate these problems.

Double cropping, or growing two crops during the year, is practiced where precipitation and the growing season are amenable. Wheat is always the winter crop and rice, soybean, vegetables, or other species are the summer crop. Double cropping gives two crops every year, but yields are sometimes hurt when one crop creates unfavorable conditions for the other or late harvest of one delays planting of the other crop.

Tillage practices for wheat range from systems of no tillage to "the more you till the more you reap." An ideal medium for wheat varies among regions but always includes a firm, moist seedbed with a loose, cloddy surface. Surface residue from the previous crop is desirable for winter wheat in arid and temperate areas to hold moisture, prevent erosion, and trap snow. It is also usually desirable for spring wheat, but in some areas it may be buried by plowing in autumn to hasten drying of the soil for planting the following spring.

Conventional tillage for wheat in regions where soil moisture is adequate generally consists of plowing to a depth of 10–20 cm, disking, and harrowing. However, plowing is increasingly being eliminated, and only disking and harrowing are done. Where moisture is limiting, a chisel or sweep cultivator is often used to leave a "stubble mulch" to retain moisture and prevent erosion and is followed by disking or harrowing immediately before planting.

Zero or conservation tillage, a practice that is growing in some regions, involves planting wheat with no prior tillage of the soil. It generally provides an excellent seedbed for rapid germination and reduces

costs and soil compaction. However, expenses for chemicals to control weeds and infestation of diseases and insects from residue of the previous crop are increased. Another form of direct seeding is practiced with the rice—wheat rotation in Southeast Asia, where wheat is sown on the soil surface after the rice is harvested. The system requires close control of soil water but gives a rapid stand of wheat without the cost and time of preparing the soil.

Farmers in some areas still till the soil intensively for wheat. In parts of South Asia, for instance, the soil is plowed 6–8 times with a tine-cultivator or disk harrow and then leveled with a wooden plank.

# **Planting**

Timely planting is critical for productivity of wheat. Winter wheat should be planted early enough in autumn to allow two to three leaves to develop before winter. Early planting is often practiced when the wheat is to be grazed by livestock, but it may promote loss of soil moisture, diseases, and insects such as Hessian fly. Delaying planting, on the other hand, gives plants little time to develop the root and shoot systems and to cold-harden, reduces accumulation of snow, and may promote erosion.

Spring wheat is sown as soon as soil conditions permit in temperate areas to encourage early plant development; avoid the hot, dry periods of summer; and in far northern areas, enable maturation before frosts occur. In milder regions, where spring wheat is planted during autumn, timely planting is also important for avoiding unfavorable summer conditions but is sometimes difficult when summer crops are harvested late.

Seeding rates for wheat range greatly from  $25 \, \mathrm{kg \, ha^{-1}}$  under arid conditions to  $200 \, \mathrm{kg \, ha^{-1}}$  for intensive management under ample soil moisture. Lower rates are sometimes used for increasing seed supplies of new varieties and higher rates for wheat that is planted late or used for grazing. The goal of varying the seeding rate is to achieve a spike density that is optimum for the moisture supply. This ranges from  $\sim \! 200 \, \mathrm{spikes \, m^{-2}}$  with  $150 \, \mathrm{mm}$  of moisture to  $600 \, \mathrm{spikes \, m^{-2}}$  with  $750 \, \mathrm{mm}$  of moisture.

The optimum seeding depth of wheat in moist soils is 2–5 cm. That depth gives adequate coverage with soil for germination and promotes vigorous roots and shoots. Deeper seeding, down to 10–12 cm, may be needed to reach moist soil, but greater depths should be avoided, especially with semidwarf varieties because of their short coleoptile.

Row spacings for wheat vary from 10 to 40 cm depending, like the seeding rate, on available moisture. Narrow spacings approach the ideal equidistant

spacing for minimum interplant competition and are used when soil moisture is ample. They are often combined with tram lines, i.e., unplanted rows for equipment travel, in intensive management systems. Wide rows are used for low moisture conditions, no-till planting, and intercropping systems.

Plants typically have more spikes and kernels and produce higher yields in east-west rows than in north-south rows. Orientation of rows perpendicular to prevailing winds apparently decreases plant stress and increases the harvest index.

Most wheat is planted with drills, air seeders, and disk seeders, and broadcasting is practiced in only a few areas. Broadcasting, or spreading the seed by hand, gives uneven distribution, poor coverage by soil, and favors weeds. Farmers in some areas, such as India, use the "desi" plow, which opens a furrow into which the seed is dropped by hand. Drills and seeders sow seed at the desired rate, spacing, and depth and cover it with soil. They range in size from three-row implements in China to equipment 10 m or longer in some areas. They are designed to make a furrow with a disk or hoe, deposit the seed, and cover it with a disk or chain. Larger drills apply fertilizer with the seed.

#### **Fertilization**

Most wheat is fertilized to provide nitrogen, phosphorus, potassium, and sometimes other nutrients. The grain contains 1.5-3% nitrogen and the plant utilizes only  $\sim 50\%$  of the element from fertilizer, so 30-60 kg t<sup>-1</sup> of grain is needed. Additional nitrogen,  $\sim$ 30 kg t<sup>-1</sup> of forage, is required if the wheat is grazed. Phosphorus is present at  $3-5 \text{ kg t}^{-1}$  of grain, and only  $\sim$ 20% of the nutrient is utilized from fertilizer the first year. Recommended levels range from 16 mg per kg of acid soil to  $50 \,\mathrm{mg\,kg^{-1}}$  of calcareous soil. Potassium content of grain is low, but the element is critical for growth of vegetation. About 150 mg K per kg of soil is adequate. Sulfur, magnesium, iron, copper, and zinc also should be supplied if deficiency symptoms or soil tests indicate that a response is likely. Lime to bring the soil pH above 5.5 and prevent aluminum toxicity may be needed, particularly after long, continuous applications of nitrogen fertilizer.

# **Pest Control and Plant Growth Regulation**

Weeds are controlled by planting weed-free seed, cultural practices, chemical herbicides, and hand harvesting. A dense, even stand of wheat is an excellent competitor with most weeds. However, sparse or uneven stands and some modern practices such as using semidwarf varieties and high rates of nitrogen fertilizer may allow weed populations to

increase. Fallowing or succeeding wheat with other crops gives opportunities to control weeds mechanically during alternate years. Working the soil before planting wheat and, in some cases, adjusting the time and depth of seeding are effective. Different herbicides can be applied before or after planting to reduce many weeds. Removing weeds by hand, while laborious, may give them some value as food or feed.

Genetic resistance controls many diseases, particularly some rusts and viruses, most economically. Cultural practices can be used to alleviate many diseases, and fungicides are available for others. Seed should be free of pathogens or treated for common bunt, loose smut, and Fusarium head blight. Eliminating volunteer wheat plants can exclude some disease vectors. Covering residue of the previous wheat crop helps to reduce the incidence of tan spot and septoria. A firm seedbed decreases crown and root rots, and delaying planting may avoid diseases transmitted by aphids and mites. Fungicides effectively control powdery mildew and rusts but, because of their cost, are usually only recommended when the potential yield is high.

Insects, like diseases, are controlled by genetic, cultural, and chemical methods. Varieties are available with resistance to aphids, Hessian fly, and some mites. Cultural methods include rotations with other crops, removal of volunteer plants and crop residue, plowing to bury the insects, delaying planting, and accelerating harvest. Insecticides are effective against many pests but are often considered a last resort because of their cost.

Plant growth regulators are applied to wheat to deter lodging. Semidwarf varieties are much less prone to lodging than tall varieties and usually do not benefit from plant growth regulators. However, under the intensive management practices of high plant densities and high nitrogen fertilizer in western Europe and New Zealand, even semidwarf varieties may lodge. Plant growth regulators act by shortening the plants and strengthening their stems.

# **Irrigation**

Many countries cannot produce wheat without irrigation because of arid conditions. Irrigation water is supplied from sprinklers, furrows, and basins. Center pivot sprinklers, which rotate about a fixed point, and tow line/sideroll sprinklers are popular because of their efficiency and low labor requirement. Other systems such as hand-move irrigators are declining because of their high labor demands. Furrows in fields typically run parallel to several rows of wheat, which may be on raised soil beds, and are supplied with water from pipes or canals. The system is versatile but often gives poor distribution of water, is labor

intensive, and complicates harvest. Basin irrigation involves flooding fields enclosed by levees and is well-suited to rice-wheat rotations. The system is efficient and gives good distribution, but initial costs are high and mechanical harvest may be difficult.

Irrigation of wheat is scheduled by two methods: depletion of soil moisture to a set level or by growth stage of the plants. Wheat utilizes  $\sim 50\%$  of the available soil moisture before yield is reduced by stress, and irrigation is applied when moisture reaches that level. Alternatively, irrigation is scheduled at critical growth stages, such as germination, booting/heading, and early grain development, when moisture deficiency is most damaging to yield.

#### **Harvest**

Much of the world's wheat is harvested by hand with serrated sickles, particularly in Asia. The scythe, sometimes with a cradle to catch the plants, has replaced the sickle in some regions. Threshing to remove the grain from the straw is accomplished through trampling by livestock; stone rollers; tractors, sometimes pulling a disk or other implement; or small machines. The grain and straw are usually separated by tossing them into the air in a breeze.

Mechanical harvesters, called binders, that cut the wheat and tie it into a bundle were once widely used but are now found in only a few areas. The bundles are arranged in stacks known as shocks for the grain to dry and are then threshed by machines called separators.

Many large farms in developing countries and most commercial farms in developed countries use combine harvesters. These machines cut the plants and remove the grain from the straw and separate them in a single pass 1-10 m wide. Usually, only the spike and part of the stem are cut to minimize the amount of straw that goes through the harvester and to leave a stubble to protect the soil. Some combine harvesters, known as strippers, remove only the spike.

Wheat is usually harvested when the grain contains less than 14% moisture and can be stored without deteriorating. In some areas, such as the northern US and Canada, wheat is cut when the grain contains as much as 35% moisture and placed in windrows to dry, a process called swathing, before it is combineharvested. In other regions, such as northern Europe and Hokkaido, the main wheat-growing island of Japan, the grain is combine-harvested when it contains 40% moisture and artificially dried. In those areas, the climate either does not permit the grain to dry naturally or causes the grain to sprout in the spike if it dries.

# **Wheat Agronomy in Major Regions**

Asia is the world's major wheat production area with China and India leading production. Another important wheat-producing country is Pakistan (Table 2). Spring wheat predominates in China, with soft and medium-hard red varieties planted in autumn throughout most of the southern provinces and hard and medium-hard red varieties planted in spring across the north. Hard and medium-hard white winter and facultative wheat occupy most of the North China Plains. Over 90% of the grain is used for noodles and steamed bread.

Cultivation of wheat is labor intensive on small plots that are often part of large fields. In the major provinces of Henan and Shandong, wheat is mostly seeded with three-row drills pulled by draft animals. Other crops – rapeseed, maize, and vegetables – are often interplanted between the wheat plots. High amounts of chemical fertilizers are applied (Table 2), and additional nutrients are commonly supplied as compost. Over 50% of the wheat area is irrigated, usually from wells and reservoirs. Much of the irrigated wheat is rotated with rice for two crops each year. Harvest begins in May in southern regions, in June in the North China Plain, and in July and August in northern areas. Most of the wheat is harvested with hand sickles and threshed with animals or small machines.

Leaf, stem, and stripe rusts; powdery mildew; common bunt; and Fusarium head blight are usual diseases in China. Aphids and armyworms are common insects. Few pesticides, mostly fungicides, are used, and weeds are usually controlled manually and fed to livestock. Drought occurs with some frequency, and declining water tables and competition for other uses may limit availability of water for agriculture in the

**Table 2** Agronomic practices for production of wheat in major countries in Asia

Practice <sup>a,b</sup>	China	India	Pakistan
Major class	Hard spring	Hard spring	Hard spring
Area (Mha)	26.65	27.49	8.46
Production (Mt)	99.64	76.37	21.08
Yield (t ha <sup>-1</sup> )	3.74	2.78	2.41
N fertilizer (kg ha <sup>-1</sup> )	120.0	112.0	106.5
P fertilizer (kg ha <sup>-1</sup> )	37.1	15.3	11.3
K fertilizer (kg ha <sup>-1</sup> )	26.6	6.1	0.5

<sup>&</sup>lt;sup>a</sup>See Table 1.

<sup>&</sup>lt;sup>b</sup>Fertilizer data from Fertilizer Use by Crop (1999) International Fertilizer Association, International Fertilizer Development Center, and Food and Agriculture Organization of the United Nations, Rome.

future. Hot, dry winds sometimes severely damage maturing wheat, and prevalent late spring and early summer rains occasionally cause severe preharvest

India's major wheat region is the Indo-Gangetic plain, particularly the states of Uttar Pradesh and Bihar in the northeast and Punjab and Harvana in the northwest. Semihard and hard red and white spring wheat planted during autumn as the "rabi," or winter crop, predominate. The wheat is often intercropped with mustard, gram, and lentil and grown in rotation with rice, maize, sorghum, and pearl millet as the "kharif" (summer) crop. Only a little durum is produced, primarily under dryland conditions in the central and western areas. Grain is harvested in March and April in the northeast and late May and June in the northwest. Most of it is used for chapatis.

Drought, high temperature, occasional freezes, diseases, insects, and nematodes are problems on wheat in India. Dryland yields are low because they often depend on residual moisture from monsoons, and irrigated wheat is threatened by declining water tables and soil salinization in some regions. High temperatures hinder crop development and promote pests during autumn and restrict grain-filling during spring. Rusts, powdery mildew, and root rots are common under the high-yield management for most of the crop. Shoot fly, armyworms, and caterpillars are occasionally damaging.

The major wheat area of Pakistan is Punjab Province with over two-thirds of the total production taking place here. Wheat is the country's primary food, providing  $\sim$ 72% of the calories and protein, mainly as chapati and "nan." Only spring wheat, either hard or semihard, is grown, and white or amber varieties are preferred.

Wheat is grown as the rabi crop in rotation with cotton, rice, maize, sugarcane, and groundnuts. The soil is worked extensively and nearly pulverized between crops, and wheat is planted by broadcasting or drilling the seed during October and November. Prompt seeding is essential to avoid stress during maturation of the crop. Use of fertilizer, while fairly heavy, is probably inadequate for the potential yields (Table 2). Most of the wheat, over 88% in Punjab, is irrigated, primarily from canals, but the water supply is sometimes inadequate and saline. Harvest begins with the dryland crop in April and ends with the irrigated crop in early June. Most of the wheat is cut with sickles and threshed by animals.

Irregular availability of water, low rainfall, and high temperatures hinder wheat production in Pakistan. Considerable areas have become too saline for crops. Several weed species, leaf and stripe rust, and loose smut are serious pests.

#### North America

Canada is the sixth leading producer and second most important exporter of wheat (Table 3). The Prairie Provinces of Alberta, Saskatchewan, and Manitoba, the major area, grow mostly hard red spring wheat. Soft red and white winter wheat, which constitute  $\sim$ 5% of the crop, are mostly grown in Ontario. Soft white spring wheat is produced in the same provinces as hard red spring wheat. Durum accounts for 15-25% of the wheat area and is mostly raised in southern Saskatchewan.

Wheat farms in Canada are large and highly mechanized. Low temperature and sparse precipitation dictate many of the agronomic practices. Most of the wheat is grown as a crop-fallow rotation or a continuous monoculture, but rotations with rapeseed and other crops are increasing. Spring wheat and durum are planted as soon as fields can be worked during spring, usually in April and May. Winter wheat is planted from late August through September, preferably directly into the stubble of the previous crop or a thin stand of rapeseed or flax to trap snow to insulate the plants from cold. Moderate amounts of fertilizer are used in keeping with yield expectations (Table 3). Leaf and stem rust, Fusarium head blight, Hessian fly, midge, and wild oat and other weeds are problems. Along with drought, heat, late frosts, and preharvest sprouting sometimes hurt the yield and quality of the grain.

Harvest of wheat in Canada begins in August and continues into October. The grain is marketed through the Canadian Wheat Board; over 70% of it is exported.

The United States is the world's third largest producer and leading exporter of wheat (Table 3). The main classes are hard red winter wheat in the southern and central Great Plains, hard red spring wheat in the northern Great Plains, soft red winter wheat in the Midwest and Southeast, white wheat in the Northeast and Pacific Northwest, and durum in the northern Great Plains and desert Southwest.

Table 3 Agronomic practices for production of wheat in major countries in North America

Practice <sup>a,b</sup>	Canada	United States	Mexico
Major class	Hard red spring	Hard red winter	Durum
Area (Mha)	10.37	21.78	0.64
Production (Mt)	26.90	62.57	3.07
Yield (t ha $^{-1}$ )	2.59	2.87	4.81
N fertilizer (kg ha <sup>-1</sup> )	45.0	70.2	183.0
P fertilizer (kg ha <sup>-1</sup> )	10.9	14.8	15.7
K fertilizer (kg ha <sup>-1</sup> )	4.2	24.3	0

a,b See footnotes to Tables 1 and 2.

Rotations for wheat depend mostly on moisture conditions. In areas that receive 350–500 mm precipitation, primarily the High Plains and Pacific Northwest, much of the wheat is alternated with summer fallow. Areas that receive 500–700 mm precipitation often follow a wheat–summer crop (sorghum, soybean, or sunflower)–fallow rotation or plant wheat every year. Higher levels of precipitation permit conventional rotations of wheat and other crops on alternate years or double cropping (two crops per year) with wheat as the winter crop and soybean, sorghum, or rice as the summer crop.

Winter wheat is seeded from late August in the northern Plains to early November in the South, and spring wheat from late autumn in the western US to early May in the North. Medium levels of fertilizer are used in most regions (Table 3). Only  $\sim 5\%$  of the crop is irrigated. Leaf and stem rusts, several viruses, and *Fusarium* head blight are major diseases. Problem weeds differ among regions and are controlled with cultivation and herbicides. Hessian fly, greenbug, stem sawfly, army cutworm, and grasshoppers are troublesome insects. Harvest begins in southern states in May and progresses northward into September. About half of the grain is exported.

Wheat in Mexico is nearly equally divided between semihard or hard spring bread types and durum (Table 3). The northwestern states of Sonora and Sinaloa, which mainly grow durum, are the most important producers, followed by the Bajio and other parts of the Central Plateau. The grain is used for breads, pasta, and tortillas.

Most wheat is planted from October to December on land that is cropped with soybean, sesame, and other species during summer. Irrigation, usually from reservoirs, is needed to establish and maintain the crop until it matures because of the arid conditions. Large amounts of nitrogen fertilizers are used, reflecting the high yield potential (Table 3). Leaf, stem, and stripe rusts; septoria; and karnal bunt are common diseases. Several aphid species and cutworms occasionally damage the crop. The wheat is harvested from April to June; all of it is consumed domestically.

#### **South America**

Argentina is the twelfth largest producer and fifth largest exporter of wheat (Table 4). The Pampas provinces of Buenos Aires, Cordoba, and Santa Fe produce most of the grain. Hard red spring wheat is the major class; durum occupies less than 1% of the crop. Wheat consumption per capita is the highest in South America, primarily for artisan breads, pasta, and cookies. About 70% of the crop is exported.

Most wheat is grown in rotation with soybean, sunflower, and maize in mixed crop-livestock systems. The climate is generally favorable for wheat; however, variable precipitation and extreme temperatures sometimes limit yields. Soils are productive, but nitrogen and phosphorus are deficient in some areas. Wheat is planted from June through August. Moderate amounts of fertilizers are used, but few pesticides are applied to wheat (Table 4). Rusts, *Fusarium* head blight, take-all, septoria, and yellow spot are common diseases, reducing yields to ~10%. Aphids and other insects are occasional problems. The grain is harvested from November to January.

Over 90% of wheat production in Brazil comes from the states of Parana and Rio Grande do Sul. All of the wheat is spring type, mostly soft to semihard, for bread (Table 4). The crop meets only one-third of the country's needs, and the balance is imported.

Wheat is mostly grown in Brazil as a winter crop in rotation with maize or soybean. It is planted from April to mid-June, often as a cover crop. Yields are low because the climate and soils are particularly unfavorable for wheat (Table 4). Rusts, *Fusarium* head blight, septoria, and mildew are often epidemic. Soil acidity causes toxicity from aluminum and fixes phosphorus. Frosts periodically damage the crop at vulnerable stages. Rainfall is sometimes inadequate but more often excessive, causing floods and promoting fungal diseases during harvest from September to November.

# Oceania

Australia and New Zealand, the two major wheat countries in Oceania, present contrasting examples of varieties, technologies, and yields. Australia is one of the world's leading wheat producers and exports over 80% of the grain (Table 5). The country's Wheat Belt extends across the states of Western Australia, South Australia, Victoria, New South

**Table 4** Agronomic practices for production of wheat in major countries in South America

Practice <sup>a,b</sup>	Argentina	Brazil
Major class	Hard spring	Semihard spring
Area (Mha)	6.07	1.25
Production (Mt)	15.10	2.44
Yield (t ha <sup>-1</sup> )	2.49	1.95
N fertilizer (kg $ha^{-1}$ )	48.7	8.0
P fertilizer (kg ha <sup>-1</sup> )	9.3	17.9
K fertilizer (kg ha <sup>-1</sup> )	0	34.0

<sup>&</sup>lt;sup>a,b</sup> See footnotes to Tables 1 and 2.

Wales, and Queensland. All the varieties are white spring types, which are usually planted during the autumn months of May and June. Six classes of wheat are produced, the most important being prime hard, hard, and standard white and durum. Output of wheat is steadily increasing as former sheep areas are cultivated and the yield potential of new varieties increases.

Production of wheat in Australia is constrained by availability of moisture, since much of the country is arid or semiarid and only 8% of the crop is irrigated. Farms are large and heavily mechanized. Fertilizer use is low because of the generally low yield potential (Table 5). Diseases – leaf, stem, and stripe rusts; crown rot, and yellow spot – are frequent problems. Late frosts sometimes damage plants from the preflowering to early grain-filling stages. Salinity from rising water tables is becoming troublesome, particularly in Western Australia. Preharvest sprouting caused by persistent rains after the white grain ripens is a major concern. The grain is harvested during October through December.

New Zealand grows wheat primarily along the eastern coastline areas of Canterbury, North Otago, and the lower North Island. Fields are generally small,  $\sim 10 \, \text{ha}$ , but the crop is managed

Table 5 Agronomic practices for production of wheat in major countries in Oceania

Practice <sup>a,b</sup>	Australia	New Zealand	
Major class	Hard spring	Semihard spring	
Area (Mha)	13.00	0.04	
Production (Mt)	23.77	0.32	
Yield (kg ha <sup>-1</sup> )	1.83	7.11	
N fertilizer (kg ha <sup>-1</sup> )	32.0	100.0	
P fertilizer (kg ha <sup>-1</sup> )	12.4	10.9	
K fertilizer (kg ha <sup>-1</sup> )	0.2	8.3	

a,b See footnotes to Tables 1 and 2.

intensively and yields are high (Table 5). About two-thirds of the varieties are facultative types that are sown in autumn (May), and the balance are spring varieties that are planted in late August to early September. Some 60% of the grain is used for bread products and 40% for livestock feed.

Most wheat in New Zealand is grown under contracts that impose strict criteria for quality and pesticide residues. High rates of nitrogen fertilizer are often split into several applications during the growing season, as are fungicides to control foliar diseases (Table 5). Much of the wheat is irrigated. Growth regulators are used to prevent lodging of the plants. The favorable conditions and intensive management commonly result in yields of 10 t ha<sup>-1</sup> or higher, particularly of feed wheat. Harvest begins in early January and continues into late February. Most of the grain is artificially dried.

# **Eastern Europe and Western Asia**

Wheat is the major cereal in the Russian Federation, the fifth largest producer in the world (Table 6). Southern Russia, including the North Caucasus, Southern Black Soils area, and Volga Valley, is the primary winter wheat region. The Don Basin, middle Volga, and southwestern Siberia grow most of the spring wheat, both bread types and durum. Winter and spring bread wheat are used for various baked products, which are important in the population's diet, and for livestock feed. Durum is used for bread as well as pasta.

Small private farms and large joint-stock farms produce wheat in Russia. Both are heavily dependent on mechanization, but economic difficulties cause much of the equipment to be obsolete and production inputs to be low. In addition, variation in weather among regions and years greatly affects production. Nevertheless, the reorganization of former state-owned farms has increased efficiency and contributed to excellent harvests during recent years (Table 6).

Table 6 Agronomic practices for production of wheat in major countries of eastern Europe and western Asia

Practice <sup>a,b</sup>	Russia	Kazakhstan	Ukraine	Turkey	Iran
Major class	Winter wheat	Spring wheat	Winter wheat	Winter wheat	Winter wheat
Area (Mha)	19.76	8.74	5.93	8.65	4.74
Production (Mt)	31.00	11.24	13.58	16.50	8.67
Yield (t ha $^{-1}$ )	1.57	1.29	2.29	1.91	1.83
N fertilizer (kg ha <sup>-1</sup> )	13.9	na	na	43.6	32.4
P fertilizer (kg ha <sup>-1</sup> )	3.7	na	na	9.9	12.9
K fertilizer (kg ha <sup>-1</sup> ) Pesticide (% of area)	2.4	na	na	0.4	0.2

a,b See footnotes to Tables 1 and 2. na = not available.

Winter wheat is planted in September and October following rotations of continuous wheat or other cereals, legumes, or flax. Winterkilling from severe cold sometimes necessitates replanting with spring wheat. Most spring wheat is sown during April and May. Use of fertilizer, while low, is increasing, but few other chemicals are applied to wheat (Table 6). Leaf rust, powdery mildew, snow mold, *Fusarium*, and aphids are major problems in addition to drought and winterkilling. Harvest begins in July in southern regions and extends into autumn in northern spring wheat areas, where it is sometimes disrupted by winter weather. The grain is presently sold to a state fund.

Kazakhstan produces both winter and spring wheat, principally on the northcentral Steppes around Kostana. Most of the crop is grown on large cooperative and joint-stock farms. Winter wheat is planted in September and October and spring wheat is planted in April and May. Drought occurs about 2 years of every five and use of fertilizer is declining, causing yields to be low (Table 6). Other problems stem from inadequate equipment, poor infrastructure, and an undeveloped market system. Harvest in the major area begins in August and continues into October.

The principal wheat area of Ukraine is in the eastern and southern parts of the country. Over 90% of the crop is hard winter wheat, and 5–10% is durum (Table 6). Most production takes place on large private associations that reformed from state and collective farms. Although Ukraine is a traditional exporter, most of the grain is consumed locally as breads or fed to livestock.

Most Ukrainian wheat is planted in September. Fertilizer use is low because credit is difficult, and other chemicals are expensive (Table 6). Little of the wheat is irrigated, and drought can occur anytime during the season. Leaf rust, powdery mildew, and septoria are often problems. Harvest during July and August can be complicated by inadequate equipment and adverse weather.

Turkey produces winter bread wheat, the major class with 50% of the area, on the Anatolian Plateau and Thrace Province (Table 6). Spring bread wheat and durum, each ~20% of the area, are mostly grown near coastal areas. The balance of the wheat is winter durum. Wheat on the Anatolian Plateau is usually alternated with fallow. It is planted during October, as is spring wheat on the coast. Medium amounts of fertilizer and other chemicals, primarily herbicides, are used (Table 6). Little of the wheat is irrigated and yields vary greatly with precipitation, which averages only 200–450 mm on the Anatolian Plateau. Moderate to severe drought every 2–4 years, yellow rust, leaf rust, root rot, and tan spot are major problems. Harvest is during June and July.

Iran grows nearly equal areas of spring and winter bread wheat (**Table 6**). The crop occupies over half of the arable land in the country, and consumption of wheat foods is among the highest in the world. Lavash, sangak, and other flatbreads; biscuits; and noodles are important products.

Precipitation varies widely across Iran and averages ~250 mm annually. Only about one-third of the wheat is irrigated, but it provides one-half to two-thirds of the crop. Much of the irrigated wheat is in eastern and southern provinces, and dryland wheat is predominately in western and northwestern provinces. The crop is planted during October and November. Moderate amounts of fertilizer are used (Table 6). Harvest begins in the southern and eastern areas in mid-April and ends in northern areas in August.

# **Western Europe**

France, like the other countries of western Europe, has a climate that is conducive to high yields of wheat (Table 7). The crop is managed intensively with high levels of inputs. The country is the major producer in the region and ranks fourth in the world. The primary wheat area is the northern half of the country, with Centre and Picardie the leading provinces. The major class is soft winter wheat, but some soft spring wheat and durum are also produced. Most of the grain is consumed as bread, and variable amounts are fed to livestock depending on the cost relative to other cereals.

Wheat is planted during October and November using high seeding rates, narrow rows, and tram lines. High rates of fertilizers and pesticides are applied, usually several times during the growing season (Table 7). Stripe rust, leaf rust, powdery mildew, and septoria are common. Harvest is in July and August.

Production of wheat in Germany differs substantially between the former east and west sectors. The former east sector has large farms formed from state cooperatives after reunification. Inputs and yields,

 Table 7
 Agronomic practices for production of wheat in major countries of western Europe

Practice <sup>a,b</sup>	France	Germany	United Kingdom
Major class	Semihard winter	Semihard winter	Semihard winter
Area (Mha)	5.12	2.61	1.85
Production (Mt)	37.05	19.62	14.87
Yield (t ha <sup>-1</sup> )	7.24	7.52	8.05
N fertilizer (kg ha <sup>-1</sup> )	155.0	147.0	192.0
P fertilizer (kg ha <sup>-1</sup> )	18.4	14.9	23.2
K fertilizer (kg ha <sup>-1</sup> )	27.4	36.5	45.7

a,b See footnotes to Tables 1 and 2.

while low relative to the west, are increasing (**Table 7**). Western farms, in comparison, are small but cultivate wheat intensively. Bayern and Niedersachsen are the major wheat states. Most varieties are semihard to hard winter wheat that are consumed as mixed bread with rye, whole wheat bread, and white bread. Considerable quantities are also fed to livestock and exported each year.

Most wheat is produced on mixed crop/livestock farms in Germany. Usual rotations with wheat include other cereals, various legumes, rapeseed, and sugarbeet. Wheat is mostly planted during October and, like in France, rates of seeding and application of fertilizer and other chemicals are high (Table 7). Rusts, powdery mildew, and septoria are major diseases. Harvest during July and August is sometimes disrupted by adverse weather, which affects both the yield and quality of grain.

Wheat is grown in the United Kingdom as far north as Scotland, but it is concentrated in southeastern England and the Midlands. Yields are extremely high because of the favorable climate and intensive management (**Table** 7). Soft and semihard winter and spring classes that are used for breads, biscuits, and pastries are grown. Much of the grain is also fed to livestock and exported.

Most wheat is grown on mixed crop/livestock farms in rotation with forages and other crops. The grain is usually planted during October and November, but sowing can be as late as January depending on the rotation and the weather. High seeding rates, narrow rows, and tram lines are used and heavy rates of fertilizer are applied (Table 7). Nitrogen fertilizer is often split among four or five treatments, and fungicides to control foliar diseases and growth regulators to prevent lodging are common. Little of the wheat is irrigated. Yellow rust, leaf rust, eyespot, tanspot, powdery mildew, and fusarium head blight are serious diseases. The grain is harvested during July and August.

See also: Cereals: Grain Diseases; Evolution of Species. Plants: Diseases and Pests; Triticale. Wheat: Breeding.

### **Further Reading**

Cook RJ and Veseth RJ (1991) Wheat Health Management, 152pp. St. Paul, MN: APS Press.

Gooding MJ and Davies WP (1997) Wheat Production and Utilization: Systems, Quality and the Environment, 320pp. Wallingford: LABI Publishing.

Hanson H, Borlaug NE, and Anderson RG (1982) Wheat in the Third World, 174pp. Boulder, CO: Westview Press.

Heisey PW, Lantican MA, and Dubin HJ (2002) Impacts of International Wheat Breeding Research in Developing Countries 1966-97, 73pp. Mexico, DF: CIMMYT.

Heyne EG (1987) Wheat and Wheat Improvement, 2nd edn., 765pp. Madison, WI: American Society of Agronomy.

Satorre EH and Slafer GA (1999) Wheat: Ecology and Physiology of Yield Determination, 503pp. New York: The Haworth Press.

USDA Agricultural Handbook No. 664 (1999) Major World Crop Areas and Climatic Profiles, 211pp. Washington, DC: US Government Printing Office.

#### **Relevant Websites**

http://www.cimmyt.org - This website of the International Maize and Wheat Improvement Center provides a wealth of information on wheat technology and production statistics, particularly in developing countries.

http://www.icarda.org - This website of the International Center for Agricultural Research in Dry Areas has information on wheat production in arid and semiarid regions of developing countries.

http://www.fas.usda.gov - The US Department of Agriculture Foreign Agricultural Service website gives up-to-date information on crop conditions and related data around the world.

http://www.awb.com.au and http://www.csiro.au -The Australia Wheat Board and Commonwealth Scientific and Industrial Research Organization web sites, respectively, contain information on production and technology of wheat in Australia.

http://www.cwb.ca and http://www.aafc.ca - The Canadian Wheat Board and Agriculture and Agri-Food Canada web sites, respectively, have information on wheat production and uses in Canada.

http://www.kswheat.com and http://wbc.agr.state. mt.us - The Kansas Wheat Commission and Montana Wheat and Barley Committee websites, respectively, provide extensive information on wheat production in those states and numerous links to wheat in other regions.

# Harvesting, Transport, and Storage

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#### Introduction

The harvesting, transport, and storage of wheat is big business worldwide because of the enormous size of

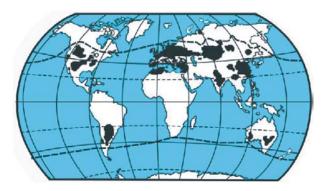


Figure 1 The main wheat-growing regions in the world; the northern and southern distribution boundaries are marked with broken lines. (Reproduced with permission from Grzesiuk S and Kulka K (1988) Biology of Cereal Kernels. Warsaw: Polish Scientific Ed. (in Polish).)

the global wheat-growing industry. Wheat cultivation covers a total of ~240 million hectares (Mha) around the world. Most of this ( $\sim$ 90%) is occupied by bread wheat (Triticum aestivum), which represents the greatest number of crop-yielding varieties of starchy grains. The numerous varieties of durum wheat (T. turgidum ssp. durum) are cultivated on the remaining area.

The geography of wheat cultivation is illustrated in Figure 1. The greater areas of wheat cultivation are in the northern hemisphere. Wheat is grown both as a winter and as a spring cereal in the moderate climate zones, and in the tropical regions in low lands and uplands alike. The distribution of wheat over the various parts of the world is related to the numerous species and varieties, and to their adaptability to particular environments. Wheat harvesting is a continuous process around the world and every month is a period of harvest in one of the wheatgrowing regions.

#### **Historical Perspective**

Wheat harvesting is a three-stage process – reaping, threshing, and winnowing. The first step of reaping involves cutting off the stalks of the wheat plant after it has become dry and ready for harvest. Before recent times of mechanization, reaping was performed by hand. The stalks were cut with a scythe or with a sickle, having a curved hook-like blade. The cut stalks were often left in the field in standing bundles with the heads uppermost, allowing the heads to dry out more, if this were necessary.

These bundles have been called shocks, stooks, or ricks. Mechanization led to the possibility that this step of cutting could be performed by machines,



Figure 2 Combine harvesting of wheat. This equipment cuts off the wheat stalks, threshes the grain from the reaped heads, and winnows away the chaff, depositing the grain in a bin. The grain is later transferred by auger (protruding above the combine to the left), into a truck driven beside the combine while it continues to harvest.

leaving the cut stalks lying in lines (swaths) if they needed to be allowed to dry more before threshing. "Swathing" is still performed in climates where harvesting is likely to be done under moist or even snowy conditions, the lying stalks being left to dry ready for the next stage of harvest.

Threshing involves removing the grain (with husks) from the stalks. Traditionally, this meant vigorous beating to separate the grain in the head from the rest of the plant parts, followed by winnowing, to free the grain from the husks, plus using some means to blow away the chaff.

Significant among the many inventions to mechanize harvesting was the reaper conceived by the American inventor and manufacturer, C. H. McCormick (1809-84). His reaper, patented in 1834, revolutionized wheat production. He went on to organize the manufacture of farm machinery on a mass-production basis. His success helped to speed up the large-scale mechanization of American agriculture. Later developments brought the modern combine harvester, which combines the three major operations of reaping, threshing, and winnowing in one machine (Figure 2).

By way of contrast, recent methods of harvesting are designed to map the harvested area according to the yield throughout the harvested area. This is done by fitting geo-positioning system (GPS) equipment to the combine. In this way, the position of the combine in the field is known at any time. The yield per unit area is recorded continuously, providing a map of grain yield throughout the field. The technique, known as "precision agriculture," is designed to identify the parts of the field that are under-producing, so that attempts may be made to remedy the problems.

This may involve taking soil samples to test for mineral deficiencies or checking for possible waterlogging in these patches of ground.

#### **Destinations for the Harvested Grain**

Historically, grain has been grown to meet the needs of the farming family, the grain being stored on the farm to provide food until the next harvest. This type of subsistence farming is still a way of life in some regions. As specialization of occupations developed, the farmer also produced grain for sale to neighbors and in nearby markets. Trade in wheat has now extended to see the movement of vast amounts of grain internationally. Furthermore, the sophistication of international trade has required that grain-quality specifications are suited to the types of process and product appropriate to each market. For example, many types of bread manufacture require hard-grained wheat that mills well to produce strong-dough properties, whereas, soft wheat giving extensible dough is required for cookie and cake production. Further specifications are required for many other products made from wheat, such as Arabic breads of many types, various kinds of noodles, pasta products, and feed grain for animals.

Many of the major wheat-growing countries produce much more wheat than can be consumed domestically. They, therefore, export a considerable proportion of their production. In doing so, they seek to maximize the returns for their grain on the international market, whilst focusing on markets that offer advantages for transport costs. The marketing organizations of the wheat-exporting countries have had to conduct extensive market research to determine what quality attributes are appropriate to targeted market destination, particularly depending on the types of products made and processing used in these regions. As a result of this knowledge, breeders have selected varieties that suit these needs, and specific varieties have been recommended for growth in appropriate regions. A range of other quality considerations must also be assessed, to ensure that the quality requirements of the specific markets are met.

# Meeting Appropriate Grain-Quality Standards

The range of quality requirements depends on the relative influences of genotype (the variety sown) and the effects of growth conditions, as indicated in **Table 1**. For example, the moisture content of the grain after harvest is solely dependent on harvest

**Table 1** Grain-quality attributes for wheat, listed according to the relative influences of genotype and growth environment on each attribute

Quality attribute	Influence of genotype (variety)	Influence of growth conditions
Variety	Sole	Nil
Starch properties	Major	Minor
Milling quality	Major	Minor
Grain hardness	Major	Minor
Protein content	Significant	Significant
Protein quality	Significant	Significant
Dough characteristics	Significant	Significant
Preharvest sprouting	Significant	Major
Defects	Minor	Major
Test weight	Minor	Major
Moisture content	Nil	Sole
Pesticide residues	Nil	Sole
Contaminants	Nil	Sole

Adapted from Wrigley CW and Batey IL (2003) Assessing grain quality. In: Cauvain SP (ed.) *Bread Making: Improving Quality*, pp. 71–96. Cambridge: Woodhead Publishing.

conditions and farm management, and the variety originally sown is not at all relevant. Moisture content is an important determinant of quality during storage, because moist grain is more susceptible than dry grain (e.g., below 12% or 13% moisture) to fungal attack and insect infestation. Similar considerations hold for the presence of weed seeds and pesticide residues, these quality factors also being wholly determined by growth and harvest conditions (Table 1).

On the other hand, aspects of grain quality such as hardness and milling quality are largely determined by the variety involved. Genotype has considerable influence on the protein content and dough properties, but so too do the growth conditions, including such factors as the level of nitrogen fertilizer and the presence or absence of frost, drought, or heat stress. For these reasons, many grades of wheat with particular quality requirements have specifications for a selection of specific varieties of similar quality, suited to the growth conditions expected for the region. Although variety is an important factor affecting quality, wheats are seldom classified on the basis of a single variety. Thus, each class consists of a group of varieties of similar characteristics for specific purposes.

The testing of a truck-load of grain for quality (**Table 1**) is ideally performed on delivery to a flour mill or storage terminal (elevator or silo). These tests must generally be performed within a short time, probably several minutes. This is because a decision must be made, while the truck is waiting, on where the grain should be tipped. In such situations, there are generally several storage cells, each holding a different



Figure 3 Delivery of harvested grain to a storage terminal may involve first stopping the truck-load at a sampling stand, so that samples of grain can be taken to assess its quality.

grade (class) of wheat, each grade being suited for distinct uses with different quality specifications.

The first step is usually to take samples from the truck's load for the tests to be carried out. Samples may be taken with a hydraulic set of probes, which are lowered into the load of grain. Alternatively, sampling may be performed manually with a spear probe, such as is shown in Figure 3. This spear consists of two long concentric cylinders, each with vertical slits. When inserted, the slits are closed. After insertion, the top of the spear is twisted, to open the slits allowing grain to flow into the inner tube from all levels of the grain load. In the case of manual sampling, an operator thrusts the spear into the grain after climbing on top of the load, or leaning over from an elevated sampling stand. Spear samples are taken at several specified points across the load, with the aim of obtaining a combined sample that is representative of the whole load. Refer to the web site of the United States Department of Agriculture (www.usda.gov/ gipsa/pubs/farm-proc/practical proc.htm) for standard sampling procedures.

## **Testing to Ensure Grain-Quality Standards**

The grading of wheat grain involves the segregation and valuation of grain consignments based on the physical and chemical properties of the grain. The scope of wheat-grain grading is related to our current agricultural knowledge, as well as to the level of organization of the local cereal market (pricing policies, grain-purchasing systems).

The systems of wheat grain grading include the following groups of grain properties:

- 1. "Stable" properties, related to genetic properties (determined by the variety), such as grain hardness, size, shape, and color (red or white). These are the factors towards the top of the list in Table 1, distinguished by being only slightly influenced by growth conditions. These quality attributes have been "built in" as a result of intentional breeding and selection. (See articles on Wheat: Genetics; Breeding.) Testing for these mainly involves varietal identification.
- 2. "Variable" properties, subject to change as a result of drying, cleaning, sorting, and transport (moisture content, contamination, damage to grain), related to climatic conditions, and management practices (e.g., bulk density). These factors, appearing lower down the list in Table 1, are described in detail in articles on Cereals: Grain Defects; Grain Diseases, and Contaminants of Grain. Specific testing must be conducted for each of these.
- 3. "Permanent" faults and defects, such as stale smell, fermented smell, foreign smell, or faults that can be rectified, e.g., washing grain contaminated with soil or foreign matter.

The "stable" properties provide a primary basis to determine the appropriate market class. They are, thus, critical for grade determination in international trading. As they are mainly determined by genotype, this is the reason for specific varieties to be nominated for premium grades and classes.

Grain hardness, a "stable" property, is an important basis for classification in trade. In relation to grading, the following definitions are applied:

- 1. Hard wheat is wheat, which, as a result of variety and breeding in combination with environmental factors during growth, has a vitreous endosperm generally considered an advantage for the production of bread-making flours.
- 2. Soft wheat is wheat, which, as a result of variety and breeding, in combination with environmental factors during growth, has a white opaque endosperm generally considered more suitable for the production of cake and biscuit flours.

The "variable" properties are secondary properties, although also important from the viewpoint of grain quality. The group of variable properties, systematized into several ranges in terms of values, constitutes grain grades, which are identical for many of the market classes. The quality grading of wheat grain sometimes includes an assessment of mechanical damage, which occurs wherever grain is subjected to the destructive action of internal or external forces.

The results of research carried out using X-ray methods have shown significant differences in grain endosperm cracks between common wheat varieties. Natural wetting of dry grain (below 15% moisture) during rainfall in field conditions is one of the main reasons for cracking. Considering the results of endosperm cracks, which can appear before harvest in some climatic regions of wheat production, special attention should be paid to this potential defect, and to the susceptibility of specific varieties to this fault.

The susceptibility of wheat to mechanical damage is determined by genetic factors (hard and soft grains), environmental effects (climatic conditions during the preharvest period), and by the conditions of grain storage (excessive humidity). The combination and selection of these properties determine further aspects of grain quality. Some of the "permanent" faults and defects are a basis for excluding shipments during grain grading, as their character is likely to disqualify grain from food use. Defined faults, which can be rectified, may make some grain lots eligible for special grades of defective quality.

## **Quality Specifications of Individual Countries**

Standards of wheat quality have been established by most wheat-growing countries. Canada and USA, as examples, have systems of wheat grading with classes based on the varietal properties expressed in the

vegetation period (spring versus winter wheats), grain coloring (red or white), and hardness (hard or soft). Within the various classes, there are specific grades (subclasses) each with their distinctive quality specifications, often relating to protein content. Five classes of wheat are distinguished in USA: Hard and Soft Red Winter, Hard Red Spring, Durum, and White (subclassified into Hard and Soft Winter White and Hard and Soft Spring White). Wheat grading in the USA additionally provides, in the subclasses, for the proportion of vitreous grains.

Groups of varieties of appropriate quality are specified for most grades of wheat. As an example, in Canada the model Hard Red Spring wheat was originally the variety Marquis. Many years ago, it was replaced successively by varieties that are genetically similar with respect to their genetic potential for appropriate grain quality, but of more value economically with respect to yield potential and disease resistance. More recent replacements include Thatcher, Manitou, and most recently, Neepawa – the "equalto-or-better-than" variety for Canada Western Red

Canada Prairie Spring Red wheat is a mediumstrength wheat suitable for the production of certain types of hearth breads, flat breads, steamed breads, and noodles. Canada Western Amber Durum specifies "any extra strong red spring wheat that is equal to or better than" the variety Hercules. In recent years, an extra class, "extra strong," has been added to the Canadian grading system, and it is made up of "any extra strong red spring wheat that is equal to or better than" the variety Glenlea. This is a good example of the use of a specific variety being used as the basis of a specific class. In this case, "extra strong" refers to the strength of the dough made from the flour, the extra-strong gluten of this class being attractive for special bread types and for blending with wheats with low and/or weak gluten content. Wheat is also produced in the eastern provinces of Canada, where the wheat is graded into various classes such as "Canada Eastern Soft White Spring" and "Canada Eastern Soft Red Winter."

In Argentina, most of wheat cultivation involves hard red winter wheat, also known as "Plate" wheat. Wheat growing is concentrated in the provinces of Buenos Aires, La Pampa, Cordoba, and Santa Fe. Argentina uses a system of bread-wheat division into subclasses based on grain hardness (hard, semihard, and soft). Export classes are named according to the ports of shipment (see Grain Production and Consumption: South America).

In Australia, the crop might be classed as spring wheat, because the conditions in all parts of the wheat belt do not provide the cold temperatures

needed for the vernalization of a winter wheat. Nevertheless, some varieties approach the winter-wheat type by having a long maturity period. A high proportion of the Australian wheat crop is exported (often  $\sim\!80\%$  of it), and it has been an important accent of marketing efforts to ensure that market requirements are understood and matched. Australia has developed a grading system based exclusively on white-grained wheats.

Prime Hard is the top-quality grade, with a protein content of 13–14%, high milling quality and processing attributes. Flour from it is used in export markets for high-protein yellow-alkaline noodles, Wanton dumpling skins, high-volume breads, flat breads, rotis, and chapatis. Its high protein content suits it to blending with lower protein wheats. Australian Hard (hard-grained varieties with a minimum protein content of 11.5%) suits European-style breads, Middle-Eastern flat breads, and Chinese steamed products such as Mantou and Pao. Premium White (minimum of 10% protein) is suitable for a wide range of products, including Hokkien, instant and fresh noodles, Middle-Eastern and Indian-style breads, and Chinese steamed bread.

Australian Standard White is a medium- to low-protein white wheat. Australian Soft Wheat (10–11% protein), a smaller part of the crop than the hard-wheat grades, is used for white-salted noodles in Japan and Korea, and for confectionary products, such as cakes, pastries, biscuits, cakes, steamed buns, and snack foods. The Australian Noodle grade is a premium class because its special starch properties make it ideal for white-salted udon noodles for the Japanese and South Korean markets. Grain that does not meet the specifications for the above grades is classed as General Purpose of Feed wheat. Australia also produces three grades of durum wheat.

In Europe, and especially in France and Germany, intensive agricultural procedures have resulted in virtually the highest grain yield per unit of area of cultivation. Traditionally, European countries have been importers of wheat, especially of strong wheats for blending, but since the 1970s, the European Community has been a significant wheat exporter. In recent years, "clubs" have been set up by wheat producers who achieve yields of 10-15 t ha<sup>-1</sup>. In this region, the dominant types are wheats of the Soft Red Winter class. The European Union countries have dozens of high-yielding varieties of very good grain quality. In Mediterranean countries, such as Italy and Spain, durum wheats make significant contributions to wheat production.

East European countries grow mainly red winter wheats, some of which have very good baking qualities. In the countries of the Commonwealth of Independent States (CIS), both spring and winter, red and white wheats are grown, as well as some durum wheats. The CIS has the largest area of wheat cultivation in the world. The leading varieties, in terms of yield and grain quality, are the winter wheats, Bezostaya 1 and Mironovskaya 808, and the spring wheat Saratovskaya 29.

Wheat grading in the CIS is based on principles similar to those followed in the USA, with the distinction that classes are replaced in the CIS by types, and subclasses by subtypes, primarily designed to take into consideration the proportions of vitreous grains. Some European countries (e.g., France, Germany) use a grading system based on protein content, bulk density, and the sedimentation test. With only slight differences in the appearance of many varieties, and with the possibility of grain-quality reduction as a result of environmental effects, grades have been developed to include, in addition, the contamination of the grain and the Falling Number test for sprout damage.

Other countries, in which wheat is grown or only imported, also use specific standards for the evaluation of the quality of wheat grain and its products. Most frequently, such systems are modifications of those presented above.

## **Transportation of Grain**

In many countries, the regions of grain production are distant from the centers of population and from the export terminals. Transportation is thus a significant expense. It is common for trucks to be used for delivering the grain from the farm to the country elevator (silo) or to the flour mill. This delivery point is the primary opportunity for testing the quality of the grain, so that best quality grain can be segregated from poorer grades. Truck delivery and sampling is illustrated in Figure 3. Country elevators are always situated near transport facilities, as is illustrated in Figures 3–6.

Rail transport is the usual means of transporting large volumes of export grain long distances from country elevators to the terminal elevators. These are generally located at harbors, ready for the loading of ships for transport by sea to importing countries. This form of transport is illustrated in Figure 7, but in this case, there will be another transfer of grain before it reaches its export destination. Figure 7 shows grain being loaded into a lake barge at Thunder Bay, Canada, for movement through the Great Lakes of North America to a further terminal where it will be transferred to a sea-going ship.

Much of the grain exported from the United States is carried by barge down the Mississippi and



Figure 4 Vertical silo storages in country Australia.



Figure 5 Circular silo, designed to hold different grades and types of grain in the separate vertical cells.

Columbia Rivers to export elevators, where it is unloaded and stored until it can be loaded onto ships. In addition, some grain is transferred directly from barge to ship, without being stored in an elevator. An export elevator can load a ship with 60 000 t of grain, worth over \$10 million, in two days. The weight certificate must thus be very accurate, due to the large sums of money involved.

By contrast with subsistence farming, in which the family consumes the grain it produces, the process of moving export grain is complex. It passes through the following stages: from the farm, to country silos, possibly via regional centers, to terminal storage elevators. At every stage, grain is subjected to checking and classification. This ranges from highly detailed to more general grading. Treatment may include improvement (drying, cleaning, sorting, and disinfestation, if required), and grouping of supplies into larger batches of uniform quality. Isolated fractions of undersize grain and lower-grade wheat may be sold on the local market.

## **Storage**

Like transport, grain storage is likely to start at the farm. Many years ago, wheat was bagged for transportation and storage, but bulk transport and storage is now usual, except in cases of special quality requirement. Some regions have a tradition that grain is delivered to a country elevator or mill immediately it is harvested, whereas in other places, onfarm storage is common. Retention of the grain at the farm may provide trading advantages for the



Figure 6 A horizontal storage silo (at right) and bunker storage (left and top center). Grain in the bunker storage is laid on layers of plastic sheet and also covered over with sheets of plastic. Note the freshly harvested field on the extreme right.



Figure 7 Loading of bulk wheat for export at Thunder Bay, Canada.

grower, permitting him to wait until prices rise before selling.

Nevertheless, most major wheat-producing countries have major storage facilities through the grain-growing regions. These may take various shapes and sizes, as shown in Figures 4-6. The vertical silos in Figure 4 are an older type, although these are being modernized by sealing them to permit insect disinfestation by fumigation, or preferably by treatment with carbon dioxide. Figure 6 shows a few forms of storage, conveniently situated beside rail and road transportation. The long, low structure at right of Figure 6 is known as a "horizontal storage." Like the vertical silos (Figures 4 and 5), it may have several separate cells to accommodate different grades and types of grain.

Stability during storage and transport are the great advantages of the grains as food sources. During storage, the rate of grain respiration is very low, and this accounts for its stability and the very low losses in the mass of the stored grain. However, these advantages depend on the condition of the grain, its moisture content, temperature, and soundness. Grain respiration may be more intense in soft grain than in hard grain. If grain moisture content is relatively low (below 14%), and if the bulk of grain of that moisture content is homogeneous, the grain can be stored for several years without significant losses. However, if proper air temperature and humidity conditions are not observed in the storage area, the increasing humidity resulting from the process of grain respiration may lead to the phenomenon of spontaneous heating of the grain, and this may render it unfit for human consumption.

In some wheat-growing regions, e.g., in the Scandinavian countries, wheat grain is harvested relatively moist and it must be dried to maintain its quality. Drying must be performed under controlled conditions of temperature and humidity. Exceeding the required temperature may lead to the loss in the baking value of the flour. To avoid damaging grain meant for baking purposes, its temperature should not exceed 35°C at moisture contents above 20%.

## **Future Prospects**

Various revolutionary approaches to grain storage and transport have been proposed and tested. One of these is the possibility of pumping a slurry of grain in liquid through a pipeline. This system has worked well for minerals in some cases, but it has not yet been adapted to any significant extent for wheat grain. One difficulty is to choose an appropriate liquid medium. Rapeseed oil has been used in trials, but the difficulty has been to remove the oil efficiently. Another possibility is supercritical liquid carbon dioxide at low temperature and high pressure; this was found to extract lipid from the grain and to be very expensive.

It is likely that conventional methods of harvesting, storage, and transport will continue with relatively slow change. However, new approaches may be directed towards improving the quality of grain consignments. This may involve more controlled harvesting, by using "precision agriculture" to confine harvesting to parts of the field that have higher protein content, thereby achieving a premium for a significant part of the harvest that would not have otherwise been possible.

A proven approach to avoid downgrading due to sprout damage involves using an immunoassay to test grain from various locations in the field, thus to determine those parts of the field that are not damaged in this way. This information can then be used to confine the travel of the combine to the sound parts of the field, and thus avoid the risk of losing a premium payment by harvesting sprouted grain with the sound grain.

For storage, new approaches to preventing loss to insects and vertebrates is a priority, because significant losses of this nature continue as a major problem, especially in developing countries.

See also: Cereals: Grain-Quality Attributes; Overview; Grain Defects; Grain Diseases. Contaminants of Grain. Milling and Baking, History. Stored Grain: Invertebrate Pests; Pest Management. Variety Identification of Cereal Grains. Wheat: Genetics; Breeding; Agronomy; Grading and Segregation; Marketing. Appendix: Test Methods for Grain and Grain-Based Products.

## **Further Reading**

Faridi H and Faubion JM (eds.) (1995) Wheat End Uses Around the World. St. Paul, MN: American Association of Cereal Chemists.

Grzesiuk S and Kulka K (1988) *Biology of Cereal Kernels*. Warsaw: Polish Scientific Ed. (in Polish).

Grundas ST (1997) Advantage of physical properties of single kernels of wheat to evaluation of its utility values. *Europe News* (Europe Section of the American Association of Cereal Chemists) July: 6–7.

Henry RJ and Kettlewell PS (eds.) (1996) Cereal Grain Quality. London: Chapman and Hall.

Hoseney RC (1994) Principles of Cereal Science and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

O'Brien L and Blakeney AB (eds.) (2000) An Introduction to the Australian Grains Industry. Melbourne: Royal Australian Chemical Institute.

O'Brien L, Morell M, Wrigley C, and Appels R (2001) Genetic pool of Australian wheats. In: Bonjean AP and Angus WJ (eds.) *The World Wheat Book*, pp. 603–640. Paris: Lavoisier.

Pomeranz Y (ed.) (1988) Wheat: Chemistry and Technology, 3rd edn., vols. I and II. St. Paul, MN: American Association of Cereal Chemists.

Pomeranz Y (ed.) (1989) Wheat is Unique. St. Paul, MN: American Association of Cereal Chemists.

Sauer DB (ed.) (1992) Storage of Cereal Grains and Their Products, 4th edn. St. Paul, MN: American Association of Cereal Chemists.

Saurer W (1996) The Single Kernel Classification System for the Characterization of Wheat. *Proceedings of the 10th International Cereal and Bread Congress*. Greece: Chalkidiki.

Stafford J and Werner A (2003). *Precision Agriculture*. Melbourne, Australia: CSIRO and Wageningen Academic Publishers.

Wrigley CW and Batey IL (2003) Assessing grain quality. In: Cauvain SP (ed.) *Bread Making: Improving Quality*, pp. 71–96. Cambridge: Woodhead Publishing.

#### **Relevant Websites**

http://www.awb.com.au - AWB Ltd, Melbourne, Australia.

http://www.cgc.ca; www.grainscanada.gc.ca — Canadian Grains Commission, Winnipeg, Canada. http://www.pi.csiro.au — CSIRO Plant Industry, Australia.

http://www.icc.or.at — International Association for Cereal Science and Technology.

http://www.seedtest.org — International Seed Testing Association.

http://www.crop.cri.nz - New Zealand Institute of Crop & Food Research.

http://www.usda.gov — United States Department of Agriculture; grain handling practices, standard sampling procedures.

# **Grading and Segregation**

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## Introduction

A key demand of flour millers and their customers is a consistent product. Unfortunately, the quality of wheat can be variable, not only between years but between shipments that depend on the medium of transport — be it an ocean vessel, a rail wagon, or a road truck. This variability is due to the effect of the environment on the inherent genetically determined quality factors, and can be traced back to quality variation within a single head of grain. Therefore, the delivery of a consistent product to customers is a considerable challenge to suppliers of wheat.

The terms grading, classification, and segregation can take on different meanings around the world, but in essence, they all describe processes that are designed to ensure that the parcel of wheat that is ultimately created meets the needs of discriminating buyers, performs in a predictable and consistent way, and, in the better developed systems, exhibits quality attributes that are ideal for the production of specific wheat-based foods. Examples are the hard red spring wheats produced in Canada and the United States that are well suited to the production of high-volume breads made by the traditional sponge-and-dough baking process, and the noodle wheats produced in Western Australia, which are well suited to the production of white salted noodles in Japan and Korea. A number of different approaches will be discussed in this article.

#### The Quality Feedback Chain

Before tackling the different approaches that are used around the world to ensure that customer quality requirements are met, it is important to first understand what we will call the quality feedback chain (Figure 1). Starting with the development process of a new wheat variety, it is assumed this will take somewhere between 8 and 15 years. New technologies now mean that the breeding component of variety development has been reduced, but there is still the need to bulk up seed crops, then there is the further delay due to the uptake of new cultivars by farmers. Depending on the attributes of the variety, the time frame of this adoption phase by farmers can vary considerably, but at least 2 years is required before significant tonnage of

seed is available for customers to use. Successful breeders have to anticipate the future quality requirements of customers not today, but in that 8–15 year period that will elapse, before their new variety is available for harvest and utilization.

Therefore, an important component of delivering a consistent product to customers is not only managing the year-to-year quality variation as a result of factors such as drought, harvest, rainfall, and/or frost, but having a product that meets processing needs, such as baking or noodle-making and eating performance. This requires an extensive working knowledge of customers' processing needs and working with them to enhance the product being delivered. An added complication in the international trade of wheat is that other suppliers are competing for a share of that customer's business. Therefore, not only do suppliers need to have ongoing quality improvement, but must also continually monitor the competitiveness of their products.

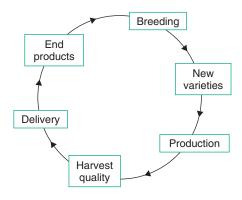


Figure 1 Wheat quality feedback chain.

## **Testing Grain Quality**

As an example of the potential for the geneticby-environment interaction, the annual harvest of wheat in Australia occurs in a 4 month period, commencing in Queensland in late September/early October, and finishing in the higher rainfall areas of NSW, Victoria, South and Western Australia in late December/early January. During this intense period of activity, each truckload of a 20–25 million ton (Mt) wheat harvest is sampled and quality tested when it is delivered to the silo network around the country. Given the large quantity of grain harvested (wheat comprising  $\sim 60\%$  of total grain production), speed of testing is essential to minimize delays and to avoid harvest-weather damage. Quick and accurate tests exist for physical or environmental characteristics such as bulk density, protein level, and cleanliness. Industry-recognized receival standards are used to determine whether wheat is suitable for milling or feed purposes from a physical-quality perspective. However, for inherent or genotypically determined quality attributes, such as milling performance, dough properties, and end product qualities, such rapid tests are not yet readily available. Instead, Australian buyers and sellers of wheat rely on the statutory variety declarations by farmers at the time of delivery to characterize genotypic quality. The success of this approach relies solely on the classification of a variety's genetic quality characteristics before it is released for commercial production. Five countries have dominated world wheat trade over the last 5 years (Table 1) and these will be discussed with respect to how they strive to match the quality expectations of their customers.

Table 1 International wheat exporters involved in world trade in wheat flour and products

Exporter	1999/00	2000/01	2001/02	2002/03	2003/04	5 year average
Argentina	11 083	11 396	11 671	5500	10 000	9930
Australia	17 124	16 682	16 494	10 500	13 500	14 860
Canada	19 373	17 351	16 758	8500	14 500	15 296
Eastern Europe	3401	2336	4151	3500	2300	3138
EU .	17 432	15 225	11 494	15 500	14 500	14 830
India	200	2357	3234	5000	3000	2758
Kazakstan	6514	3668	3780	5500	5500	4992
Other	3716	4217	6120	6570	5700	5265
Russia	518	696	4372	12 500	1000	3817
Turkey	1984	1601	558	600	800	1109
Ukraine	1952	78	5486	7500	1000	3203
United States	29 399	28 027	26 248	24 000	26 000	26 735
Total	112 696	103 634	110 366	105 170	97 800	105 933

Years are July-to-June. Volume is given in thousands of tons.

Adapted from Foreign Agricultural Service (2003) Grain: World Markets and Trade. Circular Series FG06-3 June 2003, p. 7. Washington: United States Department of Agriculture.

# Inherent Quality and Genotype Classification

Around the world, variations of the same theme are employed by buyers and sellers of wheat; i.e., the comparison of new varieties with existing varieties, whether they be targets or minimums, to determine what class or value the new variety should be given. However, the specifics of this matter vary — from the largely unregulated system which exists in the United States, to the formal process in Canada governed by both the Canada Grain Act and Seeds Act and Regulations, and the formal but more pragmatic and market-driven approach that is followed in Australia.

#### **Australia**

The major buyers (and hence classifiers of wheat) in Australia, AWB Limited and the domestic flourmilling industry have strict protocols in place to obtain a classification. These requirements focus on the provision of multiple years of quality data, from the various classification regions, for an extensive set of tests, the results of which are compared against control varieties, rather than empirical values. This recognizes the seasonal influences on wheat quality and that empirical values may vary from year to year. The classifiers also acknowledge that some parameters are essential and cannot be compromised, while less important attributes failing to reach the necessary levels can be counter-balanced by exemplary performance for other, more important quality attributes. This means that minor deficiencies do not necessarily have a negative influence on the final classification decision, provided they are compensated by other attributes.

In addition to establishing the dollar value, the classification of a variety is important because current technology does not allow for accurate assessment of the genetic attributes like milling performance, flour and dough properties, and end product suitability. Therefore, instead of performing tests at harvest, at the first point of delivery, a variety-declaration system is used in Australia. Farmers are required to make a statutory nomination of the variety being delivered, and this establishes the inherent quality profile and also the payment potential (technology allows quick and accurate measurement of physical attributes). The classes of Australian wheat are valued differently based on their quality, as illustrated in Table 2. As a consequence of this variety-declaration system, the classification process is extremely important in ensuring that the right quality is ultimately delivered to end users.

#### Canada

The Canadian variety-registration system is a formal, fee-based process, coordinated by the Variety Registration Office. Potential varieties must first be registered before being evaluated by recommending committees based on morphological, pathologic, agronomic, physiologic, biochemical characteristics, and kernel information. The significant difference to the process employed in Australia is that all nominated quality parameters must be at least matched by the new variety. In addition, each of the Canadian wheat classes "is visually distinct from the others, and the varieties within each class are visually similar. The Canadians refer to this visual characteristic as "kernel visual" distinguishability, or KVD, and it is a requirement that must be met if a wheat variety is to be registered for production" - Canadian Grain Commission, 2003. It is this aspect, combined with unwavering quality requirements, that some consider to have held back advancements in agronomic and quality performance of the Canadian wheat industry.

#### **USA**

In contrast, the system of classification is conducted on a less formal basis in the United States. Wheat is grown on a strictly regional basis. Potential varieties are considered by Wheat Quality Councils, funded by the various State Wheat Commissions, with quality testing being undertaken by four main regional laboratories of the US Department of Agriculture, central to the main class of wheat produced in the region. In addition, flour millers perform their own testing as a basis for the subsequent buying strategies. The provision of quality and agronomic information comes from trials conducted by the state-based universities and agricultural colleges, in addition to private companies.

**Table 2** Base-rate returns in \$/ton estimated as examples of value differences between specific grades

Grade of wheat	Return Aust \$/ton
Australian Prime Hard (13% protein)	243.50
Australian Hard (11.5% protein)	232.00
Australian Premium White (10% protein)	224.00
Australian Standard White (10% protein)	212.00
Australian Premium Durum (13% protein)	262.00
Australian General Purpose (10% protein)	206.00
Feed Wheat	190.00

Estimates are FOB and GST-exclusive, assuming 5% screenings and 12.5% moisture, with the exception of Feed Grade, for which protein content and screenings are not specified.

Adapted from AWB Limited, Australia.

#### **France**

With almost half of French production now destined for export, it is important for France to have a variety regulation system. The system differentiates varieties into four end product classes. These are high-grade bread making (BPS), regular bread making (BPC), biscuit wheat (BB), and wheat for other purposes (essentially for stock feed or industrial uses) than bread making or biscuit making (BU). These grades are used in recommendation lists produced by the French Department of Agriculture on which farmers base their variety choice. To be listed, varieties must go through a 2 year official testing period, in which the new variety must be equal to the standard for both agronomic and quality measurements. The key quality measurements used to differentiate between the grades are the Alveograph and bread making tests.

#### **General Philosophy**

All this attention to the quality of the wheat traded shows that successful suppliers of wheat in the international market have mechanisms in place to ensure the consistency of inherent quality characteristics. The systems used are different, although, all have a common fundamental philosophy. All classify or group wheat varieties into a commercial type or style that is recognizable for its inherent processing features. The aim of grouping wheat varieties is to produce parcels of wheat that deliver a consistent product and hence, meet customer expectations. Unfortunately, classification of varieties on inherent characteristics is only half the equation, as the growing environment has a significant influence on quality.

#### **Physical Quality – Harvest Classification**

Although testing technology for inherent quality characteristics has yet to have broad adoption by the wheat industry, continued improvements have occurred in the assessment of physical quality. It was only 30 years ago that, in Australia, wheat was classified into the broad category of fair average quality (FAQ). This was around the same time that the Canadians introduced protein measurements to assist with the marketing of hard red wheats. Without accurate and quick methods for protein and moisture, wheat was segregated on the basis of cleanliness and visual soundness. Today, near-infrared (NIR) technology, along with a host of new technologies like single kernel characterization and image analysis, allow the assessment of protein, moisture, cleanliness, and, in the not too distant future, defects like stained grains and durum vitreousness.

All the major wheat-export countries have a range of classes or grades to reflect good and bad quality (Table 3). These not only separate the clean from the dirty, but also are recognized by buyers with respect to the end use of the wheat. Therefore the systems used have the dual effect of differentiating on the basis of physical traits, such as test weight and cleanliness, while also being readily identified by customers for certain attributes over and above the physical quality.

A key feature for ensuring that the wheat is segregated appropriately is testing at harvest. This has always been, and will continue to be, a challenge because farmers must deliver their wheat as quickly as possible (to avoid any possible harvest-weather damage), yet grain-handling agents appear to be delaying the delivery process by testing and segregating. At the very least, the time taken to perform testing is necessary, simply for the fact that once a delivery is poured down the grid, it is difficult to extract it back out if it has been binned incorrectly.

#### **Australia**

In Australia, the majority of wheat is delivered from the farm to a network of silos. It is against this first delivery that individual loads are measured for the range of physical and visual attributes. Most of these silos are managed by only a hand-full of private or farmer-controlled organizations, each with its own reference laboratories. Protein content, moisture, and cleanliness are measured objectively by NIR and specifically designed automatic shakers. Repeatable measurements are important for these attributes, as farmers are paid on the level delivered. Test weight and falling number are also objectively measured, but these serve to differentiate between grades and do not have incremental payment scales like protein content and moisture. Similarly, visual assessment of frost damage, stained grains, and vitreousness determine for which grade a delivery will be eligible. However, like in North America, research in Australia is pursuing the vexed question of objective visual assessment. Whilst NIR may provide some answers for objective assessment attributes like durum vitreousness, image analysis is likely to be able to assess a wider range of defects, which may in turn reduce the costs and testing time, making such technology more attractive for adoption by grain handlers.

The harvest assessment of Australian wheat determines how much the farmers are paid and where their wheat is stored. The quality results are aggregated electronically and then used in allocation of stock. When the analytical results are combined with variety composition, AWB Limited as the bulk

 Table 3
 Wheat grades and classes of major wheat exporters

Arg	gentina	Austr	alia		Canada				Germany	France	
Grades	TWT min. (kg hl <sup>-1</sup> )	Grades	TWT min. (kg hl <sup>-1</sup> )	Classes	Grades	TWT min. (kg hl <sup>-1</sup> )	Classes	Grades	TWT min. (kg hl <sup>-1</sup> )	(grades)	
1	79	AWB prime hard	74	Canada Western Red Spring	No. 1 No. 2 No. 3	75 72 69	Hard red winter	No. 1 No. 2 No. 3	78.9 76.4 73.8	Elite (E)	E
2	76	AWB hard	74	Canada Western Amber Durum	No. 1 No. 2 No. 3 No. 4 No. 5	79 77 74 71	Hard red spring (subclasses based on vitreous kernel percentage, e.g., dark northern spring >75%)	No. 1 No. 2 No. 3	76.4 75.1 72.5	High-quality bread wheat (A)	1
3	73	AWB premium white	74	Canada Western Red Winter	No. 1 No. 2	78 74	Soft red winter	No. 1 No. 2 No. 3	78.9 76.4 73.8	Normal bread wheat (B)	2
		AWB standard white	74	Canada Western Soft White Spring	No. 1 No. 2 No. 3	76 74 69	Durum (subclasses based on vitreous kernel percentage, e.g., hard amber durum >75%)	No. 1 No. 2 No. 3	78.2 75.6 73.0	Soft wheat (K)	3
		AWB noodles	74	Canada Western Extra strong	No. 1 No. 2	75 73	Hard white	No. 1 No. 2 No. 3	78.9 76.4 73.8		
		AWB durum	74	Canada Prairie Spring White	No. 1 No. 2	77 75	Soft white (subclasses are soft white, white club <sup>a</sup> , and western white)	No. 1 No. 2 No. 3	78.9 76.4 73.8		
		AWB general purpose	70	Canada Prairie Spring Red	No. 1 No. 2	77 75					
		AWB feed	68	CW		65					

<sup>&</sup>lt;sup>a</sup>White club has the same test specifications as Hard red spring wheat.
TWT min. = minimum test weight.
Sources: Trigo Argentino Institutional, AWB Limited, Canadian Grain Commission, US Wheat Associates.

marketer of Australian wheat has a powerful tool in terms of knowing the physical quality (from harvest measurements) and intrinsic quality (from the variety composition). Grain is retested as it moves from country silos to port-loading facilities. Prior to shipment, wheat is again thoroughly tested to further refine the quality of stocks to meet customer's contract specifications. The final testing of the wheat occurs during loading, when representative samples are collected and then tested accordingly to customer requirements. In some instances, this may require milling and actual end-product assessment, and/or residue testing for chemical or pesticides according to government import requirements.

#### Canada

At the opposite end of the harvest quality-testing regime is the system used in Canada. Owing to the fact that the majority of storage is on-farm, the Canadian system, for ensuring that export wheat meets customer requirements, has a strong focus on measuring the quality of rail car lots from the elevator network as they begin their movement to port for export. It is commercial companies that assemble the wheat received from farmers to meet the requirements of the Canadian Wheat Board (CWB) through their quota system. Ensuring that the testing is performed to industry standards, the Canadian Grain Commission (CGC) licenses each silo operator and monitors their testing proficiency.

The unique aspect of the Canadian system is the reliance on KVD to segregate most farmer deliveries. Protein assessment occurs at the country silo for the top grades of the Canadian western red spring (CWRS) class, with other classes being tested at export terminals for protein. Generally, achieving the necessary protein level is not a problem in Canada, unlike in Australia where inherently poor and very old soils mean that to achieve higher levels, farmers need to actively manage their crop. When the KVD is done, the silo operator also determines the cleanliness of the delivery and whether or not cleaning is required to meet export standards. This is different from Australia, where the customer receives the aggregated cleanliness of the wheat as delivered by farmers, as wheat is not cleaned as it moves through the supply chain. Canadian farmers' payments are adjusted based on this "dockage" measurement.

Wheat is then moved to export terminals where CGC inspectors confirm the quality of the wheat being unloaded. Again, the higher grades of the CWRS class are protein analyzed. In addition to cleaning facilities, wheat can be dried if required. As occurs in Australia, a cargo sample is collected,

upon which the CGC issues a "Certificate Final" which guarantees the quality and the weight of the wheat in the cargo. The regulated nature of the Canadian systems means that the CGC has strong governance of the product ultimately sold by the CWB whereas in Australia, AWB Limited relies on contracted third parties to load their wheat.

#### **USA**

Country elevators also play a key role in the movement of wheat in the United States. There are some 10 000 privately owned elevators throughout the wheat-growing areas of the United States. Either large grain-exporting companies, farmer cooperatives, flour milling companies and or other business enterprises own the country elevators. As occurs in other countries, a sample is taken of the delivery to determine its grade, moisture, and dockage. A division of the United States Department of Agriculture called Grain Inspection, Packers and Stockyard Administration (GIPSA) manages the government quality standards. The grade is determined on the basis of appearance, moisture level, and cleanliness as outlined in the US Grain Standards. If required, protein and falling number tests can be performed as required under customer contracts.

From the country elevator, wheat is transported by either rail, road, or river barges to larger central storage facilities that serve both domestic and export customers. At terminal facilities, wheat can be blended to achieve the specific quality requirements of different customers. Again, the United States has facilities that can clean wheat to lower specifications than those that apply to grain received at the country elevator. GIPSA representatives or licensed state and private agencies inspect all wheat exported as it moves through the supply chain from country silo to export terminal.

Unlike Australia or Canada, any individual or company meeting certain criteria can export US wheat. According to US Wheat Associates, "the export grain companies that merchandise US wheat are generally of three types:

- large, privately-owned, vertically integrated, multinational companies which have offices or representatives in most importing countries;
- smaller, privately-owned multinational companies which do not own or operate significant grainhandling facilities themselves, but maintain an international network of agents or representatives in importing countries; and
- cooperatively-owned firms or farmer-owned cooperatives that can compete as viable exporters.

Each type of exporter has access to the same sources of wheat supplies and all three types maintain well trained, technically competent staff who are able to coordinate the many facets of logistics, finance and governmental regulations necessary to transact international grain sales and deliveries."

As a large central quality-assurance agency, GIPSA is active in pursuing new technologies to be used in their inspection services. For example, prior to becoming part of GIPSA, the Federal Grain Inspection Service as it was known and Agricultural Research Service developed a method of differentiating wheats on the basis of hardness. This technology known as the "single-kernel characterization system" allows for an objective measurement of a grade sample to determine whether it is hard, soft, or mixed; rather than relying on visual and subjective assessment. On the pursuit of testing technology, Steve Tanner, Director, Technical Services Division with GIPSA, made the comment in 2002 that "the United States official system of grain-quality measurement technology is in transition. Currently, the system is comprised of a mixture of electronic, chemical, mechanical, and visual methods for assessing grain quality. Less costly and more objective methods are being developed. The cost of standardization and quality control must be minimized. Future goals must be: (1) rapid, accurate, low-cost, simple, easily standardized, and widely available testing methods and (2) new methods to measure functionality and value-added traits."

#### **Europe**

Unlike other exporters that differentiate between hard and soft wheats using grades or classes (akin to bread and biscuit wheats), the wheat classification system in Europe takes greater effort to differentiate between common and durum wheat. Given the environmental conditions and the prevalence of rain during harvest, the assessment of sprouted kernels and the use of falling number measurements have important roles in grading. Many European countries, in addition to measuring protein level, also focus on gluten level and quality.

The basis for differentiating wheat in Europe is strongly influenced by each country's baking methods. While France, Germany, and Italy have four classes, the regional preferences become evident with respect to baked products (e.g., the French baguette) and testing equipment (e.g., the Alveograph for France) when the quality specifications of each are examined (Table 4).

Looking at one of the major exporters,  $\sim 10\%$  of French cereal production is retained on farms with the rest sold to collectors who clean, segregate, store, and

 Table 4
 Comparison of European wheat-classification systems

Quality					Quality Ciè	asses (F)	Quality classes (France = F, Germany = G, and Italy = I)	ny = G, and Italy	() =			
specifications	F	В	/	F	G	_	F	В	1	F	В	/
	Elite (E)	Elite (E) Elite (E) Improver	Improver	1	High-quality	High	2	Normal bread Normal	Normal	B	Soft	Confectioner
					bread wheat quality (A)	quality		wheat (B)	bread wheat		wheat (K) products	products
Protein (%)	>12.0 13.8	13.8	14.5	11.0-12.5	13.2	13.5	13.5 10.5–11.5	12.8		<10.5	12.4	
Flour yield (%)		9/		74	74			74			92	
Water absorption (%)		56.9			55.9			53.7			52.6	
Farinograph stability (min)			15			10			5			
Falling number (s)	220	285	250	220	255	220	180	255	220	Not	235	240
										specified		
Alveograph (W)	> 250		300	160 - 250		220	According		160	Not		115
							to contract spec.			specified		
Alveograph (P/I)			-			9.0			9.0			0.5
Loaf volume (ml/100 q)		710			650			290			260	

Sources: (1) Veron-Delor G, Leygue JP, Magdelaine V, Martin G, and Verjux N (2003) Wheat Trade: The organization and Perspectives of the French Market. Proceedings of International Wheat Quality Conference, Manhattan, Kansas, May 20–23, 2001, pp. 345–356. (2) Lászitity R (2002) Quality Assurance of Cereals – A European View. Proceedings of International Association for Cereal Sciences and Technology Conference 2002 – Novel Raw Materials, Technologies and Products; New Challenges for the Quality Control. pp. 7–12. Budapest.

market the grain. With increasing production, more wheat is available for export and up to half of France's annual production may be exported. It has been estimated that there are 1100 of these collectors in France, with  $\sim 300$  of them cooperatives managing 65% of cereal production. The remaining collectors are grain merchants and industrial users, such as for animal feeds and for starch manufacture, holding shares of 31% and 4%, respectively. The collectors have silos at over 7000 locations throughout France. These primary locations then can transfer grain to secondary sites in preparation for large export consignments or directly to domestic users, noting that some domestic mills have their own storage facilities.

To ensure that customers are familiar with the evolving French wheat crop, the Office National Interprofessional des Cereales (ONIC) in association with the Institut Technique des Cereales et des Fourrages (ITCF) and the French National Millers Association, arrange for annual quality testing. Over time, these results have shown the strength of French wheat to be increasing and a higher proportion of sowings to involve the better quality varieties.

#### **Argentina**

Faced with increasing competition in the international market, the Argentine industry has recently taken a new approach to quality. With customers demanding security on quality, for the first time, protein content is now being regularly tested at harvest. This is, in addition, to an expanded testing regime to profile the overall quality of the wheat produced in the eight Argentine wheat regions. Farmers, elevator companies, and millers work together to collect representative samples based on nominated tonnages. These are aggregated to allow for testing of physical, milling, and dough properties by various laboratories around the country. This joint industry approach has now produced four Institutional Quality Reports, all of which have been published on the internet (www.trigoargentino.com.ar) starting with 1999–2000 crop.

#### **General Comment**

The publications of crop reports that describe the physical and intrinsic quality of a country's wheat are now available for all the major exporters. The common goal is to demonstrate the general quality of the various wheat types available, but given the diminishing number of customers worldwide, such reports are becoming more of a historical reference point. Nearly all suppliers will meet with their customers postharvest to discuss the quality of the wheat available in the forthcoming shipment period, and at this time identifying any possible quality issues. Such meetings are not in isolation, with AWB Limited, the Canadian Wheat Board, and US Wheat Associates regularly meeting with customers both abroad and at home to discuss quality expectations and needs. That said, as a historical reference, the crop reports are ideal for tracking how the overall quality of a particular style of wheat may have changed over time.

#### Conclusion

It is anticipated that into the future, customers will become more demanding with respect to continuity of quality. Therefore, the quality systems of the major exporters will continue to evolve. This may occur due to new technologies that can, for example, quickly determine the dough strength of an individual delivery at harvest, perhaps removing the need to have a variety declaration. With ongoing reviews on trade barriers, changes may also occur with the industry structures of exporter countries, and this in turn could change the way in which quality is delivered. Needless to say, customers will pay for the quality delivered and if they do not like what they receive, this opens the opportunity for another supplier. The challenge is to understand customers' future requirements, and to have flexible systems of segregation and transport (both in terms of inherent and physical quality) to ensure consistency of grain quality.

See also: Animal Feed. Cereals: Overview; Grain Defects; Grain Diseases; Grain-Quality Attributes. Contaminants of Grain. Variety Identification of Cereal Grains. Variety Registration and Breeders' Rights. Wheat: Breeding; Harvesting, Transport, and Storage; Marketing.

#### **Further Reading**

Canadian Grain Commission (2003a) Official Grain Grading Guide. Winnipeg, Canada: Canadian Grain Commission.

Canadian Grain Commission (2003b) Improving Canada's Grain Quality Assurance System: A Discussion Paper on the Use of Variety Eligibility Declarations. Winnipeg, Canada: Canadian Grain Commission.

Canadian International Grains Institute (1993) Grains and Oilseeds: Handling, Marketing, Processing, 4th edn. Winnipeg, Canada: Canadian International Grains Institute.

Faridi H and Faubion JM (eds.) (1995) Wheat End Uses Around the World. St. Paul, MN: American Association of Cereal Chemists.

Foreign Agricultural Service (2003) Grain: World Markets and Trade. Circular Series FG06, p. 7. Washington: United States Department of Agriculture.

Lásztity R (2002) Quality Assurance of Cereals -A European View. Proceedings of International Association for Cereal Sciences and Technology Conference 2002 - Novel Raw Materials, Technologies and Products; New Challenges for the Quality Control, pp. 7-12. Budapest.

Levgue I and Martin G (1997) French Perspective and Needs for International Standards. Proceedings of International Wheat Quality Conference, Manhattan, Kansas, May 18-22, 1997, pp. 425-434.

Tanner S (2003) United States Grain Quality Measurement Technology - Present and Future. Proceedings of International Association for Cereal Sciences and Technology Conference 2002 – Novel Raw Materials, Technologies and Products; New Challenges for the Quality Control, Budapest, pp. 129-136.

Veron-Delor G, Leygue JP, Magdelaine V, Martin G, and Verjux N (2003) Wheat Trade: The Organization and Perspectives of the French Market. Proceedings of International Wheat Quality Conference, Manhattan, Kansas, May 20–23, 2001, pp. 345–356.

#### **Relevant Websites**

http://www.awb.com.au - AWB national pool estimated pay grade base rate return 2003, AWB Limited. http://www.uswheat.org - Buyer information overview "US wheat: quality-variety-dependability" 2003, US Wheat Associates.

http://www.grainscanada.gc.ca - Canadian Grain

http://www.cigi.ca - Canadian International Grains Institute.

http://www.cwb.ca - Canadian Wheat Board. http://www.cma-exportservice.com - Central Marketing Organization of German Agricultural Industries. http://www.cfia-acia.agr.ca - CFIA (2000) procedures for the registration of crop varieties in Canada, 2000, CFIA.

http://www.fas.usda.gov - Foreign Agricultural Service Online, Grain and Feed Division.

http://www.usda.gov - Grain Inspection, Packers and Stockyard Administration.

http://www.arvalisinstitutduvegetal.fr Technique des Cereales et des Fourrages.

http://www.onic.fr - L'Office National Interprofessionnel des Céréales.

http://www.trigoargentino.com.ar - Organization and Methodology 2003, Trigo Argentino Institutional.

http://www.uswheat.org — Quality and Inspection — Wheat Class Brochure 2003, US Wheat Associates. http://www.usda.gov - United States Department of Agriculture.

## **Dry Milling**

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#### Introduction

Wheat may be used whole in various ways for human food but usually it is ground and/or fractionated in preparation for further processing. Roller milling is the most flexible and widely used procedure for grinding and fractionating wheat. Products of roller milling exhibit diverse composition and functionality, suitable for preparation of an almost limitless array of human foods.

The main fractions of wheat roller milling are flour (or semolina when milling durum wheat), bran, shorts, and germ. Bran consists of large flakes that are comprised of outer layers of the kernel and adhering aleurone. Shorts are finer bran particles, and usually contain some endosperm and germ.

Properties of roller-milling products are determined by roller-milling conditions and by intrinsic properties of the wheat being milled. Modern roller milling is a gradual process involving successive grindings and separations. Each flour stream has unique composition and functional properties. Judicious blending of specific flour streams allows millers to produce diverse custom flours of differing refinement and functionality.

The two main classes of wheat cultivated today are common wheat, often referred to as bread wheat, and durum wheat. To achieve best results, millers select wheat carefully on the basis of intrinsic quality and physical condition, and make process adjustments to mitigate variations in quality among wheat lots. Physical damage incurred by poor growing conditions and improper postharvest handling and storage, influences wheat-processing potential. Plant breeding has developed common wheat types of variable kernel hardness, protein content, and dough properties with quality traits suited for specific end products. Durum wheat, an ancestor of common wheat, is harder than the hardest common wheat and is usually milled into a granular flour, referred to as semolina, for manufacture of pasta and couscous.

## **Historical Background**

Wheat milling is an ancient craft, dating back thousands of years to the dawn of civilization. Ancient wheat was hulled, unlike free-threshing modern

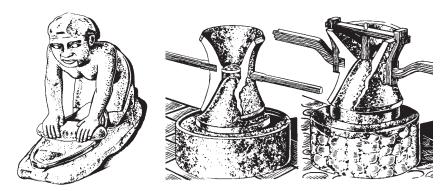


Figure 1 Saddlestone widely used in ancient Egypt (left) and hourglass stone mills used in Roman times (right). (Adapted from Sarkar AK (1993) Flour milling. In Bass EJ (ed.) *Grains and Oilseeds: Handling, Marketing, Processing*, 4th edn., vol. 2, pp. 603–653. Winnipeg, MB: Canadian International Grains.)

wheat. Primitive cultures  $\sim 10\,000-12\,000$  years ago used a mortar and pestle to crush wheat to separate ground kernels from hulls. Implements evolved from primitive ones like stones and hollowed tree trunks, to more sophisticated objects designed and shaped to improve performance. In ancient Egypt, saddlestones were widely used (Figure 1). An upper stone was moved, back and forth by hand, over a lower stationary stone.

A great development was the invention of the millstone in Roman times. Rotary motion enabled the use of humans or animals to power the mill by traversing a circular path (Figure 1). Over time, advancements were made in stone shape and size. Other means of power were used to drive the millstones such as flowing water and wind, and eventually steam power. Initially, stone milling comprised a single intense grinding. A multistage grinding process with intermediate sifting came into being in Europe in the Middle Ages, which allowed recovery of relatively white flour.

The demand for white flour grew. The roller mill, purifier, and plansifter were developed in the eighteenth and nineteenth centuries, and led to the gradual break and reduction system. The addition of mechanical conveying allowed mechanization, and flour milling became a continuous manufacturing process. The basic process remains similar today but advances in design of equipment for cleaning and milling have allowed continuous improvement in reliability, production, sanitation, and overall efficiency. A major advance ~1945 was pneumatic conveyance of mill stocks. Today most flourmills feature computer control, and some are virtually fully automated.

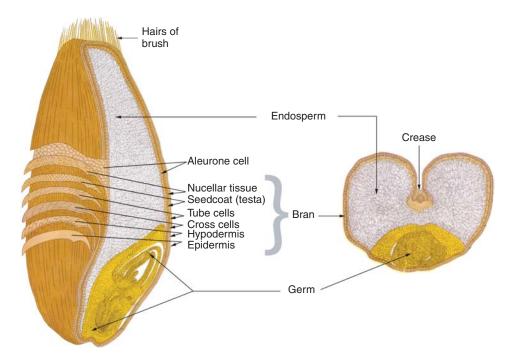
## Structure and Composition of the Wheat Kernel and Flour

Wheat-kernel structure is shown in Figure 2. The aleurone layer is part of the endosperm, but some

remains attached to bran following milling. White flour originates from starchy endosperm, which comprises  $\sim\!85\%$  of the wheat kernel. In practice, even the most efficient roller mills fall short of that theoretical maximum yield of highly refined flour, because the crease, which runs along the length of the grain and reaches almost to the middle, makes perfect separation of endosperm impossible.

Wheat-kernel composition is heterogeneous. Starchy endosperm is much lower in mineral content than other constituents. Accordingly, flour ash content (mineral matter left behind after incineration) is a widely used index of flour refinement, low ash content being indicative of highly refined flour containing almost pure endosperm. Gluten proteins, which give flour dough viscoelastic properties essential for high-volume bread and firm pasta, are found in the endosperm. Germ is particularly rich in protein. The proteins in germ and bran are nongluten proteins that have better nutritional value than gluten proteins, but are not beneficial to processing properties. Bran contains most of the fiber in the kernel, also an important nutritional consideration.

As flour yield (or extraction rate, the proportion of wheat converted into flour) increases, contamination of flour by bran and germ increases. As a result, the nutritional value of flour improves as flour yield increases. However, flour-processing quality and shelf life diminish at higher yield. The presence of bran disrupts the gluten protein matrix, weakening dough properties and reducing gas-holding capacity of dough during fermentation. The presence of bran also results in darker end products, detracting from aesthetic appearance. Germ reduces dough strength because it contains low-molecular-weight sulfydryl compounds that break disulfide cross-links, reducing the size of gluten-protein polymers that impart elasticity to dough. Germ also reduces flour shelf life due to oils and enzymes that promote rancidity.



**Figure 2** The structure of the wheat kernel sectioned longitudinally (left) and transversely (right). There are six bran layers. The crease, which runs the full length of the kernel prevents fully efficient separation of bran from endosperm. (Courtesy of North American Millers Association, with permission.)

## **Wheat Reception and Storage**

Wheat may arrive at mills by truck, rail, or ship (Figure 3). Wheat is weighed, and a representative sample is taken for analysis prior to storage. Wheat analyses may include content of foreign material and damaged grains, test weight (weight per unit volume), protein content, and moisture content. The lot is then transferred to a bin, where wheat of comparable physical condition and similar end-use potential is stored, assuring that quality characteristics are preserved, and used to the best advantage.

On the way to storage, wheat generally undergoes precleaning. First, a magnet removes ferrous metals. Then a high-capacity grain cleaner, with shaking or rotating screens, removes rubble, and fine dust. This cursory cleaning protects equipment downstream, makes more efficient use of storage space, and improves wheat-storage stability.

#### Wheat Blending

Wheat may be milled individually, or as a blend. The objective of wheat blending is to meet quality requirements at minimum cost. Blending can create quality attributes lacking in individual wheats. Having the right quality is critical to maintain customer satisfaction and market share. Blending of inexpensive low-quality wheat with the high-quality variety can achieve the desired quality at reduced cost.

Ideally, blending is performed following cleaning and conditioning. Different wheat lots may have different kernel size, making cleaning individually more efficient. Wheats of varying hardness have differing optimum milling moisture and conditioning time. Convenience, cost, and storage limitations often necessitate blending prior to cleaning and conditioning.

## **Wheat Cleaning**

Efficient wheat cleaning prior to milling is critical. Foreign material such as stones, metal, unthreshed grain, badly damaged kernels, and foreign seeds either adversely affect flour quality, or are hazardous to milling equipment. Most impurities are quite easily separated from wheat on the basis of size, shape, density, or magnetism (Table 1).

Figure 3 shows how cleaning machines would be arranged in a typical wheat-cleaning scheme. A magnet removes ferrous materials that may cause serious damage to subsequent equipment. Magnets are also used throughout cleaning to remove any "tramp" metal which surfaces from equipment or is introduced inadvertently.

It is important to remove impurities as early as possible to reduce load on subsequent equipment and increase cleaning efficiency. A grain separator uses sieves and aspiration to remove impurities. The top screen removes large impurities, such as

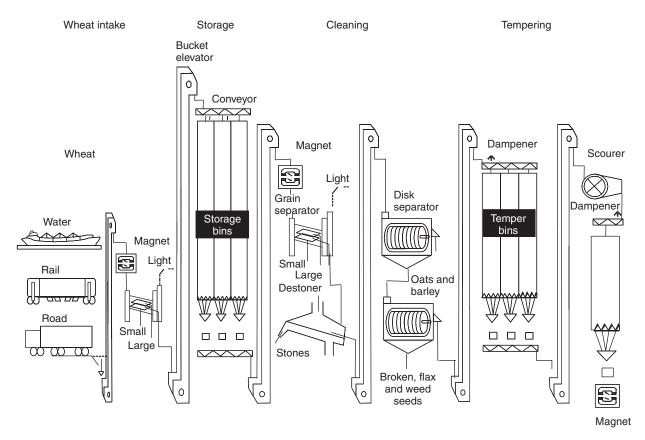


Figure 3 A simplified wheat intake, cleaning, and conditioning diagram.

Table 1 Examples removing impurities from wheat on the basis of physical properties

Physical properties	Foreign material	Cleaning equipment
Size	Larger than wheat: large stones, wood, corn, peas Smaller than wheat: rapeseed, flax, sand, dust	Grain separator or millerator
Density	Similar size and shape but heavier: small stones, mud balls, glass	Destoner, combinator, gravity table, gravity selector
Shape	Similar size and shape but lighter: ergot, damaged light wheat Similar cross-section but shorter: broken wheat, wild buckwheat	Disk separator or indented cylinder
	Similar cross-section but longer: oats, barley, wild oats, ergot	
Air resistance	Lighter than wheat: chaff, husk, light dust, thin kernels, insects, damaged light kernels	Aspiration
Magnetism	Ferrous objects and alloys containing iron: nuts, bolts, washers, nails, shavings	Magnets

Adapted from Sarkar AK (1993) Flour milling. In: Bass EJ (ed.) *Grains and Oilseeds: Handling, Marketing, Processing*, 4th edn., vol. 2, pp. 603–653. Winnipeg, MB: Canadian International Grains Institute.

maize, soybeans, and unthreshed wheat. Small weed seeds and broken kernels pass through the bottom sieve. Wheat falling off the bottom sieve is aspirated to remove chaff, dust, and shriveled kernels.

A destoner removes materials that can cause unnecessary wear and tear, such as stones of similar size to wheat. An upward air current is passed through a sloped oscillating metal screen. Wheat floats on

an air cushion and moves down the slope by gravity. Denser materials, such as mud balls and small stones, make more contact with the screen. They are propelled up the slope and are collected separately.

Disk separators remove remaining impurities that differ in length to wheat kernels, but are of similar density and cross-section. Disks revolve in a vertical plane through the wheat. The surfaces of the disks have numerous pockets that pick up objects that are short enough to lodge in the pockets. There are generally two units. The first picks up wheat while rejecting kernels longer than wheat, such as oats and barley. The second picks up small seeds and rejects wheat.

A recent innovation is to separate wheat into a heavy stream ( $\sim 70-80\%$  of total) and a light stream following the grain separator. The heavy stream, after removal of stones, goes directly to conditioning, whereas the lighter stream passes through the remaining cleaning equipment. Savings in capital investment and operational cost savings are realized because the bulk of the cleaning equipment is required for only  $\sim$ 20–30% of the wheat.

## **Wheat Conditioning (Tempering)**

After cleaning, wheat is conditioned by addition of tempering moisture, and moist wheat is rested in bins (Figure 3). Conditioning optimizes separation of bran from endosperm during milling. Bran is toughened, reducing bran powdering, and lessening bran contamination in flour. Endosperm hardness is also reduced, facilitating reduction into flour.

Achieving optimum moisture level for milling is critical. Too much tempering moisture reduces flour yield because complete separation of bran from endosperm is more difficult to achieve, and sieving efficiency is reduced. Too little tempering moisture results in bran powdering, which contaminates flour.

Spray nozzles add tempering water in an enclosed screw conveyor. Modern tempering equipment features electronic control and vigorous mixing action to ensure that precise and uniform addition of moisture is achieved. Online moisture measurement, operating in feedback mode, allows moisture addition to be continuously regulated to the desired level.

Optimum tempering moisture varies among wheat types. Soft wheat is tempered to lower moisture than hard wheat. A tendency for soft-wheat stocks to be sticky and more difficult to sift than hard-wheat stocks is exacerbated by higher moisture content. Following tempering, wheat is stored in bins to allow penetration and uniform distribution of moisture. Optimum rest time varies from several hours to more than a day depending on wheat moisture content and hardness. Soft wheat does not need to rest as long before milling as hard wheat. Soft wheat endosperm is more porous than hard wheat endosperm, so water penetrates into soft wheat endosperm more quickly.

Wheat is often tempered in two stages with resting time between stages, particularly when a large amount of tempering moisture is added. Addition of tempering water is limited to  $\sim$ 3% in conventional tempering equipment. Modern intense mixing equipment allows higher moisture levels to be added, and sometimes eliminates a two-stage tempering requirement.

After conditioning, wheat is scoured. Scourers use steel beaters to throw wheat against a wire screen. Dirt and loosened bran, which would contaminate flour, pass through the screen. Commonly, wheat is sprayed lightly immediately before milling. Up to 0.5% water is added, and the sprayed wheat is held in bins for  $\sim 30$  min. The purpose is to toughen bran, and reduce bran powdering. The wheat is then weighed, and passed through a magnet to remove ferrous metal fragments prior to milling.

## Mill Equipment

The most important equipment in modern flourmills are roller mill, plansifter, purifier, and bran duster. Equipment from different suppliers varies in design, engineering, reliability, and price, but all have similar working principles.

Stock is ground by a pair of closely spaced grinding rolls (Figure 4). In each roller mill, two pairs of rolls are housed back-to-back within a cast iron frame. One pair of rolls is accessed on each side of the roller mill. Each roller pair is driven separately and has separate feeding and adjustment mechanisms, allowing different stocks to be fed to each roll pair. Recently, roller mills with four pairs of rollers have become available. Two pairs are stacked vertically, with the upper pairs feeding the lower pairs directly. They are gaining in popularity because they allow an economical way to increase milling capacity.

Stock to be ground is distributed evenly over the entire length of grinding rolls by a feed control device. The feed control device consists of a balanced feed gate and a pair of feed rolls. The balanced feed gate regulates flow of stock. Feed rolls facilitate smooth flow of stock precisely into the "nip" of the grinding rolls.

Grinding rolls are cast iron cylinders with a hard "chilled" surface to resist wear. Rolls are either 225 or 250 mm in diameter, and lengths are usually 800– 1000 mm. Each pair rotates inwardly in opposite direction and at different speed. A transmission belt drives the fast roll, and the slow roll is driven from the fast roll by either a gear or chain drive. The ratio of roller speeds, known as differential, imparts shear to grinding action. The slower roll holds the stock, while the faster roll compresses and shears it. For most effective performance, break rollers have a differential of  $\sim$ 2.5:1, compared to 1.25:1 to 1.5:1 for reduction

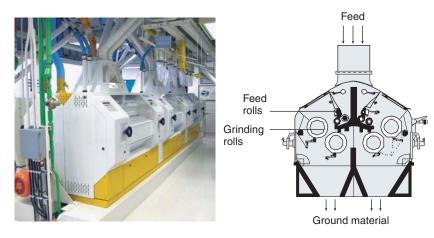


Figure 4 Modern roller mills. External view of roller mills (left) and schematic view of a roller mill (right).

Reduction rolls are commonly smooth with a frosted (slightly rough) surface to impart some shear to the grinding action. Break rolls are corrugated, corrugations becoming finer (more rows per cm) with each break as stock becomes finer. Corrugations are cut in a spiral pattern along the length of the roller. Spiral prevents rolls from locking and imparts a scissor-like cutting action.

Sifting is performed by plansifters, commonly known as sifters (Figure 5). Plansifters are suspended from girders by canes and are driven by a rotating vertical shaft with attached balanced weights. Rotation is on a horizontal plane in a circular motion, such that a pencil fastened to the bottom of the sifter traces a circle. The diameter of the circle is termed the "throw." Sifters have a throw from 75 to 100 mm and rotate from 180 to 225 rpm.

Plansifters may have four, six, or eight sections. Each section has a stack of 14-30 layers of sieves. Each section is independent and has its own feed inlet at the top of the sifter. Sieves consist of a wooden frame with a coarse wire grid bottom to hold "cleaners," which are plastic or cotton pads. Cleaners keep the underside of the sieve clothing clean, and help keep openings clear. Sieves are stretched and secured to frames by staples or glue. Apertures of sieve clothing range from  $\sim 1600 \, \mu m$  to less than 100 µm. Coarser clothing is composed of wire mesh while finer clothing is composed of nylon or silk. Sieve frames are inserted into outer frames with collecting trays. Coarse material tails over the top of sieves, and fine material passes through the sieves and is collected in the trays.

Purifiers take advantage of the greater density of pure endosperm particles compared to bran-rich or germ-rich particles of comparable size (Figure 6). A purifier has two sections, each section consisting of two or three layers of oscillating sieves. Sieve frames



**Figure 5** A plansifter with doors on the nearest two sections open for viewing. In the second section, the sieve stack has been pulled forward and in the first section some of the sieves have been pulled. The canes supporting the plansifter are visible in the foreground.

are mounted with a slight downward slope, and an eccentric drive generates longitudinal oscillation. A controlled air current passes upwards through the sieves.

Graded stock from a sifter enters one section of the purifier through a feed inlet. Stock is distributed



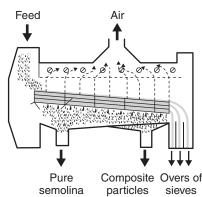


Figure 6 A triple deck purifier. External view (left) and schematic diagram (right).

uniformly across the width of the sieve. The frame is clothed with four sieves of progressively coarser aperture from head to tail. Sieve openings are kept clear by brushes, which move back and forth on guide rails under the sieves.

The sloped oscillating sieve promotes downward movement of stock in a layer. Controlled air currents drawn up through the stock stratify stock layers so that light bran-rich particles rise to the surface. Heavier endosperm particles fall to the bottom of the layer and pass though the sieve if the aperture is large enough. Composite particles with bran or germ attached remain in the middle of the layer. Thus, air flotation and sieving action combined allow separation into fractions that are progressively coarser and more contaminated by bran and germ from head to tail.

Bran finishers use impact to remove endosperm attached to broad bran at the end of the break system (Figure 7). The same machine, when used to remove endosperm from shorts (fine bran from the end of the break system), is commonly referred to as a shorts duster. Bran or shorts is fed into a horizontal cylinder. The lower part of the cylinder is perforated. Endosperm is scraped from bran or shorts by rotating finger beaters attached to a central shaft. Endosperm passes through the perforations and bran or shorts passes through the cylinder and is collected separately.

## **Hard Common Wheat Milling**

Figure 8 is a simplified typical hard common wheat flourmill flow, designed to give a flour yield of  $\sim$ 75%. The main stages of milling are the break system, intermediate processing, which includes purification and sizing, and the reduction system.

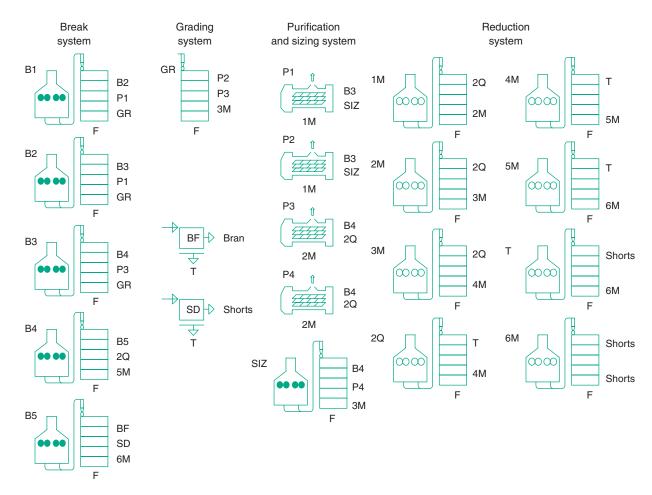
The purpose of the break system is to separate bran from endosperm as efficiently as possible, although





**Figure 7** Bran finisher. View with front cover plate removed to show the metal screen (top), and view with screen removed to show finger beaters (bottom).

a small amount of flour is produced. The entire break system usually consists of four or five passages. The first break opens the wheat kernels, and ground material proceeds to the first break sifter, where particles are separated by size. The largest particles, consisting of wheat bran and adhering endosperm, are conveyed



**Figure 8** A simplified hard common wheat flour mill flow diagram. B, break; BF, bran finisher; GR, grader; M, middling; P, purifier; Q, quality; SD, shorts duster; SIZ, sizing; T, tailing. Corrugated rollers are closed and smooth rollers are open. (Adapted from Sarkar AK (1993) Flour milling. In Bass EJ (ed.) *Grains and Oilseeds: Handling, Marketing, Processing*, 4th edn., vol. 2, pp. 603–653. Winnipeg, MB: Canadian International Grains.)

to the second break rollers. Ground stock is once again sifted and the coarsest fraction sent to the third break passage. Each successive break and sifting passage separates more bran from endosperm. The coarsest fraction of bran from the last break proceeds to a bran finisher, and shorts proceed to a shorts duster, to remove the last traces of adhering endosperm.

Finer stocks from break sifters consisting of semolina (coarser particles), middlings (finer particles), and some flour are separated by a sifter referred to as a grader. Semolina and middlings are conveyed to purifiers that separate pure endosperm particles from bran-rich material. Essentially, pure endosperm particles are sent to the beginning of the reduction system where they are reduced into flour.

The coarser less-pure materials from purifiers proceed to the sizing (scratch) system. The primary objective of sizing is not to make flour, but to remove bran adhering to middlings. This is accomplished by

light grinding on finely corrugated rollers. Ground stock is then sifted and purified before continuing on to the reduction system.

The reduction system is the heart of the flourmilling process. Carefully sized and purified middlings are reduced into flour gradually by successive grinding and sifting. Germ is flattened rather than reduced by smooth reduction rolls, and can be recovered as a coarse fraction by sifting.

Reduction grinding conditions are carefully controlled to produce as much highly valued primequality flour as possible. If grinding is too severe, bran contamination is increased, reducing flour brightness. Severe grinding can also excessively damage starch granules, adversely affecting flour functionality.

#### **Divide Milling**

A flourmill may produce single or multiple grades of finished flour from a given wheat or wheat mix.

Table 2 An example of typical yields and composition of flour streams (in ascending order of ash content) from milling of hard common wheat of about 13.5% protein content

Stream	Yield (%)	Ash (%)	Protein (%)	Bread volume (cc
Middling 1	21.2	0.33	11.5	1000
Middling 2	13.0	0.40	13.0	1060
Sizing 1	3.4	0.40	12.6	1050
Sizing 2	4.6	0.40	11.6	910
Middling 3	8.4	0.44	11.9	850
Break 1	4.4	0.47	13.8	1020
Break 2	3.4	0.48	15.4	1020
Break 3	3.2	0.57	18.1	1060
Middling 4	4.2	0.82	13.6	810
Middling 5	2.3	1.00	13.5	650
Break 4	1.5	1.23	20.9	890
Middling 6	2.8	1.49	14.2	440
Bran finisher	1.7	2.61	21.1	620
Straight grade	74.1	0.55	13.2	1060

Adapted from Izydorczyk MS, Symons SJ, and Dexter JE (2002) Fractionation of wheat and barley. In: Whole-Grain Foods in Health and Disease, pp. 47-82. St. Paul, MN: American Association of Cereal Chemists.

Individual flour streams exhibit variable composition and processing quality (Table 2). Flour millers may combine all flour streams to produce "straight-run" or "straight-grade" flour. A more complex alternative is to produce several flours with different properties by judicious blending of streams. This is known as "divide" or "split-run" milling, and allows millers with demanding clients to increase return by closely targeting specific processing requirements. The whitest, most bran-free, reduction flours may be combined to produce highly refined "patent" flour, often marketed as household flour. Remaining patent flour, when blended with early break flours, gives a strong bakery flour of high protein content. Late break flour, late reduction flour, and bran finisher flour are heavily contaminated by bran and makeup lower quality "clear" flour.

Figure 9 shows how flour streams leaving sifters can be directed to any one of three flour conveyors to make divide flours. Divide boards can be used to further blend divide flours to make custom flour blends. In the upper example, a portion of the first patent flour is diverted on a divide board and blended with the second patent to make a strong industrial bakers' flour. In the lower example, the divide board is positioned to blend all three divide flours together to create a straight-grade flour.

#### Flour Additives

Additives are commonly used to improve flournutritional value or to improve processing quality (Table 3). Endosperm, the main component of flour, is poor in vitamins. Many countries have legislation requiring addition of vitamins and minerals to improve flour-nutritional value.

Flour can be made whiter by bleaching agents, like benzoyl peroxide. Other additives, known as improvers, include various enzymes of either fungal or grain origin and agents to improve dough strength and baking performance. Additives are available in powder form and are dispensed directly into flour conveyors using powder feeders.

## **Soft Wheat Milling**

Soft wheat breaks down more quickly than hard common wheat. More flour is produced on the break system for soft wheat than for hard wheat, requiring greater sifting capacity for the former. Endosperm of soft wheat adheres more strongly to bran, reducing flour extraction rate expectations by up to 2% unless sifting capacity is increased. Yield of semolina and middlings is lower, so purifiers are of less importance, and often are absent. Stock is stickier and fluffier, which makes it more difficult to sift than hard wheat stock. Soft wheat is fed more slowly to the mill to facilitate sifting, and to ensure that stock flows freely through the mill.

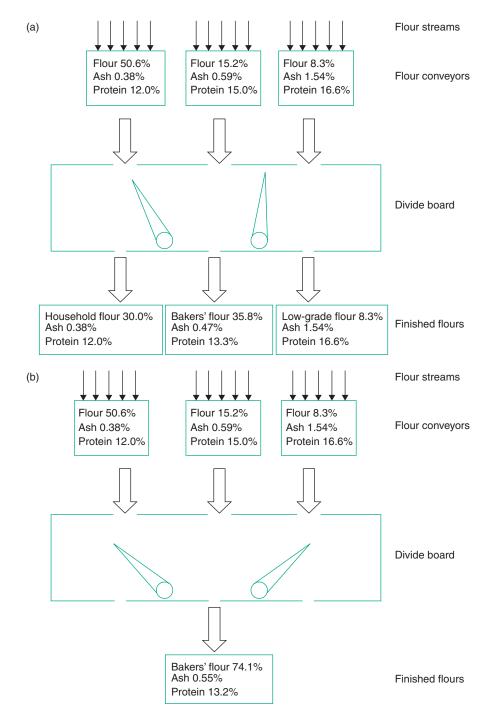
## **Durum Wheat Milling**

Durum wheat mill flows are different from common wheat mill flows, because the product preferred for premium pasta and couscous is uniformly sized semolina. Durum wheat flour that is unavoidably created as a by-product of semolina milling is of lower value. A typical durum wheat semolina mill flow is shown in Figure 10.

Durum wheat is very hard, which facilitates high yield of semolina. The break system for durum wheat is extended to allow gradual breakdown of kernels to achieve maximum production of semolina and minimum production of flour. Purified semolina from the break system is uniformly sized and freed from adhering bran by repeated sizing, grading, and purification. Most semolina is from sizing purifiers, making durum mills readily recognized by the large number of purifiers.

## Milling By-Products

By-products of milling are an important economical consideration in flour milling. These include impurities from the cleaning house (screenings), mill feed (bran and shorts), and germ. Screenings, with the exception of metal, stones, and mud balls, are usually ground and sold for animal feed. Bran and shorts are



**Figure 9** Examples of divide milling, where three custom flours are being produced from a single wheat mix based on streams shown in **Table 2**. Streams are collected to yield high-quality patent, second patent, and low-grade flours. In (a), part of the first patent is diverted by a divide board to blend with the second patent to make a bakers' flour. The remaining first patent would find use predominately as household flour. In (b), the divide board is positioned to blend the three divide flours to create a straight-grade bakers' flour.

also primarily used as animal feed. Depending on market conditions, bran also finds use for foods such as breakfast cereals and high-fiber specialty wheat products. Wheat germ is highly valued because of its excellent nutritional composition, and many mills have sophisticated germ recovery systems.

## **De-Branning (Preprocessing)**

Wheat de-branning, or preprocessing, removes bran layers sequentially by friction and abrasion stages in modified rice polishers prior to milling. De-branning is gaining acceptance for durum wheat semolina

Table 3 Some commonly used flour additives

Additive	Physical state	Approximate usual rate of application (ppm)	Purpose
Vitamin-mineral premix	Powder		Nutritive supplement
Folic acid		1.5–2	
Iron		30-45	
Niacin		35-65	
Iron		30-45	
Thiamin		4.5-8	
Riboflavin		3.5-5	
Potassium bromate <sup>a</sup>	Powder	10-20	Oxidation
Azodicarbonimide <sup>a</sup>	Powder	2–20	Maturing, oxidation
Ascorbic acid	Powder	70	Baking improver
Benzoyl peroxide <sup>a</sup>	Powder	50	Bleaching
Chlorine <sup>a</sup>	Gas	1000-1400	Bleaching, maturing
L-cysteine hydrochloride	Powder	30	Shorten dough mixing time
Fungal α-amylase	Powder	As required	Increase yeast gas production

<sup>&</sup>lt;sup>a</sup>Banned or restricted use in many countries.

Adapted from Sarkar AK (1993) Flour milling. In: Bass EJ (ed.) Grains and Oilseeds: Handling, Marketing, Processing, 4th edn., vol. 2, pp. 603-653. Winnipeg, MB: Canadian International Grains Institute.

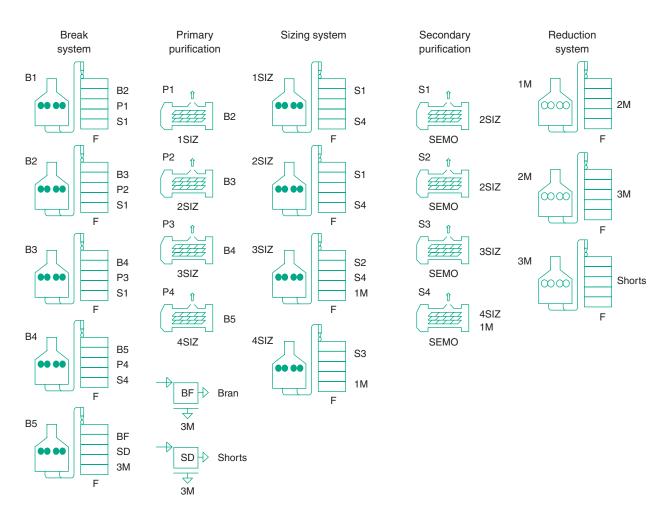


Figure 10 A simplified durum wheat semolina mill flow diagram. B, break; BF, bran finisher; M, middling; P, purifier; SD, shorts duster; S or SIZ, sizing. Corrugated rollers are closed and smooth rollers are open.

milling. Yield and refinement of semolina are significantly improved by de-branning. Top quality durum wheat can be milled more efficiently, or, alternatively, lower quality durum wheat can be used to produce semolina within customer specification. Evidence that de-branning improves milling performance of common wheat is less conclusive.

De-branning lowers capital investment because mill flow is shortened (the break system is almost eliminated), permitting more compact plants for a given capacity. During de-branning, individual bran layers are stripped off in sequence, whereas all bran layers are removed together by conventional roller milling. Each bran layer has distinct physico-chemical and nutritional properties, giving de-branning by-products great promise as novel food ingredients.

## **Milling Process Monitoring and Control**

All electrical motors driving equipment are sequence controlled and interlocked to provide ease of operation, control, and protection. This allows sequential starting and stopping of motors and stopping of the process in a fail-safe manner. Process control functions may allow the entire process to be automated, depending on the extent of various types of sensors, switches, and high-level and low-level indicators. Conventional control systems have mimic display diagrams of the process, and a hard wired electro-mechanical relay control system for control functions.

Since the early 1980s, there has been a transition to mill automation. Mimic panels with small pilot lights are being replaced by computer graphics for visual display of the process status. Programmable logic controllers (PLC) are replacing hard-wired control relays. Computerized process control offers the flexibility of changing control functions, if required, through simply making programming changes rather than changing hard wiring. It also allows full integration of critical functions relating to process control and performance evaluation, such as product yield calculations and automated roll gap adjustment. Computerized process control has reduced labor requirements in flourmills. Some mills have so-called "lights out" operation, where the mill runs for extended periods without staff present in the mill building.

Another important advance is on-line quality monitoring. Automated on-line quality monitoring systems continuously monitor factors such as moisture content, protein content and flour color, allowing flour millers to efficiently meet customer specification.

See also: Barley: Milling and Processing. Grain, Morphology of Internal Structure. Maize: Dry Milling. Milling and Baking, History. Wheat: Harvesting, Transport, and Storage; Grading and Segregation; Wet Milling.

## **Further Reading**

- Bass EJ (1988) Wheat flour milling. In: Pomeranz Y (ed.) Wheat: Chemistry and Technology, 3rd edn., vol. 2, pp. 1–68. St. Paul, MN: American Association of Cereal Chemists.
- Bizzarri O and Morelli A (1988) Milling of durum wheat. In: Fabriani G and Lintas C (eds.) *Durum Wheat: Chemistry and Technology*, pp. 161–189. St. Paul, MN: American Association of Cereal Chemists.
- Cornell HJ and Hoveling AW (1998) Wheat: Chemistry and Utilization. Cambridge UK: Woodhead Publishing Ltd.
- Dexter JE and Wood PJ (1996) Recent applications of debranning of wheat before milling. *Trends in Food Science Technology* 7: 35–741.
- Gradenecker F (2003) NIR on-line testing in grain milling. *Cereal Foods World* 48: 18–19.
- Hoseney RC (1994) *Principles of Cereal Science and Technology*, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.
- Izydorczyk MS, Symons SJ, and Dexter JE (2002) Fractionation of wheat and barley. In: *Whole-Grain Foods in Health and Disease*, pp. 47–82. St. Paul, MN: American Association of Cereal Chemists.
- Matz SA (ed.) (1991) The Chemistry and Technology of Cereals as Food and Feed, 2nd edn. McAllen, Texas: Pan-Tech International.
- National Association of British and Irish Millers (1976) *The Practice of Flour Milling*, Vol. 1. Richmond, UK: Dimbleby Printers.
- Owens GA (2001) Wheat, corn and coarse grains milling. In: Owens GA (ed.) Cereals Processing Technology, pp. 27–52. Cambridge UK: Woodhead Publishing Ltd. Palmer GH (ed.) (1989) Cereal Science and Technology. Aberdeen, UK: Aberdeen University Press.
- Posner ES and Hibbs AN (1997) Wheat Flour Milling. St. Paul, MN: American Association of Cereal Chemists. Sarkar AK (1993) Flour milling. In: Bass EJ (ed.) Grains and Oilseeds: Handling, Marketing, Processing, 4th edn., vol. 2, pp. 603–653. Winnipeg, MB: Canadian
- Sarkar AK (2003) Grain milling operations. In: Chakraverty A, Mujumdan AS, Raghavan GS, and Ramaswamy HS (eds.) *Handbook of Postharvest Technology: Cereals, Fruits, Vegetables, Tea and Spices*, pp. 253–325. NY: Marcel Dekker.

#### **Relevant Websites**

International Grains Institute.

http://www.atma.asn.au — Australian Technical Millers Association (ATMA), with members from Australia, New Zealand, and Papua New Guinea.

This website contains news and information on milling and milling training courses, and also provides useful links to other milling associations and industry organizations.

http://www.grainnet.com - Grainnet. This website provides news and information for the grain, milling, feed, and seed industries.

http://www.aomillers.org - International Association of Operative Millers (IAOM). The IAOM is an international organization devoted to advancement of technology in the flour milling and seed processing industries.

http://www.nabim.org.uk - National Association of British and Irish Millers (NABIM). The NABIM website contains useful downloadable information articles, and information on training courses.

http://www.namamillers.org - North American Millers Association (NAMA). This association has members from Canada and the United States. The website provides industry news and background information on industry issues.

http://world-grain.com - A grain and grain processing information site. Contains many useful links to industry, including many flour milling associations world-wide.

# Marketing

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#### Introduction

Wheat marketing is conducted in many formats around the world. At one extreme is a highly regulated central government control of wheat movement from field to the flour mill and even beyond, with controls over flour to bakeries and other processors. At the other extreme is a total "free market" system of supply-and-demand forces determining production, price, quality levels, movement, and availability. This article presents information on the major players in global wheat trade and differences in their wheat marketing systems. Evolutionary changes in the last few years that have had significant impact on wheat marketing are commented upon. Durum wheat is considered as part of the discussion on wheat marketing, with mention of unique characteristics of durum marketing where necessary.

#### **Global Wheat Production and Trade**

Annual global wheat production during 1990s has averaged ~570 million tonnes (Mt) but global wheat trade (defined as commercial export or import from one country to another) is less than 20% of that level, averaging just over 100 Mt. There are only five major wheat producing regions that contribute to over 80% of that trade. According to the Canadian Wheat Board 2002–03 statistical tables, based on a ten-year average (1993-2002), the leading wheat exporters have been (with their percentage market shares): United States (27.7%), Canada (16.3%), European Union (EU 15.0%), Australia (13.7%), and Argentina (8.2%).

The major wheat producers (with ten-year (1993– 2002) average annual production) are China (93.9 Mt), EU (91.7 Mt), India (68.8 Mt), US (53.3 Mt), Russian Federation (46.9 Mt), Canada (20.6), and Australia (19.4 Mt). China, India, and the Russian Federation, although major producers, tend to be virtually self-sufficient. However, these countries can have swings in production, either due to weather impact or political decision, that can turn them into major importers or exporters. When this occurs, there is a tendency for this kind of unusual supply or demand to impact on prices and movement from the more traditional exporters.

There are some regions or countries that are strong traditional buyers of wheat. In the past, this included the former USSR and China, but they have recently improved their infrastructure to require less imported wheat. With the breakup of the Soviet Union, the individual countries from that region have started to adopt more of a free market mentality that has had an influence on global trade, to be discussed later, but for the most part tend to deal with intertrade between themselves. China went through a period in the late 1990s where government policy encouraged build up of reserves of wheat. Over the last few years however the policy has changed to promote different agricultural priorities and the reserves are being depleted, to the point where China may again become a major buyer of wheat over the next few years. Currently (2003), the major buyers of wheat who have made annual purchases of greater than 3 Mt (based on ten-year average annual purchases during 1993-2002), are Brazil, Egypt, Japan, Algeria, Iran, Russian Federation, China, South Korea, Indonesia, and EU.

Feeding the people of a nation is a priority for many governments, obviously necessary to avoid unrest and to encourage progress. Therefore, agricultural policy in numerous countries introduces factors that can impact or distort free trade activities. There are many

examples of state-owned buying agencies that are totally responsible for purchase of agricultural goods from farmers within the country as well as for imported commodities. These are slowly reducing in number but are still a significant part of world trade. In some cases, liberalization takes place as the state-owned buying works in parallel with private trade during a phasing-out period. Examples of importing countries where central purchasing and controls are still in effect include China, Egypt, Japan, Algeria, and Iran.

On the exporter side there are two levels of intervention.

- 1. For Canada, Australia, and Argentina there has been direct action through government-legislated marketing agencies that have differing levels of influence in the sales of wheat from those countries. Recently, Argentina has moved to a private system. In Australia, although the marketing has been privatized, government legislation still provides the marketing agencies with single-desk authority over all export wheat sales. Canada also maintains single-desk authority through legislation for the majority of the wheat-growing area of the country.
- 2. The US and EU assume a more indirect influence by providing wheat price supports, deficiency payments and set-aside payments directly to farmers to subsidize income levels when returns are lower. In addition, governments in these regions provide further market-distorting tools such as export subsidies, government buying programs and other support mechanisms. In general, marketing activity is conducted by private trade and the governments provide direct payments to farmers through the income support structures.

A great deal of global wheat trade is conducted by major multinational companies that buy and sell a number of agricultural and other commodities. The scale of activity from these companies ranges from a local office buying wheat from a few farmers and brokering it to a nearby flour mill all the way up to a several thousand tonne sale from a major exporter to a major importer. Besides making the sale, these companies also arrange shipment, often by chartering the ocean vessel, and can arrange all the financial transactions including credit arrangements, letters of credit and securing all the inspection and certification requirements.

Some of these organizations are vertically integrated, involved in the processing of the same commodities they trade. Examples are Cargill Inc., Bunge and Louis Dreyfus. Many of these companies have existed for several years, having grown and diversified

to take on additional activities. There have been considerable changes in terms of movements, takeovers, and acquisitions of various companies in the area of grain trading.

## **Marketing Overview**

International trade in wheat is conducted by three basic marketing methods:

- 1. Government agencies or delegated representatives call for *public tenders* for a specific time period, referred to as a position. The call for tender outlines the terms for the purchase, including quantity, class, grade, and quality specifications, delivery period, payment terms, discharge port, and other details necessary for the bidder to make an educated and appropriate price offer. Major importers that use public tenders include Japan, Egypt, Tunisia, Taiwan, Philippines, and Algeria.
- 2. Private milling or end-use companies call for a private tender. Some government agencies may also use a private tender. The buyer privately calls for a bid from an exporting company or agency to meet desired specifications. There is often a pre-existing relationship between buyer and seller with knowledge of the quality available from the supplier and of the terms and delivery conditions from the buyer. Flour mills in the EU, Latin America, and many parts of Asia usually conduct private tendering.
- 3. Trade can take place in an open marketplace, referred to as a spot market, where wheat of specific quality and quantity is traded on a position basis. The Rotterdam market along with a few other EU ports are the only true open spot markets actively trading at present. Wheat from many different origins owned by private traders is offered for immediate or future delivery with prices reflecting market conditions. Many smaller flour mills in the EU purchase their milling wheat requirements in this type of open market.

For any of the marketing methods it is critical that there is a clear understanding of quality and other contractual expectations between buyer and seller. Misunderstanding and negligence in establishing clear specifications are the principal cause of controversy and legal recourse in wheat trade. Items such as protein content need to be specified in terms of moisture basis and whether the required level is an absolute minimum or an average level throughout the shipment. Other quality terms will typically include such factors as moisture content, test weight (grain

density), sprout damage level, typically expressed in Falling number units and dockage or impurity level.

Delivery conditions are another important component of a contract. A seller may quote a price on a free-on-board (FOB) basis, which specifies to the buyer that the seller is quoting on the basis of a loading port. For deliveries on the basis of an unloading port, prices will be quoted on a cost—insurance—freight (CIF) or a cost-and-freight-only (C&F) basis, therefore the seller is looking after some or all of the delivery terms.

In the past there tended to be the need for contract performance guarantees, or some type of performance bond, to ensure fulfillment of the contracted product. Today, where there is a relatively small grouping of buyers and sellers, a letter of credit from the buyer's bank is the principal requirement of the supplier. When a new buyer or seller enters the marketplace, stricter financial credentials are needed.

## **Major Wheat-Exporting Nations**

The movement of wheat from the farmer in one country to the flour mill and processor in another can take many paths. Exporting agencies or marketing boards, private grain traders and importing agencies can all be involved. Where wheat exports are controlled by an agency or a board, they may deal directly with the importing agency or private company and look after all the necessary credit, freight logistics, and certification requirements. Or they may just conduct the sale and hand off some or all of the intermediate activities to private trading companies. Of the 110 Mt of world wheat trade ~31% (Canada and Australia) use export marketing boards and perform a mix of these activities. On the import side, nations that have government importing agencies using public or private tenders account for  $\sim$ 40% of the volume moved. There is a slow trend for importing countries to privatize their wheat import purchasing by eliminating their government import agencies.

#### **United States**

The US federal government, through the Federal Grain Inspection Service (FGIS) of the United States Department of Agriculture Grain Inspection Packers and Stockyards Administration, controls the grading standards for wheat and many other grains, oilseeds, and other field crops.

The US wheat grading system encompasses five classes and six numeric grades segregated by different physical properties. For some classes there is further classification by protein content. By law, all export

**Table 1** The classes of US wheat, with 5-year average production (1999–2003) and percentage values

Wheat class	Production (Mt)	Percent of total
Hard red winter	23.62	41.6
Hard red spring	12.41	21.9
Soft red winter	11.09	19.5
Soft white	6.94	12.2
Durum	2.7	4.8
Total	56.76	

Source: US Wheat Associates, 2003 publication (http://uswheat.org).

shipments of wheat are inspected by FGIS who issue both official grade and weight certifications.

According to a 2003 publication of US Wheat Associates, the classes of US wheat, with five-year average production (1999–2003) and percentage values, are as provided in Table 1. There is also an emerging "hard white" class that has averaged  $\sim 300\,000\,\mathrm{t}$  of production from 2001 to 2003 and has the potential to grow much larger. This class has been adopted as an official sixth class of wheat in the US system.

New wheat varieties have been developed mainly by state agricultural colleges and universities. Improvement by scientists is guided primarily by the need of farmers for high-yielding wheats that resist drought and disease, but also by end-use quality requirements. Registration of wheat varieties into the various classes is unregulated although there is a peer review system for evaluation of agronomic, disease resistance and end-product quality. Uptake of new wheat varieties by farmers is based on agronomic and disease performance with improvements to quality characteristics being a desirable, but not obligatory, part of the system.

Although the US is considered to operate under totally free market conditions, there are two factors that can influence or distort wheat trade and marketing. First, the US government, as mentioned earlier, is involved in numerous programs and schemes that provide support and subsidies to aid farmers or provide customers with beneficial credit arrangements or discounts. Although controversial and open to debate, there is a general feeling that these programs do not allow world markets to function on a level playing field. The other influence comes from the concentration that exists in the major private grain companies that vie for the farmer's product. As recently as 1998, there was a US Department of Justice investigation into the merger proposal of two of the country's largest grain trading businesses. After the investigation was completed in 1999, Cargill Inc. took over the commodity marketing operations of Continental

Grain and the merger was only approved on conditions of sales of a number of their handling facilities. The government felt that this was necessary to avoid concentration in certain key regions that could lead to fewer marketing choices and lower returns for farmers.

On-farm storage accounts for  $\sim 60\%$  of the amount of grain grown in the US; therefore, the majority of farmers must at some point make a choice on moving the wheat to market. When a farmer is ready to deliver wheat there are generally two marketing choices for commercial sale. As one choice, the product can be sold to either a local cooperative or an independent grain company. The second choice for a farmer is to make the wheat available to the government through a wheat loan. In the former case, the farmer negotiates a final price directly with the buyer. If selling through the government wheat loan program, the farmer receives the deficiency payment that has been established by legislation and compensates farmers for marketplace shortfall. In either scenario, the wheat is delivered to the country elevator where the grade is established. Country elevators may also provide drying and conditioning services and may offer a variety of transport and payment terms to the

Wheat delivered to the government under the wheat loan program can be used by the government to put into the federal Food Security Reserve or for delivery to export customers through the Commodity Credit Corporation. This latter organization authorizes the sale of agricultural commodities to other government agencies and to foreign governments and the donation of food to domestic, foreign, or international relief agencies. The Commodity Credit Corporation also assists in the development of new domestic and foreign markets and marketing facilities for agricultural commodities.

Country elevators, especially those in wheat-producing regions, increasingly ship grain directly to ports, often using large shuttle trains. They also ship by truck or rail to processors, feedlots, and to larger river and rail-terminal elevators. River elevators usually ship grain by barge to port elevators, although their grain may also move to processors. Rail terminal elevators ship to processors and port elevators in large shipments up to 100 rail cars. About 50% of movement of wheat from inland to export position is by barge along major river systems such as the Mississippi.

Export of wheat from the US is from the port facilities of four regions — Gulf of Mexico, Pacific northwest, Great Lakes or through Atlantic ports. Port elevators usually combine grains of different grades, protein levels, and other characteristics to

meet buyer specifications, and they may also clean, dry, or condition the grain to meet required specifications.

The majority of sales of US wheat to importing nations is conducted by very few companies. Cargill/Continental together account for 40% of all US grain exports. Exporting marketers operate large overseas networks of elevators and trading offices through which the companies attempt to arbitrage differences in grain prices, buying grain at times and locations where prices are low, and selling at times and locations where prices, net of transport and storage costs, are high.

The US has three major commodity exchanges that deal in futures transactions for wheat, with each trading a separate type of wheat. "Soft red winter" wheat is traded on the Chicago Board of Trade, "hard red winter" wheat is traded on the Kansas City Board of Trade, and "hard red spring" and "white" wheat are traded on the Minneapolis Grain Exchange. There is no commodity exchange that trades durum wheat. In global wheat trade, these three commodity exchanges are used to establish base prices for equivalent wheat types around the world.

#### Canada

The Canadian Grain Commission (CGC) is the official federal government agency that exercises independent control over quality aspects, class designation and grades, under authority of the *Canada Grains Act*. There are seven designated classes of wheat in each of western Canada and eastern Canada, although they are not identical. Sale of wheat from Canada is most often based on CGC official grade designation with additional grade specification rarely needed. All export shipments of wheat are accompanied by a CGC "Certificate Final," which is a certification of the class, grade, and protein level (where applicable) of the parcel of wheat.

There are two major wheat-growing regions in Canada and production from these is marketed in significantly different ways. In eastern Canada, wheat, principally "soft red winter," is grown in Ontario with smaller amounts in Quebec and the Maritime provinces. The area of western Canada produces a much larger amount, principally "hard red spring" and durum wheats. Table 2 provides the production for 2003, noting that the eastern Canadian production was ~75% greater than normal, due to ideal growing conditions.

Wheat from eastern Canada is generally sold through private traders, with price determination based on negotiation and official CGC grades to meet customer requirements.

Table 2 Production for 2003

Region		Production (thous	sand metric tons)	
	Winter wheat	Spring wheat	Durum wheat	Total wheat
Eastern Canada	2081.0	365.6		2446.6
Western Canada	751.1	16 074.7	4279.6	21 105.4
Total Canada	2832.1	16 440.3	4279.6	23 552.0

Source: Statistics Canada - Catalogue No. 22-002XPB, Field Crop Reporting Series, Vol. 82, No. 8.

Table 3 Production of seven wheat classes

Wheat class	Production (Mt)	Percent of total
Canada Western Red Spring	20.93	75.8
Canada Western Amber Durum	4.19	15.2
Canada Prairie Spring Red	1.40	5.1
4 minor wheat classes	1.09	3.9
Total	27.61	

Source: CWB, Weather & Crop Surveillance Department, November 2003.

For the 90% of Canadian wheat production from the western region, authority to market wheat on behalf of farmers has been legislated to the Canadian Wheat Board (CWB). All farmers who grow wheat for human consumption for domestic and export markets must market through the CWB. Although wheat grown for domestic feed, seed, and inter-farm trade in western Canada is outside the control of the CWB, any feed wheat for export markets is under their authority.

Production of the seven wheat classes from western Canada for the five-year period 1999–2003 is presented in Table 3.

Varieties within the classes go through a formal registration process, controlled by the Canadian Food Inspection Agency of the federal ministry Agriculture and Agri-Food Canada. Varieties are evaluated for agronomic, disease resistance, and quality characteristics by a committee of experts in the field and must meet functional expectations based on check varieties within each class.

The CWB is controlled by a 15-member board of directors that is two-thirds elected by farmers and the balance appointed by the federal government. The CWB single-desk authority has been challenged on many occasions, especially by US interests, as being nontransparent and outside the spirit of open trade. The debate on this issue continues and the US has been successful in imposing an import tariff on Canadian "red spring wheat" (not durum), although this is being appealed through the North American Free Trade Association (NAFTA) tribunal process.

The CWB determines the available quantity and quality of the various wheat classes soon after harvest and then devises a sales strategy to sell it to customers to make the best return possible for farmers. As sales are made, the CWB acquires ownership of wheat from farmers, paying them an initial price that is set at  $\sim 70-80\%$  of the expected return for that quality of wheat for the marketing year. Farmers' wheat is made available to the CWB through a system of delivery quotas and contracts calculated to share market opportunities among farmers and to call forward particular qualities and quantities of grain as required to meet the sale.

The initial price is a guaranteed price by the government of Canada, so even if there is a downward swing in prices, the initial price is protected for farmers. At the end of the crop year, after all sales are made, prices are pooled for wheat and durum; normally, the final realized price is well above the initial price and the farmer benefits from a final payment after the CWB has covered its operating costs. When prices move up significantly through the marketing year, farmers receive interim payments.

About two-thirds of sales by the CWB are made directly to buyers with the balance being sold through an approved list of accredited exporters acting as agents for the CWB. In all cases, the CWB establishes the sales price for the given quantity and quality of wheat to the permitted destination. The CWB is the single desk authority of wheat sales and does not sell wheat to third parties who could then resell to end users.

Farmers in Canada for the most part have sufficient on-farm storage to carry their total production for the year. This gives them the opportunity to move wheat and other field crops into the marketplace at the time that they feel will give them the best value. Farmers contract with the CWB for the specific quantity of each class and grade they produce and agree to deliver a minimum of 85% of that quantity when called for by the CWB to meet sales obligations through the crop year.

Rail movement is the principal means of getting wheat from the primary elevator system to port for

export delivery or to customers within North America. For export,  $\sim 65\%$  of wheat is moved to the western ports of Vancouver or Prince Rupert. About 30% is moved by rail to the port of Thunder Bay at the head of the Great Lakes, from where it is transported by lake vessel to terminal elevators in ports along the St. Lawrence River. A small amount of wheat is moved during a brief three-month period out of the northern port of Churchill on Hudson's Bay.

The strong dependence on rail movement for grain and other major commodities in the heartland of Canada has been an issue of political and economic pressure in the Canadian system. A major rail transportation subsidy was removed in the mid-1990s, and railway companies continue to lobby for improved rail transportation rates claiming poor margins in dealing with the vast distances and often harsh climatic conditions in moving product to port. It can cost a farmer more to move grain by rail from his farm in central Saskatchewan to Vancouver than from Vancouver to a flour mill many thousands of kilometers away in South America or Asia. The average distance to move wheat from the western growing region to port facilities is ~1350 km. Producers in the eastern wheat-growing region are much closer to port facilities, averaging  $\sim 150$  km.

#### **European Union (EU)**

There are no formal classes, grading system, or variety registration requirements within the various countries making up the European Union. In some countries there is an approved list of wheat varieties that meet specific functional qualities but even within this loose classification system wheat is generally traded through agreed specification between buyer and seller.

Production of wheat by the 15 member countries of the EU in the ten-year period of 1993-2002 averaged ~93 Mt, making it the second largest wheat-producing region of the world after China. Internal consumption is significant and over the same time period only ~16 Mt annually was available for export. The surplus is purchased by private grain traders and wheat processors or by governments under the EU's Common Agricultural Policy (CAP). This policy establishes minimum quality specifications and, if a farmer's wheat can meet these, the government is required to accept delivery and pay an annually established "intervention" price. Due to the strong farm lobby in EU nations, this price is a major negotiation issue and is calculated to provide farmers with incomes equivalent to incomes in the non-farm sector. Since the intervention price is generally higher than

world market prices, the EU is often challenged as subsidizing farmers to a level that distorts world trade.

EU domestic wheat buyers pay prices to farmers that are significantly higher than world prices. This results in a significant level of support from the nonfarm sector as consumers are ultimately paying these higher support levels. In addition, for exported wheat the EU budget supports CAP prices to farmers for wheat that brings a lower value on the world market. Agricultural reform within the EU is attempting to modify the support level on a more direct basis rather than through commodity price support but the social, economic, and political pressures provide challenges in achieving this.

Due to the two-price system in the EU, the Brussels government must control export wheat price levels and volumes. On a weekly basis, private trading companies compete for the established EU export program. As the support level changes through agricultural reform, export subsidies are evolving as having less impact in determining export prices.

Very little wheat is moved by rail within EU countries. Due to the relatively short distances to various processors or port facilities, most wheat is moved by truck or by barges through the well developed canal systems of many of the countries.

#### Australia

Wheat classes, grading standards, and the variety registration process in Australia are highly controlled by AWB Limited (formerly Australian Wheat Board), now a private company that has a governmentlegislated authority over all bulk export sales of wheat. When a new wheat variety is introduced, AWB designates for which of the seven classes it will be eligible. On an annual basis, AWB establishes receivable standards for wheat to be delivered based on growing and harvesting conditions, crop expectations, and customer requirements. Wheat delivered into the system that does not meet receivable standards for the seven major classes can be graded as Australian General Purpose, Australian Feed or other special designations. AWB's commercial grain testing laboratory provides certification on the quality of export or domestic shipments of wheat.

Average wheat production in Australia for the tenyear period 1993–2002 was 19.4 Mt, with a range of 8.9-24.8 Mt. Production can often be affected by severe drought, with Australia having had devastating drought years in 1982/83, 1994/95, and 2002/03. Due to the small population in Australia,  $\sim 75\%$  of wheat production is exported, with average wheat exports of 14.4 Mt for the same ten-year period.

Table 4 Percent by class of receivals, 1998-2002

Class	1998–99	1999–2000	2000–01	2001–02	2002–03
Australian Prime Hard	5	6	3	5	2
Australian Hard	14	16	3	15	20
Australian Premium White	31	31	34	32	34
Australian Standard White	32	31	26	28	20

Source: AWB Ltd. Crop Reports, 1998-99 to 2002-03.

Although AWB does not publish production or sales by specific wheat classes, their annual crop report does provide an indication of receivable percentages for the major classes, as outlined in Table 4. The other wheat classes include Australian noodle, Australian soft, and Australian durum.

AWB operates in a manner similar to the CWB in Canada; however, there is one very important distinction here, and that is, since privatization, AWB no longer benefits from government underwriting or any other form of support. Within Australia, there is a "dual market" system as domestic processors can buy wheat either from private traders or through AWB. Also, bagged or container sales of wheat to export markets can be made through private traders.

Farmers have a choice of various innovative pricing options when they make their wheat available to AWB. These include pricing on the wheat futures market, foreign exchange market, or on the basis of contracts. All of these are self-managed options for the farmer. Farmers may also accept a simple pooled pricing formula that gives them 80% of the expected pool price upfront and a final payment after all sales are pooled.

AWB direct sales to buyers represents ~70% of all wheat sales. The balance of sales are made through approved grain trading partners. Farmers deliver wheat to local Bulk Handling Authority (BHA) storage facilities. These complexes used to be mainly farmer-owned cooperative facilities but many of them have now privatized. AWB has recently become a competitor to the BHAs, as they have built their own storage facilities. Since there is limited farm storage, representing only ~9 Mt capacity from a total grains production of 41 Mt, the majority of wheat is moved directly to a BHA after harvest. At delivery, the receivable standard grade is assigned and the farmer chooses the pricing option desired.

The wheat-growing region in Australia follows the coast line in a crescent around the east, southeastern and western coasts and the average distance from production regions to port being  $\sim 350 \, \mathrm{km}$ . Wheat is moved from BHA storage to export position by truck or by rail as called forward and needed by AWB's sales program.

#### **Argentina**

The Ministry of Agriculture in Argentina introduced a grading and classification system in the late 1990s. There are three classes, plata, prime, and "soft winter" and each is graded into four quality levels. The ministry also controls the registration system to ensure that new varieties meet quality expectations as well as agronomic and disease resistance attributes. For export wheat shipments the ministry also issues grade certification, verifying conformity with the established grade standards.

Until 1991, all wheat marketing was under state control through the National Grain Board (NGB). The NGB had significant wheat storage facilities, both inland and at port positions that were subsequently sold off to farmer cooperatives or the private sector. Wheat production increased with deregulation of the agricultural economy and elimination of the NGB and wheat export taxes. In the early 1990s, wheat production averaged under 10 Mt per year but from 1998 to 2002 production increased to an average of 14.4 Mt. This has allowed Argentina to increase wheat exports and it is currently the fifth largest exporter, with average exports over the last five years of close to 10 Mt. Since Argentina is in the Mercosur trading agreement along with Brazil, Uruguay, and Paraguay, these countries, principally Brazil, account for  $\sim 65-70\%$  of Argentine wheat exports. The Mercosur agreement is a protectionist umbrella for Argentine farmers as wheat from origins outside the Mercosur countries must incur significant tariffs, which are used to compensate Mercosur farmers. Since Argentina is by far the largest wheat producer in the region, their farmers get the most advantage of this system.

On-farm storage is minimal in Argentina and wheat competes strongly with other important crops, especially soybeans and maize, for commercial storage space. This means that farmers can often be forced into "price to be fixed" contracts in order to take advantage of exporters' and millers' storage facilities. In addition, farmers in Argentina tend to have a much poorer working capital level relative to those from the other major exporting nations and access to credit is

limited. This puts farmers at a disadvantage when negotiating prices at harvest time and there tends to be a lot of pressure for exporters to make sales close after the harvest period in order to maintain turnover in storage facilities. In Argentina the harvest pressure occurs generally in March, which is outside the time range of harvest availability from the other exporters from late summer to early winter (July—December). Demand tends to come from non-Mercosur destinations, especially those that have lower quality expectations, such as Iran, Iraq, and African nations.

The rail transportation system in Argentina is in poor repair, having been built in the nineteenth century by British, French, and German interests, with each system having incompatible track gauges. Therefore, truck transport is most often used to get wheat from the growing regions to port. The average distance from country position to an export port is  $\sim 300 \, \mathrm{km}$ . There is also use of barges and ships along the major Parana river system, with continuing effort to increase draft levels to allow larger ships to move further inland.

## **Minor Wheat Exporting Nations**

During the early 1990s through early 2000s, the major exporters have accounted for trade as presented in Table 5.

It is significant that the "others" category has increased over the last two crop years, mainly due to poor growing and harvest conditions for the traditional exporters and bumper crop conditions in other parts of the world. This includes countries and regions such as Russia, Kazakhstan, Ukraine, Eastern Europe (Black Sea exporters) as well as Pakistan and India. Over the last two calendar years (2001–2002) even China was entering the

export market for wheat. These countries have traditionally been self supporting and only tend to enter the world trade arena in a minor way. Surpluses in the last two years accompanied by shortages from the traditional exporters have resulted in the private grain trade moving wheat from unusual origins to service need around the world. Importers have needed to review their quality expectations and blending capabilities as wheat has come from nontraditional sources. The infrastructure of minor wheat exporters has been challenged by the huge amount of wheat and other grain that has moved through their system.

It can be expected that these regions of the wheat-growing world will continue to make improvements in transportation and storage infrastructure as well as grading and quality selection in order to be able to command increasing share of world trade. Traditional exporters will need to respond to the challenge from these newer exporters by improvements in their own marketing strategies, perhaps turning away from lower value "commodity" wheat and toward supply of higher value, identity preserved selection of wheat types that meet specific end-use functional quality.

#### Conclusion

The various wheat marketing systems of the major exporters have been presented, showing there has developed a well-recognized and traditional international trading environment that is affected by geo-political influences. Governments can have a significant impact on trade characteristics, especially where subsidies allow farmers to produce products that would not normally be grown if the subsidies were not available. Recently, newer players have entered the wheat marketing environment and created

**Table 5** Exports of all wheat, semolina, and flour by principal exporters, 1993–94 to 2002–03 (July–June) (thousand tonnes and percentages in parentheses)

Crop year	United States	Canada	European Union	Australia	Argentina	Others	Total
1993–94	33 111 (32.5)	19 304 (19.0)	20 066 (19.7)	12 751 (12.5)	4511 (4.4)	11 988 (11.8)	101 731
1994-95	32 541 (32.1)	20 771 (20.5)	17 110 (16.9)	7 786 (7.7)	7869 (7.8)	15 430 (15.2)	101 507
1995-96	33 795 (34.1)	16 198 (16.3)	13 242 (13.3)	12 086 (12.2)	4448 (4.5)	19 479 (19.6)	99 247
1996-97	27 298 (26.2)	19 366 (18.6)	17 835 (17.1)	18 157 (17.5)	10 079 (9.7)	11 296 (10.9)	104 031
1997-98	28 151 (27.0)	19 996 (19.2)	14 196 (13.6)	15 398 (14.8)	9827 (9.4)	16 794 (16.1)	104 362
1998-99	29 001 (28.4)	14 723 (14.4)	14 589 (14.3)	16 104 (15.8)	9199 (9.0)	18 344 (18.0)	101 960
1999-2000	29 399 (26.1)	18 313 (16.2)	17 432 (15.5)	17 124 (15.2)	11 083 (9.8)	19 345 (17.2)	112 696
2000-01	28 027 (27.0)	17 108 (16.5)	15 225 (14.7)	16 682 (16.1)	11 396 (11.0)	15 196 (14.7)	103 634
2001-02	26 244 (23.8)	16 206 (14.7)	11 494 (10.4)	16 494 (14.9)	11 671 (10.6)	28 253 (25.6)	110 362
2002-03 <sup>a</sup>	22 970 (21.3)	9 191 (8.5)	16 000 (14.8)	10 946 (10.2)	6276 (5.8)	42 395 (39.3)	107 778
10-year average	29 054 (27.7)	17 118 (16.3)	15 719 (15.0)	14 353 (13.7)	8636 (8.2)	19 852 (19.0)	104 731

<sup>&</sup>lt;sup>a</sup>CWB, Weather & Crop Surveillance Department, November 2003. Source: Canadian Wheat Board 2002–03 Statistical Tables, http://www.cwb.ca.

new opportunities for importers and challenges for traditional exporters.

Wheat remains a major food source and is a unique agricultural product due to the vast range of consumer products that can be produced from it. As developing nations improve their economies they can be expected to demand a greater variety of foodstuffs and wheat will become a greater part of their diet. This is expected to increase world trade in wheat as these nations do not tend to be traditional producers. The dynamics of wheat marketing systems around the world will have to develop to meet this new demand.

See also: Grain Production and Consumption: Africa; Asia; Europe; Cereal Grains in North America; Oceania; South America. Variety Registration and Breeders' Rights. Wheat: Breeding; Agronomy; Harvesting, Transport, and Storage; Grading and Segregation.

# **Further Reading**

CWB, Weather & Crop Surveillance Department, November 2003.

Statistics Canada — Catalogue No. 22-002XPB, Field Crop Reporting Series, Vol. 82, No. 8.

AWB Limited Crop Reports, 1998-99 to 2002-03.

### **Relevant Websites**

http://www.cwb.ca - Canadian Wheat Board 2002-03 Statistical Tables.

http://uswheat.org – US Wheat Associates, 2003 publication.

http://www.awb.com.au — This website provides information on pooling, marketing, trading, financing, risk management, and logistical management of Australian wheat.

# Wet Milling

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### Introduction

Starch produced from wheat was identified as a binder and stiffening agent early on; it was used in the making of historical textiles and paper-made utensils in Egypt and Greece. J. Beccari developed the first aqueous process for the separation of starch and gluten in 1745, this formed the basis for modern technology. Wheat starch has had an unparalleled use for purposes of stiffening and finishing until modern times. However, the introduction of starches from potatoes and especially from maize in the course of the last century diminished its importance significantly. Utilization of wheat starch was reduced mainly to application in food, in particular, for baking purposes. Political decision making of the European Community in the 1990s drastically changed the situation again. Modern production of wheat starch is closely connected with its most important dried co-product – vital dry gluten. This product consists of waterinsoluble high-molecular-weight protein particles, which are able to recreate the viscoelastic structure typical for wheat flour dough. In many countries worldwide, only beneficial commercialization of vital dry gluten enables economic production of wheat starch.

Based on figures presented in 1999, a valuable estimate for the actual situation can be given (Table 1).

As a result of different economic reasons and requirements, wheat starch and gluten production show significant imbalance in size in different world regions. The indisputable leading position of the European community (approximately two-thirds of world production) is followed by the Americas and the Asian and Pacific area with about one-third of total production, both with an equivalent ratio. Eastern Europe, in comparison, plays a minor role.

In Europe wheat starch is traditionally used in confectionery production, but substantial amounts are converted to sweeteners. More recent developments allow for utilization in various sections of nonfood industry too. Dry vital gluten is a substantial product in fortification of low-protein flours, but is also used in production of pet food and aquatic food. The advantage of this product results from the ring- or flash-drying process that retains its functional properties

**Table 1** Worldwide production of wheat starch and gluten by regional distribution

Region/production (10 <sup>3</sup> Mt)	Starch	Gluten	
European community (8 of 15 countries)	1964	373	
Eastern Europe	113	22	
North and South America	596	114	
(USA, Canada, Mexico, Argentina)			
Asian and Pacific Area	570	109	
Others	26	5	
In total	3269	623	

# **Technological Developments**

Developments in starch extraction from wheat were the result of numerous variations in process engineering. Wheat kernels as well as wheat flours of differing composition have been used as raw material. The indicated processes allowed recovery of the main wheat components — A starch, B starch, and gluten—in different quantity and quality. Protein extraction from kernels produced only in few processes the water-insoluble wheat protein fraction as the high priced and therefore interesting vital gluten. Finally, those processes that yielded the high-quality vital dry gluten from wheat flour were successful.

Changes in process engineering, in particular the switch from the original flour-based dough systems to dispersions of varied dry substance concentration, found their expression in the selection of respective source materials. In dough-processing techniques, like the once-popular Martin process, protein-rich and strong gluten-producing flours were in general prerequisites with respect to satisfying dough formation and successive extraction by intensive and water-consuming washing procedures. In contrast, in Europe recently introduced processes are based on differentially defined centrifugal separation techniques, allowing use of wheat flours of reduced protein content and protein quality as well. In this respect, even softer wheat cultivars can be applied in starch factories that must ascribe to the limiting range between bread wheat and feeding wheat. This situation allowed a favorable adaptation to market developments, where gluten prices especially could no longer contribute in the usual manner to the necessary revenue of wheat starch production.

In 1999, Maningat and Bassi presented an overview of the state-of-the-art wheat starch production. Their presentation highlighted the situation in the Americas in particular, where the modified Martin, hydrocyclone, Alfa-Laval/Raisio, and the Westfalia threephase decanter processes are standard procedures in the industry. In contrast, technologies based on highpressure agglomeration of gluten-forming proteins and three-phase decanting (Westfalia process) or the modified Martin process, were indicated as predominant in European industry. Another alternative exists with the Tricanter® process. Driving in process development was the necessary reduction in freshwater consumption in relation to wheat flour (Table 2). The consumption occurs as water is used for dough or batter preparation, as well as in the following extraction/separation of starch, gluten,

**Table 2** Water/flour ratios requested according to process variants in wheat wet milling

Technology	Water/flour ratio	
Martin process	15:1	
Modified Martin process	6:1	
Batter process	5-7:1	
Hydrocyclone process	4-5:1	
Decanter process	4:1	
HD/Westfalia	2-3:1	

fibers, and further components. While a water/flour ratio of up to 15:1 was applied for the Martin process in its traditional form, the modified Martin process as well as the batter process required less than half the quantity of water consumed earlier. Technological improvements and consistent recycling of process water led to this important reduction. At the very beginning, a concentrated flour-water system, similar to a baker's bread dough, is prepared for extraction. For other process proposals, which used hydrocyclones or decanters as primary separations systems, further reductions in the ratio of flour to water were reported. In more recent developments such as the Westfalia process, which represents efficient lump-free hydration of flour particles, mechanical agglomeration of gluten forming proteins by application of high pressure, and downstream three-phase separation in a decanter, the procedures induced further reduction towards a minimum ratio of approximately 2.1:1.

They also described in detail the modified Martin process, the hydrocyclone process, and the Alfa-Laval/Raisio process as reported by Bergthaller and Kersting. For decisive improvements in process technology, only effective water regime will not suffice. As there are substantial developments in initial flour hydration and subsequent separation of components from each other in early stages of a selected process unit operations are necessary.

# Obstacles of Wheat as Substrate

### Starch Particle Size Distribution/B Starch

Scanning electronic microscopy of wheat flour particles (Figure 1) illustrates the central problem of isolating the starch from other flour constituents. Besides the main portion of big-sized granules, many tiny granules are visible and all of them are closely embedded in a matrix composed essentially of protein particles. Following the characteristic particle size distribution of wheat starch that can be described as bimodal (Figure 2), in wet milling the starch is classified into two products, high-grade starch (called A

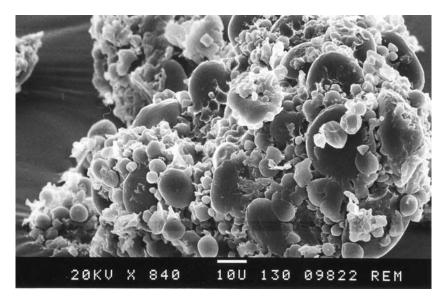


Figure 1 Scanning electron microscopy of starch granules and protein bodies embedded in cells of a flour particle.

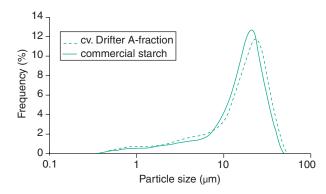


Figure 2 Wheat starch particle size distribution.

 Table 3
 Upper limits for impurities of commercial wheat starch according to general quality requirements

Criteria	A Starch	B Starch
Moisture content (%)	14	14
Protein <sup>a</sup> (% i.s.)	0.5	5
Minerals (% i.s.)	0.3	1.0
Lipids (% i.s.)	0.1	0.5
Sulfur dioxide (mg kg <sup>-1</sup> i.s.)	50	50

<sup>&</sup>lt;sup>a</sup>N conversion factor = 6.25.

starch, as it comprises big-sized granules for the most part) and lower-grade starch (commonly called B starch or "tailing" starch, which mostly consists of small granular starch (SGS)). This fraction contains so far granules that are associated in general with bigger amounts of proteins, lipids, and pentosans. The much higher specific surface area of B starch granules and resulting differences in classifying, washing, concentrating, and drying make it particularly difficult in processing them further.

As a result of processing, commercial wheat starch products differ significantly in purity. Their quality requirements are described in general by criteria represented in Table 3.

### **Pentosans**

Another group of grain components, the pentosans, causes serious problems in wet milling of wheat flour.

The main and most disadvantageous property with respect to starch separation is the extreme water-binding capacity that seriously changes the viscosity of suspensions. The pentosans represent water-soluble and water-insoluble arabinoxylans of differing molecular weight. The presence of this fraction results in a reduced recovery rate of gluten, due to an incomplete agglomeration of gluten. Furthermore, impeded separation of fractions is induced during initial centrifugal operations applied to water/flour suspensions as well as classification steps in mill starch streams. High concentration of pentosans neutralizes differences in density and particle size. Existing differences cannot contribute effectively in relevant separation procedures.

A potential application of pentosan-degrading enzymes in wheat wet milling, xylanases, is to break down pentosans in order to reduce viscosity of flour slurries.

### **Modern Wet Milling Processes**

### **The Modified Martin Process**

The modified Martin process, the modern variant of the traditional procedure after necessary improvements and modifications, still exists for wet milling of wheat starch worldwide. Necessary improvements were for the most part a result of reducing freshwater consumption through increasing process water recycling to the maximum acceptable extent. Process modifications concerned in general the substitution of equipment towards more efficient separation of starch and gluten. The modified Martin process is described in detail by the flow diagram presented in Figure 3.

Flour milled in the factories or purchased from outside according to local potentials is mixed in a continuous mixer with water to a stiff dough in order to develop the gluten proteins. Water temperature is preferably set at 32°C (90°F). Mixing results in a cohesive dough that is allowed to rest for completion of hydration of flour particles and gluten, in

particular. The fully developed dough is then mixed vigorously with additional water under turbulent agitation to accelerate segregation of viscoelastic gluten mass from suspended starch. Separation of gluten from milky starch suspension is done while pumping the mixture into a gluten washer constructed as a long, slanted rotating cylinder equipped with 40 mesh screens. Through nozzles, water is sprayed onto gluten and screens to wash away starch from the gluten and to prevent plugging of the screen. Then, gluten is conveyed to a gluten washer where it is mixed with excess water to remove residual starch. The purified, viscoelastic gluten mass is dewatered and after remixing with dry gluten powder, it is reduced to small pieces of suitable size and is sent for a quick final drying in a flash drier. Drying conditions are adapted to maintenance of maximum vitality, which means instantaneous recovery of viscoelastic behavior when hydrated. After fine grinding, dry vital wheat gluten is till today an economically important byproduct of wheat wet milling used for ~80% in flourmills and bakeries for flour improvement.

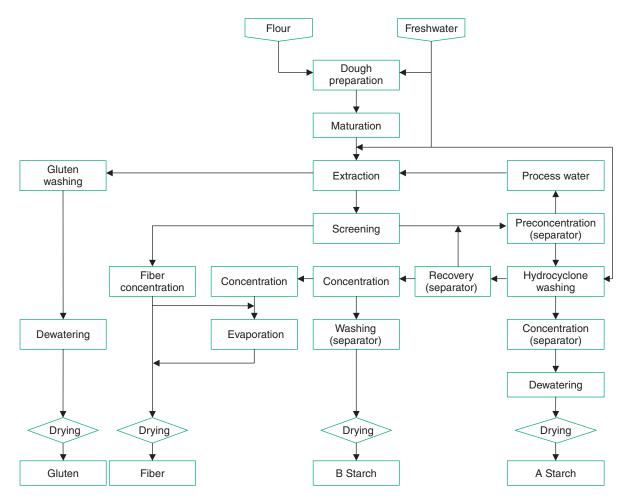


Figure 3 Flow scheme of the modified Martin process.

The starch suspension (starch milk) coming from gluten separation goes through a purification process consisting of a succession of sieving, centrifugation, and hydrocyclone washing. First, small gluten particles are removed by a rotating sieve, shaker sieves, or sieve bends. Bran particles, fibers (mainly of cell wall materials), and gritty endosperm pieces are removed, too. The resulting starch suspension is refined further in nozzle centrifuges where small-size starch (B starch), soluble components consisting mainly of soluble carbohydrates (pentosans and sugars), proteins, and minerals are removed with the overflow. A starch is concentrated the same time to  $\sim$ 35% and leaves the separator via nozzles. Final purification in a counter-current washing process is accomplished using a multistage hydrocyclone system that concentrates starch solids to 40%. After dewatering by means of vacuum drum filter, peeler centrifuge, or alternatively pressure filtration (with 60% starch solids), the starch cake is dried in a flash or drum drier.

### **The Hydrocyclone Process**

A new direction in starch/gluten separation was set with the use of hydrocyclones. Initial stages resemble the modified Martin process. Then, the water/flour dough is conveyed to a maturation tank for 10-20 min of rest and afterwards fed into a dilution tank. There, the dough is mixed with excess water to produce a homogeneous suspension. While passing through a multistage hydrocyclone system, the applied shear induces spontaneous agglomeration. In the hydrocyclone process, separation of agglomerated gluten from starch results from even small density differences between both components. The addressed densities differ, with  $1.05-1.1 \text{ kg l}^{-1}$  for agglomerated gluten and 1.4-1.5 kg l<sup>-1</sup> for starch. Gluten is collected together with B starch as the lighter portion in the first section of the multistage hydrocyclone system (in general four stages) as overflow, then purified from B starch, bran, and fibers (cell wall materials) by washing and sieving. After dewatering, gluten is dried. At the same time the starch stream undergoes counter-current washing and purification in an eight-stage hydrocyclone system and before final concentration (up to 40%), residual bran and fiber particles are removed by two-step sieving, for example, through a 75 µm sieve bend and a 50 µm rotating screen. The starch slurry is dewatered then and the resulting cake dried as indicated with the modified Martin process.

### The Alfa-Laval/Raisio Process

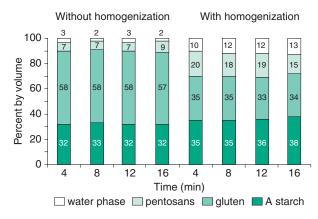
With introduction of the Alfa-Laval/Raisio Process, which was developed on the basis of a thick wheat

flour/water batter instead of dough, centrifugal separation represented by a decanter-type centrifuge was introduced as a new principle. The batter is first treated in a disk-type disintegrator to achieve a homogeneous suspension that is separated then into a starch fraction having a protein content of  $\sim$ 1% and a gluten fraction of  $\sim$ 40% protein. Fine fibers are removed by sieving the starch fraction over rotating conical screens, and final purification and concentration to 55% solids occurs by washing in counter-current mode in subsequent decanters. Drying in a flash or drum drier results in starch of high purity (0.3% protein). Gentle stirring matures the gluten fraction and highly agglomerated gluten is formed. For completing agglomeration, gluten is disintegrated again and vibration screens separate gluten lumps formed once more from residual starch and bran particles. Wet gluten is dried as usual to receive vital wheat gluten. The filtrate coming from the gluten screen still contains B starch, some A starch, and soluble substances (pentosans). While A starch is recycled, all insoluble residues, in particular B starch, are first collected via decanting. B starch is concentrated, dewatered, and dried.

### Westfalia Three-Phase Process or Tricanter Process

In successful recent processes, centrifugal separation of starch and gluten in decanter machines is combined with previous rigid segregation of wheat flour into its components – starch, gluten strains, fibers, and pentosans. The application of high pressure with a homogenizer proved to be a very effective prerequisite for splitting flour components in a three-phase decanter. There, mechanical strain, shearing forces, friction, and cavitation produce tissue disruption within the specific valve of a homogenizer. The technique was successfully taken over from studies of improved maize starch extraction after pretreatment of ground, de-germinated maize. By application of this technique ahead of decanter separation, specific wheat protein fractions, in particular the high molecular gliadines and glutenines, are prepared to agglomerate finally to voluminous aggregates. Separation into distinct and characteristic layers (starch, gluten, pentosans, and process water) and its efficiency as investigated in spinning tests strongly depend on the time of rest after homogenization ( $\sim$ 16 min) (Figure 4). Because of the lower density compared to starch, gluten agglomerates leave decanters primarily with lower density phases in the middle phase.

The principal process of continuous preparation of highly concentrated flour/water slurry and its separation into three distinct phases by applying three-phase decanters exists in varied designs and different distributions. The dominant processes applying this principle are Westfalia process, Flottweg Tricanter process, and Decanter-based Weipro process (Figure 5). In most modern versions, the concentrate leaving the decanter consists almost entirely of A



**Figure 4** Effect of homogenization and maturation time on gluten formation and separation efficiency after centrifugation of a pretreated and diluted flour/water mixture (determined by percent volume distributions of its fractions.

starch (less than 1% protein). Recovered concentrates are further processed by screening, multiple washing, and concentration. Washing and concentration is then realized in a combination consisting of a centrifuge and a multiple stage hydrocyclone unit or a sequence of three-phase separators to produce commercial grade wheat starch (protein content  $\sim 0.3\%$  d.b.). The middle phase consists of gluten, SGS, tailings, and some fiber. Gluten is recovered from this stream by rotary or bend screens, subsequent washing in a gluten washer, dewatering, and air drying. The filtrate coming from gluten separation consists, in general, of some SGS, the main part of tailings. The A starch is recovered and added to the main A starch stream for improvement of the recovery rate. Sieving over rotary cone screens or horizontal vibration sieves of adapted mesh size separate fibers and B starch from one another. The B starch stream is then concentrated and dewatered and finally dried by application adapted drying procedures, e.g., drum drying to receive a pregelatinized product. The light phase contains mainly pentosans and soluble compounds and depending on agglomeration potential eventually some finely distributed gluten. The latter

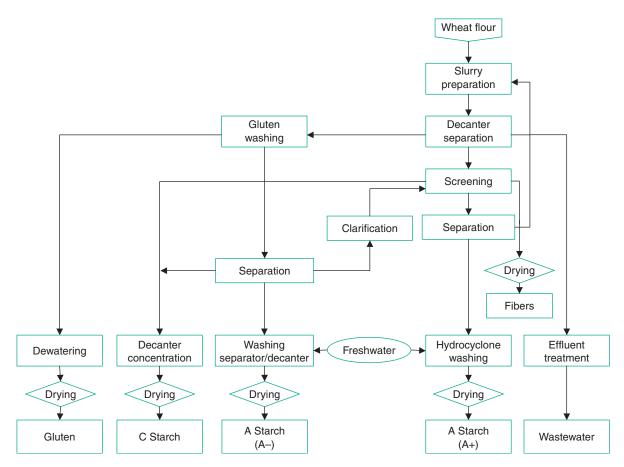


Figure 5 Flow scheme of the Westfalia process for wheat wet milling.

is recovered by filtering the third phase through fine screens. Pentosans can be used as liquid or dried products in animal feeding. In this respect, bran can be mixed with concentrated process water and soluble material together with pentosans in preparing high-value feed. Such products are known as wheat gluten feed and may have protein contents between 17% and 21%.

The Westfalia separator three-phase decanter process designed in the early 1980s underwent various improvements to reduce, in particular, the water regime and recover SGS as A-minus starch. Instead of B starch, a C starch fraction is produced. The new concept is strictly based on the use of three-phase decanters for principal separation of concentrated flour/water mixtures into three phases as described previously and following processing, classification, and washing with three-phase nozzle separators. This concept is described, in principle, in Figure 6. However, even this standard will be further developed to find an even more economic solution.

### **Selection of Wheat for Wet Milling**

Substrate selection refers to grain and flour characteristics as well. The suitability of wheat flour nowadays focuses more on flour data. The selection is based on applied technology and potential recovery rate, in particular, the level of protein, which determines the yield of the most important by-product, dry

vital gluten. Two principal technologies are presently applied and they require different demands.

### **Wheat Grain Characteristics**

Specifications widely used in the past for grain indicate a minimum protein content of 12.0-12.5% on dry substance as the key factor in evaluation of suitability. As for wheat, in general, the nitrogen conversion factor is 5.7. Falling number and amylograph consistency ought to reach medium to high level, while endosperm hardness should be low. Falling number and amylograph consistency describe mainly the integrity and quality of starch but also the enzyme status present in grains. Endosperm hardness is closely connected with grain behavior in milling. Greater softness offers not only potential cost reductions in the milling process but also increased A starch yields and reduced starch damage as a result of less pronounced breakage and deformation. Besides the already mentioned characteristics, general requirements for milling wheat ought to be adequate, in particular, its sanitary status and limits for the presence of besatz.

### Wheat Flour Characteristics

Processing ability of flours was evaluated analytically in the context of the initial principle in separating starch and gluten. Following conditions of the Martin process, Schäfer described a laboratory procedure for the first time that allowed selective separation of the

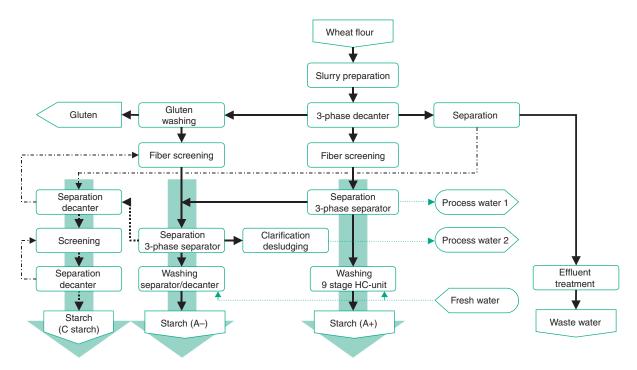


Figure 6 A new concept for A starch and small-size granular starch (C starch) recovery according to a modified Westfalia process.

**Table 4** Extended catalog of requirements for wheat flour properties relevant in modern starch manufacture

<sup>&</sup>lt;sup>a</sup>To be determined by the "mixer/wash test."

main products similar to common wheat wet milling and, finally, evaluation of the flour's wet milling potential.

Diverse corresponding testing procedures were developed on a small-scale basis for identification of new suitable varieties, which use high-speed mixing in imitating mechanical strain and shearing forces applied in decanter processes. Tests of this kind (e.g., the mixer/wash test analysis) allow yield evaluation of moist gluten, dry gluten protein, total dry starch (in case of additionally desired information, split into A and B starch), fibers, and soluble material. However, a spinning test administered as a quick procedure may provide suitable information about separation ability of wheat flours into distinct layers of starch, gluten, pentosans, and process water.

Based upon previously fixed characteristics and extended by additional quantities, **Table 4** presents a new catalog of wheat flour properties as optimum requirements relevant for modern starch manufacture. The key information is represented by the amount of recoverable granular white starch based on wheat flour dry substance and addressed here as "starch potential."

### **Outlook**

A new development in wheat wet milling concerns production of organic gluten and starch. Targeting organic products requires specific efforts in providing the concept of identity-preserved products, which deals first in receiving suitable substrates from organic production and second in applying a process that replaces biocides used for maintaining hygiene by rigid cleaning systems. Today sulfur dioxide is still the common biocide in use for suppression of microbial growth and maintenance of process security. In contrast, application of cleaning systems means regular interruption of today's continuous production which functions 7 days of the week, throughout the year.

During interruptions, the alternative measure uses intensive cleaning cycles lasting several hours, as this is the practice in manifold food production processes. This expensive alternative procedure produces starch and gluten of a high hygienic standard.

Besides, production of organic gluten and starch requires wheat varieties with a high protein quality. In Germany, maximum level (E-quality grade) or high levels of A-quality grades guarantee, in general, satisfactory expression of high molecular gliadin and glutenin proteins as prerequisites of adapted segregation of starch and protein and agglomeration of protein bodies in initial steps of modern production. Processing ability of flours can be tested by small-scale extraction procedures based on centrifugal separation of high-speed mixed water/flour systems.

See also: Gluten and Modified Gluten. Starch: Synthesis. Wheat: Breeding.

### **Further Reading**

Bergthaller WJ (1997) New uses of wheat gluten and nonstarch wheat components. In: Steele JL and Chung OK (eds.) *Proceedings International Wheat Quality Conference*, pp. 285–301. Manhattan, KS: Grain Industry Alliance.

Bergthaller WJ, Witt W, and Seiler M (2002) Wheat quality and testing with regard to modern wet milling technology. In: Steele JL and Chung OK (eds.) *Proceedings of the Second International Wheat Quality Conference*, pp. 381–401. Manhattan, KS: Grain Industry Alliance.

Brautlecht CA (1953) Starch – Its Sources, Production and Uses. New York: Reinhold.

Cornell HJ and Hoveling AW (1998) The wet milling of wheat flour. In: Wheat Chemistry and Utilization, pp. 79–125. Lancaster, Basel: Technomic Publishing.

De Baere H (1999) Starch policy in the European community. *Starch/Staerke*, 51: 189–193.

Gordon I (1999) Starches from different sources – supply, demand, price formation. *Starch/Staerke* 51: 193–196.

Kempf W and Roehrmann C (1984) Verfahren der industriellen Weizenstaerkegewinnung auf Rohstoffbasis Weizenkorn (Processes of industrial wheat starch production on source material basis whole wheat). Starch/Staerke 36: 1-7.

Kröner G (2002) Erfahrungen bei der Herstellung von Biogluten und Biostärke aus Weizen (Experiences in the production of organic gluten and starch from wheat). *Getreide Mehl und Brot* 56: 118–122.

Lindhauer MG and Bergthaller WJ (2002) Characteristics for the assessment of starch extractability from wheat. In: Yuryev VP, Cesàro A, and Bergthaller WJ (eds.) Starch and Starch Containing Origins: Structure, Properties and New Technologies, pp. 375–382. New York: Nova Science Publishers.

Maningat CC and Bassi SD (1999) Wheat starch production. In: Tumbleson M, Yang R, and Eckhoff S (eds.) *Proceedings of the International Starch Technology Conference*, pp. 26–40. Urbana, IL.

Meuser F (1994) Wheat utilization for the production of starch, gluten and extruded products. In: Bushuk W and Rasper VF (eds.) Wheat Production, Properties and Quality, pp. 179–204. Glasgow, London: Blackie Academic and Professional.

Witt W (1997) Modern methods of separating the components of wheat. In: Steele JL and Chung OK (eds.) *Proceedings International Wheat Quality Conference*, pp. 231–248. Manhattan, KS: Grain Industry Alliance.

Zwitserloot WRM (1989) Production of wheat starch and gluten: historical review and development into a new approach. In: Pomeranz Y (ed.) Wheat is Unique, pp. 509–519. St. Paul, MN: American Association of Cereal Chemists.

# Ultrastructure of the Grain, Flour, and Dough

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### Introduction

The wheat grain is unique. Only wheat flour is capable of producing dough with rheological properties that permit the baking of leavened bread. These properties are also needed for the many other food products made from wheat, namely, noodles, pasta, Chinese steamed breads, Arabic flat breads, pastry products, cakes, and cookies. All these products are distinct in the grain-quality characteristics needed for their production. For these reasons, international trade in wheat involves a wide range of wheat grades, each suited to specific groups of food product.

These unique aspects of wheat quality make it a special product in its marketing and processing. As a result, wheat has become much more than a bulk commodity. It has become an object of intense research activity, the "prize" being knowledge about the structure, composition, and function of the grain that can be applied to increase the market value of specific types of wheat.

The resulting knowledge includes insights into the physical structure of the grain and of dough at the macroscopic and microscopic levels. This knowledge has obvious applications in flour milling and in

baking, with respect to the respective ultrastructure of the grain and of dough. These aspects of research outcomes are described in this article.

### The Anatomy of the Wheat Grain

### **External Features**

The wheat grain is botanically a single-seeded fruit, called a "caryopsis" or "kernel" (Figure 1). It develops within floral envelopes (the "lemma" and "palea"), which are actually modified leaves. Viewed from above (the dorsal side — the same side as the germ), the grains of different varieties may appear to be oval, ovate, elliptical, elongated, or truncated (short). These characteristics can be useful in attempting to identify varieties by aspects of grain shape. In addition, dimensions may be helpful, but they vary with growth conditions. The wheat kernel averages  $\sim 2.5-3.0$  mm thick (or high as it stands on its base), 3.0-3.5 mm wide, and 6.0-7.0 mm in length. Wheat kernels average  $\sim 30-40$  mg in weight.

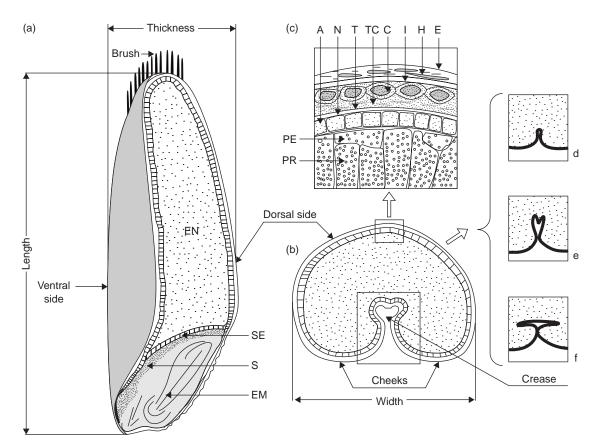
Wheat kernels are rounded on the dorsal side, with a longitudinal "crease" (a deep groove) running the full length of the ventral side. The shape of the groove is a characteristic feature of some species and varieties. The presence of a wide and deep crease is undesirable, because it contributes to making a low bulk density (test weight) for the grain. The main inner volume of the grain is taken up by the starchy "endosperm," which becomes the white flour that is released and crushed to fine particles by the flour miller.

The "embryo" (called the "germ" by millers) forms an irregular patch at one end of the dorsal side of the kernel, near the point of attachment of the kernel to the plant. This point is called the "hilum." The tip of the opposite (distal) end of the kernel is covered with small hairs ("trichomes"). This feature, known as the "brush," can also be a useful varietal characteristic, as wheats may differ in the length of the brush hairs. Several other features of the grain's anatomy are illustrated and labeled in Figures 1 and 2.

### The Bran

The wheat grain is enclosed in a series of layers, collectively called the "bran" (Figure 2). This layer is visible by light or scanning electron microscopy as the outermost collection of cells surrounding the grain (Figures 3 and 4). The fluorescence micrograph of Figure 5 shows a section of the bran layers that has become separated from the underlying aleurone-cell layer.

The "pericarp" (fruit coat) surrounds the entire seed and consists of two portions, the outer pericarp



**Figure 1** The main morphological components of the wheat kernel: (a) longitudinal section of kernel; (b) cross-section of kernel; and (c) segment of bran layer with aleurone and endosperm cells. Abbreviations to segment (c): E, epidermis; H, hypodermis; I, inner pericarp; C, cross cells; TC, tube cells; T, testa; N, nucellar layer; A; aleurone layer; En, endosperm; PE, peripheral cells of endosperm; PR, prismatic cells of endosperm; SE scutellar epithelium; S, scutellum; E, embryo; d, e, and f, segments with typical shape of creases. (Reproduced from Encyclopedia of Food Sciences and Nutrition, 2nd Edition (2003), p. 6139, Elsevier Ltd.)

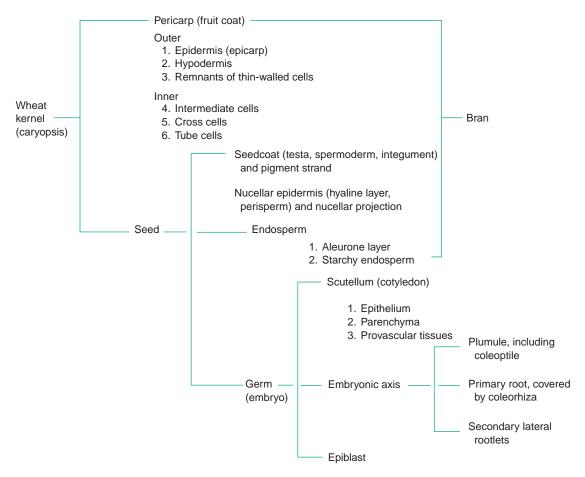
and inner pericarp. The outer pericarp has the following layers; the "epidermis" (epicarp), the "hypodermis," and the innermost layer, called the remnants of thin-walled cells. These thin-walled cells, which have a discontinuous cellular structure, form a natural plane of cleavage. Removal of the outer pericarp, which millers call "beeswing," also aids movement of water into the kernel. The inner pericarp, adjacent to the remnants, is composed of intermediate cells — a single layer of cross cells and tube cells. The cross cells are long and cylindrical ( $\sim 12.5 \times 20 \, \mu m$ ), and have a long axis perpendicular to the long axis of grain. They are tightly packed, with little or no intercellular space.

The tube cells are similar in size and shape to the cross cells, but they have their long axis parallel to the long axis of grain. The tube cells are not packed tightly and do not form a continuous layer; thus have many intercellular spaces. They are only recognizable in the mid-dorsal region of mature grains. The next layer inwards is the seedcoat ("testa" or "integument"), which is firmly joined to the tube cells on the

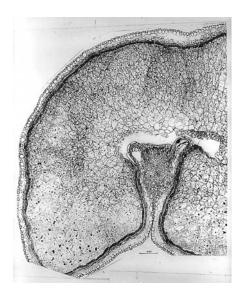
outside and the nucellar epidermis on the inside. The seedcoat of red wheat consists of a thick outer cuticle layer, which is strongly pigmented, and a thin inner cuticle layer. The seedcoat in white wheat has cell layers containing little or no pigments. Grain color, usually red or white (although purple is also known), is related to pigment in the testa.

Tightly bound to the internal surface of the seed-coat is the nucellar epidermis ("hyaline layer," "perisperm"). The thickness of the seedcoat varies from 5 to 8  $\mu$ m. The nucellar epidermis is  $\sim$ 7  $\mu$ m thick and closely attached to both the seedcoat and the aleurone layer. The total pericarp has been reported to comprise  $\sim$ 5% of the kernel volume.

The "aleurone" layer, which is generally one cell thick in wheat, completely surrounds the kernel, covering both the starchy endosperm and the germ, except for that adjacent to the scutellum (Figure 2). Although the aleurone layer is anatomically a part of the endosperm, the miller regards the aleurone as the innermost layer of the bran. The majority of the mineral matter located in bran is found in the



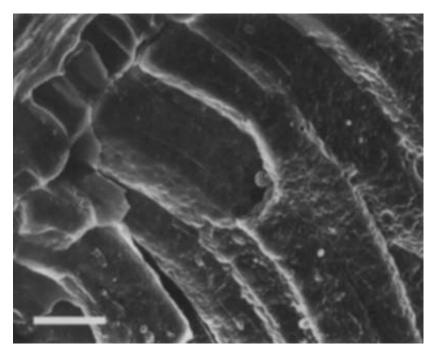
**Figure 2** The parts of the wheat kernel, grouped according to their positions in the kernel. (Reproduced with permission from Hoseney RC (1994) *Principles of Cereal Science and Technology*, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.)



**Figure 3** Light micrograph of wheat-grain ultrastructure. A thin section was cut near the center of the grain, at right angles to the crease, which appears on the right. The section was fixed with Spurrs medium and stained with toluidine blue. (Courtesy of B Campbell.)

aleurone layer, which also contains one-third of the grain's thiamine content. The cytoplasm of the cells contains many small (3–4  $\mu$ m), round aleurone granules surrounded by lipid droplets.

The aleurone granules contain two types of inclusions: type I contains phytin and type II contains protein, carbohydrate, and bound nicotinic acid, which is largely unavailable for human nutrition. The phytin granules are the main source of mineral matter. Hence, the degree of aleurone (or bran) contamination of flour is frequently evaluated by an ash analysis. In addition, thiamin and riboflavin are higher in the aleurone layer than in the other parts of the bran, and enzyme activity is high. Over the embryo, the aleurone cells are modified, becoming thin-walled cells that may not contain aleurone granules. The thickness of the aleurone layer over the embryo averages  $\sim 13 \,\mu\text{m}$ , or less than one-third the thickness found elsewhere. The aleurone cells are heavy-walled, essentially cube-shaped, and free of starch. They can vary in thickness from 30-70 µm within a single kernel and have thick  $(6-8 \, \mu m)$ , double-layered cellulosic walls.



**Figure 4** Scanning electron micrograph of the cut surface of a vitreous kernel of wheat, showing the subaleurone region with peripheral and prismatic cells. Note that a fracture has taken place at the interface between the cell contents and endosperm cell wall. The length of the bar at the lower left is 50 μm. (Reproduced with permission from Grundas S (ed.) (2003a) Wheat: grain structure of wheat and wheat-based products. In: *Encyclopedia of Food Science and Nutrition*, pp. 6130–6137. Elsevier.)

### The Endosperm

The starchy endosperm, excluding the aleurone layer, is composed of three types of cells: "peripheral," "prismatic," and "central." The peripheral cells are the last to be initiated during grain filling and they tend to be smaller than the other endosperm cells (60  $\mu$ m in diameter and 20–60  $\mu$ m radially). In addition, they have thicker cell walls (8  $\mu$ m). Several rows of elongated prismatic cells are found inside of the peripheral cells. They extend inward to about the center of the cheeks and are  $\sim$ 150–200  $\mu$ m in length. The central cells are more irregular in size and shape than are the other cells. They are located inside of central endosperm.

The central endosperm cells are the first to be formed and they have thin walls ( $2 \mu m$ ). Cell-wall thickness also appears to vary among cultivars and between hard and soft wheat types. The differences between hard and soft wheat may be the result of selection: hard wheats (bread wheat) have been selected for high water absorption. The endosperm cell walls are composed of pentosans, other hemicelluloses, and  $\beta$ -glucans, but not cellulose. The pentosans in them absorb large amounts of water. The endosperm cells are packed with starch granules embedded in a protein matrix. Starch is the major component of wheat endosperm, comprising  $\sim 75 \%$  of milled endosperm.

Generally, the starch granules in wheat are classified into two size groups: large, lenticular (lensshaped) A granules of up to 40  $\mu$ m across the flattened side, and small, spherical B granules up to 10  $\mu$ m in diameter. The B granules are formed later in the grainfilling process than are the A granules. The number ratio of small to large granules is  $\sim 3:7$ .

In bread wheats, the endosperm texture varies both in texture (hardness) and appearance (vitreousness). In general, high-protein hard grains are vitreous, whereas low-protein soft grains tend to be opaque. Some wheat grains are vitreous or translucent in appearance, while others are opaque, mealy, or floury. In vitreous kernels, with no air spaces, light is diffracted at the air—grain interface and it then travels through the grain without being diffracted again and again.

With vitreous kernels, the protein shrinks but remains intact, giving a denser kernel. As expected, the presence of air spaces within the endosperm makes the opaque grain less dense. The air spaces are apparently formed during the drying of the grain. If grain is harvested immature and freeze-dried, it becomes entirely opaque. This shows that the vitreous character results during intensive drying in the field. It is also well known that vitreous grain wetted and dried in the field, or for that matter in the laboratory, will lose its vitreousness. In durum wheat, which is much harder

than common hard bread wheat, a much larger number of starch granules are broken when the grain is fractured compared to bread wheat.

### The Germ

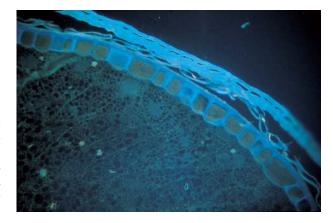
The wheat germ is composed of two major parts, the embryonic axis (rudimentary root and shoot) and the scutellum, which functions as a storage organ (Figure 2). The scutellum is adjacent to the endosperm and contains the remaining two-thirds of the grain's thiamine content. The germ is quite rich in vitamin E (total tecopherol) and in B-vitamins. It contains many enzymes. The germ is a rich source of protein (25%), sugar (18%), oil (16% of the embryonic axis, and 32% of the scutellum are oil). The sugars are mainly sucrose and raffinose. On incineration, the germ gives a high level of ash (5%). The wheat germ comprises 2.5–3.5% of the kernel. Recovery of the germ during the milling process is an important step because of its value in the food and pharmaceutical industries.

# Techniques for Studying Grain Ultrastructure

Many cereal scientists and technologists have successfully employed the three main branches of microscopy — light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) — to study the structure and composition of wheat and wheat-based products. Recently, X-ray techniques have been used to detect kernel cracks. The objective of the studies involves gaining fundamental information on the accumulation of cellular constituents in the developing wheat grain, as well as providing information that can improve the understanding of differences in processing ability and in the overall quality of wheat and wheat-based products.

### **Light Microscopy**

The main stages in the preparation of samples for examination by LM are fixation, embedding, sectioning, and staining. The aims of fixation are to preserve samples from attack by enzymes or microorganisms, to render some constituents insoluble, and to strengthen the sample, thus improving its structural integrity during sectioning. The most commonly used fixative is aqueous, buffered glutaraldehyde, but specialized fixatives have been developed for specific applications (e.g., fixation of lipid-rich samples). Baked samples, which have been heat-fixed, may not require chemical fixation. Embedding, to provide additional support during sectioning, commonly involves aqueous gums for cryostat microtomy,



**Figure 5** Fluorescence microscopy of the outer layers and outer endosperm of a section of wheat grain. The effect of autofluorescence provides contrast for the cell walls of the aleurone layer. (Courtesy of B Campbell.)

synthetic resins, or special waxes. Resins are generally used where thinner sections are required and are cut using glass knives. Cryostat microtomy and sectioning of wax-embedded samples are carried out using steel knives. For examination, with transmitted bright-field illumination, stains are commonly used.

The ultrastructure of the wheat grain is illustrated in Figure 3. This micrograph was obtained by cutting a thin section from the grain, fixed with Spurrs medium. The section was stained in toluidine blue before viewing. The outer bran layers can be seen as distinct from the endosperm cells, further inside the grain. The crease is very obvious, extending well into the outer circle of the grain. The vascular bundle is seen at the innermost extent of the crease. Magnification by LM is generally less than is provided by electron microscopy, as can be seen by comparing the light micrograph of Figure 3 with the scanning electron micrograph of Figure 4.

Fluorescence microscopy has been widely used and may rely on auto-fluorescence or the application of fluorescent dyes, often coupled to specific antibodies or lectins. Other coupled antibody techniques have been developed whereby colored reaction products are produced. Figure 5 shows an example of the use of auto-fluorescence to highlight the outer layers of the endosperm, with special contrast being provided for the cell walls of the aleurone cells.

Polarized light can be used to study starch gelatinization or to provide detail of cell-wall structure. Native starch granules viewed under polarized light show a cross-shaped structure, known as the "Maltese cross" phenomenon (Figure 6). It is indicative of the crystalline internal structure of the granules in their native state. This feature is not seen after starch granules have gelatinized due to the effects of moist heat

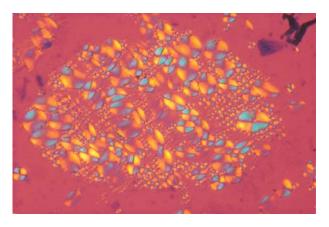


Figure 6 Starch granules viewed under polarized light, showing the "Maltese cross" effect on the granules. (Courtesy of R Moss.)

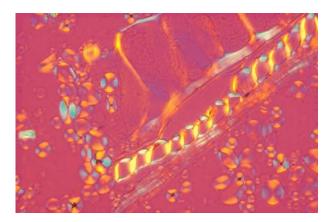


Figure 7 Bran layers of wheat viewed under polarized light, showing the effects of polarized light on bran fragments. Some starch granules are also visible. (Courtesy of R Moss.)

on their crystalline structure. This technique is thus a useful means of determining the status of starch granules, with respect to their crystalline state. Viewing under polarized light also produces distinctive pattern for bran particles, making them easier to detect their presence in white flour (Figure 7). One of the major changes that can take place during the cooking of baked products is the gelatinization of the starch granules. The extent to which this has occurred is easily followed using polarized light microscopy.

In Figure 7, light macro-photography has been used with a video camera to examine the wetting of wheat endosperm with water. The three frames of Figure 8 show parts of this sequence. Water, added to the section of endosperm, has started to form strands of gluten, which are seen streaming away from the otherwise dry section of endosperm.







Figure 8 Successive frames from a video of wheat endosperm being wetted with water. Strands of gluten can be seen forming and streaming away from the dry endosperm mass at left. (Courtesy of J Bernardin.)

### Scanning Electron Microscopy (SEM)

SEM has a greater depth of focus than LM and therefore it is not necessary to section samples prior to examination. Samples are normally coated with a thin layer of an electrically conducting material, usually gold, platinum, or carbon, prior to

examination. Samples of dry wheat (<12% moisture) or similar products only require air-drying over a desiccant prior to coating. Samples containing more moisture may be rapidly frozen prior to dehydration, using freeze-drying or critical-point drying. The development of cold stages in SEM has obviated the need for drying and this technique, together with freeze-etching, has been successfully used to examine cereal foods in which moisture is high, forming an integral part of the structure.

Endosperm hardness influences the manner in which grains fragment during milling, and this can also influence the yield of white flour. The efficiency with which floury endosperm is removed from overlying bran and the degree to which bran is powdered or otherwise damaged are both influenced by the manner in which grain fractures during milling. If fracturing occurs at the boundary between the endosperm cell wall and cell contents, the endosperm is efficiently removed from the bran, but the bran is fractured into small pieces. Figure 4 shows the cut surface of a wheat grain. Some signs of rupture between cells are visible.

When the endosperm is fractured intracellularly, bran clean up is poor, but large pieces of bran are produced. The manner in which the grain is fractured is determined by both the inherent hardness of wheat grain and its moisture content. Therefore, these two factors are carefully monitored by the flour miller. Grain of known hardness is sought for milling into specific types of flour, and grain is "conditioned" to appropriate moisture content prior to flour milling.

### **Transmission Electron Microscopy**

Samples for TEM require fixation and thin sectioning. Initial fixation is usually with glutaraldehyde followed by fixation with osmium tetroxide. The fixed tissue blocks are rinsed, dehydrated, and embedded with a resin prior to sectioning. Heavy metal salts are used to stain the sections and enhance contrast; more recently, antibody staining has been developed whereby the antigenic sites are located at TEM level by the presence of colloidal gold particles, which are coupled to the antibodies.

### **Image Analysis and Other Techniques**

During the 1990s, the value of all the above work was enhanced by high-tech methods, such as magnetic nuclear resonance, laser, and X-ray methods. More recently, the application of stereology techniques and automatic image analysis of grain have been used. Furthermore, user-safe X-ray techniques have proven to be highly suitable for visualization of mechanical damage of grain, especially inner cracks in kernels or insect infection (Figure 9). Mechanical damage occurs

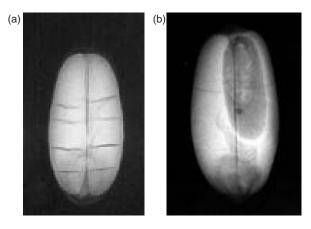


Figure 9 The X-ray images of wheat kernels: (a) kernel with inner cracks and (b) kernel with a weevil larva inside it.

wherever grain is subjected to the destructive action of internal or external forces, causing internal cracks.

The results of research carried out by using X-ray method showed significant differences in grain endosperm cracks between common wheat varieties. Natural wetting of dry grain (below 15% of moisture content) during rainfall when wheat is standing in the field is one of the reasons of its cracking. The susceptibility of wheat to mechanical damage is determined by genetic factors (e.g., grain hardness), environmental effects (climatic conditions during the pre-harvest period), and by the conditions of grain storage (especially excessive humidity). The combination of these properties determines the quality of grain material.

Radial cracks in the endosperm of a grain are shown in Figure 9, using X-ray microscopy to reveal these inner faults. X-ray detection makes it possible to identify the position of cracks and also to quantify cracks inside the kernel, and thus to evaluate the physical condition of the grain endosperm. Also shown in Figure 9 is the presence of a weevil larva that could not otherwise be detected. In this way, the stage of development of insect larvae living inside the kernel may be monitored, permitting the detection of insect infestation that is not visible by normal external examination.

# The Ultrastructure of Wheat-Based Products

### Flour Milling

The aim of the flour-milling process is to remove the endosperm from the crushed grain, separating it from the other anatomical parts of the grain, namely, the germ, bran, and scutellum. The particular aim is to obtain a maximum yield of white flour with the

minimum contamination of nonendosperm material. Fluted rollers are used to break open the grain and scrape the endosperm from the bran; smooth rollers are used to reduce the endosperm particles into flour. The moisture content of cleaned wheat is adjusted to between 15% and 17% prior to milling. This process ("conditioning") facilitates the separation of bran and endosperm and toughens the bran, thereby reducing the amount of ash in the flour.

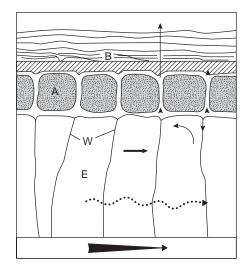
Conditioning can be completed in ~6 h for soft wheat, but hard wheat may require 24 h or longer. X-ray microscopy has shown that added water moves rapidly through the bran layers, but may remain at the aleurone—endosperm interface for several hours. The rate of water penetration through the endosperm is dependent on protein content, initial moisture, and grain hardness. The air spaces that are present in the endosperm of soft wheat allow water to move more rapidly and hence the conditioning time is less than that required for hard wheat.

Of the morphological factors that can influence the yield of white flour, the shape of grain and the amount of endosperm within the grain are particularly important. Grain size has been shown to be significant; larger grains have a higher potential flour yield. Image analysis has been used to measure a large number of morphological parameters, and extraction has been shown to correlate with grain-length parameters.

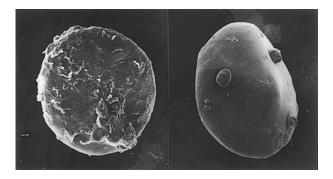
In addition to the amount of endosperm contained in the grain, the efficiency with which bran and endosperm can be separated ("bran clean-up") is also a factor influencing flour yield. Because of differences in the structure of hard and soft grains, they must be milled differently. The cell structure of soft wheat is very weak and readily broken. In addition, the endosperm of soft wheat appears to adhere strongly to the bran. Durum wheat is used to produce semolina, a granular material analogous to the farina or flour middlings of the hard-wheat milling process. Granular products such as semolina and farina are used for pasta, baby foods, and specialty foods.

Endosperm hardness influences the manner in which grains fragment, and this can also influence flour yield. An important difference between hard and soft wheats is the boundaries of endosperm fragmentation. In hard wheat, it takes place mainly at the boundary between adjacent cells as the contents of the cells are more firmly bound together by the continuous matrix protein. Thus, hard wheat endosperm can be removed more efficiently from bran as the shear forces imparted by the fluted rollers are directed along the boundary between adjacent endosperm cells towards the bran (Figure 10).

When they reach the bran, some of the forces are deflected along the endosperm interface, facilitating

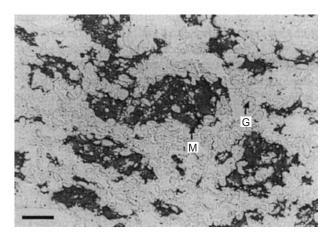


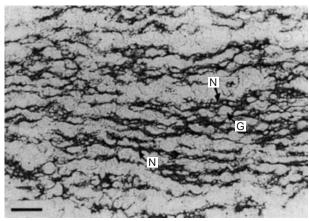
**Figure 10** Diagrammatic representation of the forces acting on one endosperm cell of a wheat grain during milling. The large arrow at the bottom of the diagram indicates the direction of the shear force caused by the differential speed of the break rollers. In hard wheat, the contents of the cell behave as one unit, and hence the cell is cleanly torn from the overlying aleurone layer. In soft wheat, the shear force passes through the content of the cell, as indicated by the dotted arrow. B = bran layer. A = aleurone layer. W = endosperm cell wall. E = endosperm. (Reproduced with permission from Macrae R, Robinson RK, and Sadler MJ (eds.) (1993) Wheat: Structure of wheat and wheat-based products. *Encyclopedia of Food Science, Food Technology and Nutrition*. London: Academic Press.)



**Figure 11** Scanning electron micrographs of starch granules released during flour milling from a hard wheat (left) and from a soft wheat, showing the much greater amount of adhering storage protein on the starch granule from the hard wheat.

separation of the endosperm from the bran. Of course, as the kernel is reduced to flour size, the hard wheat cell contents are also fractured. In soft wheat, the discontinuities in the protein matrix allow endosperm contents to break apart easily and cleavage takes place intercellularly. Thus the shear forces are dissipated within the endosperm are not redirected towards the bran. Figure 11 shows how





**Figure 12** Light micrographs of a cryostat section of an under-developed bread dough (left) and of a fully developed dough (right). The stain was Ponceau 2R. For the under-developed dough, the coarse gluten masses (M) that do not surround the majority of the starch granules (G), as they do, by contrast, for the developed dough which has become more interconnected and forms a continuous network (N). The bar is 60 μm long. (Reproduced with permission from Macrae R, Robinson RK, and Sadler MJ (eds.) (1993) Wheat: structure of wheat and wheat-based products. In: *Encyclopedia of Food Science, Food Technology and Nutrition*. London: Academic Press.)

the starch granules are released from a soft wheat, clean of adhering interstitial protein, which remains adhering to the starch granules from a hard wheat.

Microscopy can also be used to study the mode of action of different items of mill equipment and the effect of processing variables on flour quality. Starch damage is regulated by the amount of pressure applied by the smooth reduction rollers. The physically damaged granules absorb more water and are more susceptible to enzyme attack. A controlled level of starch damage is required for bread flours.

# **Dough Formation**

When water is added to flour and energy is imparted to the dough by a mixer, the gliadin and glutenin proteins interact to form elastic, cohesive gluten. The tendency to form viscoelastic gluten can be seen even without mixing. Figure 8 shows a few frames from a video of allowing water to come in contact with a section cut from wheat endosperm, lying on a microscope slide. Immediately the gluten-forming proteins start to form fibrils that stream out from the endosperm section, carrying starch granules with them.

The mechanism and rate of gluten development depend on the level of water addition and rate of work input. In bread dough, the level of water addition (55-62%) is sufficient to cause the mixer initially to pull the gluten away from the starch granules and form coarse, poorly connected masses (Figure 12). As mixing proceeds, these masses are gradually stretched out and become more interconnected and eventually form a uniform, continuous, extensible network,

which surrounds the majority of the starch granules in the dough. This gluten network gives dough a smooth external appearance, in turn leading to the production of well-risen bread of uniform crumb structure.

The level of water addition in noodle or pasta dough is considerably less (30–33% addition) than that in bread, and no protein pullback occurs during mixing. The function of mixing in these processes is to insure uniform distribution and hydration of ingredients. The continuous gluten matrix is formed by sheeting rollers in the case of noodles and by high-pressure extrusion in the case of pasta.

### Conclusion

Thorough knowledge of the ultrastructure of the wheat grain, and of the products made from it, is essential to intelligent manipulation and optimization of wheat processing in all its forms. Much of this knowledge has already been translated into greater efficiencies, especially in milling and dough processing. Also needed is the integration of the knowledge of ultrastructure with chemistry and genetics. This overall picture will help in combining the efforts of technologists, chemists, and breeders, thereby to provide vertical integration from the original "design" of better varieties through to the final product and to the most important person in the business chain — the consumer.

See also: Wheat: Genetics; Grading and Segregation; Dry Milling; Dough Rheology; Grain Proteins and Flour Quality.

### **Further Reading**

Faridi H and Faubion JM (eds.) (1995) Wheat End Uses Around the World. St. Paul, MN: American Association of Cereal Chemists.

Fitzsimmons RW, Martin RH, and Wrigley CW (1983) Australian Wheat Varieties: Identification according to Plant, Head and Grain Characteristics, 2nd edn. Melbourne: CSIRO.

Grundas S (ed.) (2003a) Wheat: grain structure of wheat and wheat-based products. In: Encyclopedia of Food Science and Nutrition, pp. 6130-6137. Elsevier.

Grundas S (ed.) (2003b) Wheat/Grain structure of wheat and wheat-based products. In: Encyclopedia of Food Science and Nutrition, pp. 6137-6146. Elsevier.

Hamer RJ and Hoseney RC (eds.) (1998) Interactions: The Keys to Cereal Quality. St. Paul, MN: American Association of Cereal Chemists.

Hoseney RC (1994) Principles of Cereal Science and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Macrae R, Robinson RK, and Sadler MJ (eds.) (1993) Wheat: structure of wheat and wheat-based products. In: Encyclopedia of Food Science, Food Technology and Nutrition. London: Academic Press.

Pomeranz Y (ed.) (1989) Wheat is Unique. St. Paul, MN: American Association of Cereal Chemists.

### **Relevant Websites**

http://www.awb.com.au - AWB Ltd, Melbourne, Australia.

http://www.cgc.ca; www.grainscanada.gc.ca Canadian Grains Commission, Winnipeg, Canada. http://www.pi.csiro.au - CSIRO Plant Industry, Australia.

http://www.icc.or.at - International Association for Cereal Science and Technology.

http://www.seedtest.org — International Seed testing Association.

http://www.crop.cri.nz - New Zealand Institute of Crop & Food Research.

http://www.usda.gov - United States Department of Agriculture; grain handling pratices, standard sampling procedures.

# Dough Rheology

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# Introduction

Rheology is defined as the study of the flow and deformation of materials, conventionally applied to

**Table 1** Typical shear viscosities of materials

Material	Viscosity (Pas)
Bitumen	108
Polymer melts	$10^4 - 10^5$
Dough	$10^3 - 10^4$
Golden syrup	10 <sup>2</sup>
Molten chocolate	10 <sup>2</sup>
Set yogurt	$3 \times 10^{1}$
Liquid honey	10 <sup>1</sup>
Tomato sauce	10 <sup>0</sup>
Glycerol	10 <sup>0</sup>

materials that flow within normal experimental timescales. Liquids and soft solids have viscosities in the range 0.1–10 000 Pa's (e.g., viscosity of wheat flour dough is  $\sim 1000-10\,000\,\mathrm{Pa}\,\mathrm{s}$ ) (Table 1). Given enough time, most of the materials will flow, e.g., ice flows over decades in glaciers and rocks flow over millennia in the earth's crust, but these are generally regarded as brittle, elastic materials over normal experimental timescales (a few minutes) and are generally described by solid mechanics principles. The Deborah number (named after the biblical prophet Deborah, who said "The mountains flowed before the Lord") defines the flow of a material with respect to time. It is defined as the ratio of the time taken by a material to flow or relax and the time of observation. If the material takes a long time to flow, it will have a large Deborah number. The lower the Deborah number, the more fluid is the material. Dough relaxes very quickly, over a matter of a few minutes, and hence will have a low Deborah number.

To measure rheological properties, deformation or strain is applied to a material in a controlled and quantifiable manner, and the resultant forces or stresses are measured to give an indication of the stiffness, modulus, or viscosity of the material. For materials which flow readily, shear stresses are commonly applied using standard viscometer geometries such as rotating parallel plates, cone and plate, and concentric cylinders. For complex fluids such as doughs, especially ones with long polymer molecules, the rheological properties measured in shear are very different from those measured in extension; therefore, extensional deformation is often applied by the imposition of a stretching flow.

Rheological measurements on dough have long been used to define its physical properties. The primary aims of these measurements are:

- to obtain a quantitative description of its mechanical properties;
- to characterize and predict its performance during processing and end use; and

 to obtain information related to its molecular structure and composition.

Rheology can tell us how a dough will behave under a given set of conditions and can be used to characterize and predict its performance during deformation, e.g., during mixing, sheeting, proving, and baking of dough. Rheological data are an important tool to process design, for example, of mixers and sheeters, or to predict the behavior of expanding bubbles within proving doughs, and to predict end-use quality ultimately. At the heart of this work lies the long-held belief within the baking industry that the rheological properties of dough, traditionally assessed by kneading and stretching the dough by hand, have a strong relationship with its eventual baking quality. This belief has been strengthened by centuries of empirical evidence, and there is no reason to doubt its validity. However, results from both conventional industrial methods of assessing dough rheology and modern fundamental rheological test methods have given disappointing relationships with baking quality. This is mainly because many of these tests are carried out inappropriately under deformation conditions far removed from those occurring during baking. This article will review rheological test methods applied to doughs and assess their usefulness for predicting end-use performance.

### **Historical Background**

There has always been an intuitive feel for rheological testing, for example, in tactile and visual assessments of material properties such as hardness, stiffness, flexibility, and viscosity, and their relation to end-use quality characteristics. The quality of solid foods is often instinctively assessed by gently squeezing them, or liquid viscosity is assessed by gently rotating the liquid in its container, and these tests are often applied on the factory floor as a crude measure of quality. These intuitive assessments gradually became formalized into quantitative descriptions of material properties by scientists such as Newton (1687), Boyle (1662), Pascal (1663), Hooke (1678), Young (1807), and Cauchy (1827). Modern rheology as an independent discipline can be traced back to 1929, when The Society of Rheology was set up by a number of scientists working in complementary fields to secure an absolute standard for viscosity, and the name rheology was proposed by Bingham and Reiner to describe the study of flow and deformation of all forms of matter. Since then rheology has grown rapidly as a science and contributed to a number of applications such as colloids, suspensions and emulsions, polymer processing, extrusion, and polymer

modeling. Recent developments in polymer rheology have established a quantitative link between the molecular size and structure of polymers and their rheology and end-use performance. Rheological measurements are increasingly being used as rapid, sensitive indicators of polymer molecular structure and predictors of end-use performance.

### **Rheological Test Methods**

There are many test methods used to measure rheological properties, and the reader is referred to general reviews of rheology, rheological testing of foods, and cereal doughs given at the end of the article. It is common to categorize rheological techniques according to the type of strain imposed (e.g., compression, extension, shear, torsion, etc.) and the relative magnitude of the imposed deformation (e.g., small or large deformation) as well. The main techniques used for measuring cereal dough properties have traditionally been divided into descriptive empirical techniques and fundamental measurements.

### **Descriptive Rheological Measurements**

Within the baking industry there is a long tradition of using descriptive empirical measurements of rheological properties, with instruments such as the penetrometer, texturometer, consistometer, amylograph, farinograph, mixograph, extensograph, alveograph, various flow viscometers, and fermentation recording devices (Table 2). Empirical tests are easy to perform and are often used in practical factory situations, providing data which are useful in evaluating performance during processing and for quality control. The instruments are often robust, capable of withstanding demanding factory environments, and do not require highly skilled or technically trained personnel. They have provided a great deal of information on the quality and performance of doughs such as consistency, hardness, texture, etc. However, these measurements do not fulfill the requirements of a fundamental rheological test since (1) the sample geometry is variable and not well defined and (2) the stress and strain states are uncontrolled, complex, and nonuniform. It is therefore impossible to define any rheological parameters such as stress, strain, strain rate, modulus, or viscosity. Therefore, these tests are purely descriptive and dependent on the type of instrument, size and geometry of the test sample, and the specific conditions under which the tests were performed. For example, empirical tests are used to characterize the behavior of bread doughs during processing, using instruments such as the extensograph, farinograph, and mixograph. Many of these

Table 2 Rheological methods used for cereal products

Methods	Products	Property measured
Empirical methods		
Mixers:	Dough	Mixing time/torque
Farinograph		Apparent viscosity
Mixograph		
Reomixer		
Extensograph	Dough	Extensibility
TAXT2/Kieffer RIG	Dough, gluten	Extensibility
Alveograph	Dough, gluten	Biaxial extensibility
Amylograph, RVA	Pastes, suspensions	Apparent viscosity
		Gelatinization temp.
Consistometer	Sauces, fillings	Apparent viscosity
Flow cup	Fluids, sauces, batters	Apparent viscosity
Falling ball	Fluids	Apparent viscosity
Flow viscometers	Fluids, pastes	Apparent viscosity
Fermentometers	Dough	Height, volume
Penetrometers	Semisolid foods, gels	Firmness, hardness
Texturometer, TPA	Solid foods	Texture, firmness
Fundamental methods		
Dynamic oscillation	Fluids, pastes, batters, doughs	Dynamic shear moduli
Concentric cylinders	Parallel plates	Dynamic viscosity
Tube viscometers:		
Capillary	Fluids	Viscosity
Pressure, extrusion	Sauces, pastes, dough	Viscosity
Pipe flow		In-line viscosity
Transient flow:	Semisolid (viscoelastic) materials	Creep, relaxation
Concentric cylinders		Moduli and time
Parallel plates		
Extension:		Extensional viscosity
Uniaxial, biaxial	Solid foods, doughs	Strain hardening
TAXT2 dough inflation system		
Lubricated compression		

are used as "single point" tests, where a single parameter is often arbitrarily selected from a whole range of data acquired during the test as, for example, in selecting the peak torque from a mixing trace and then using this to correlate with performance. This, however, neglects a large part of the recorded data, and is appropriate only to the set of conditions under which that test was performed, and which are generally not applicable to any other deformation conditions. Since dough experiences a wide range of conditions of stress states and strain rates during processing and baking, and the rheological properties of dough are dependent both on time and strain, there is often a discrepancy between such single-point-type tests and actual performance on the plant, where conditions of strain and strain rate may be poorly defined and very different to those in the laboratory test. It is impossible to compare results between different testing machines, or to extrapolate the results to other deformation conditions.

Doughs are viscoelastic and therefore their properties depend on how quickly the test is performed (the strain rate or frequency). This is important in many

aspects of dough processing: if the dough is deformed quickly, for example, in mixing or sheeting, its rheological properties will be very different when measured at the typically slower rates of deformation found in conventional testing machines. Alternatively, performing a test under small deformation shear will give very different results to the large deformation extension conditions that dough experiences during sheeting or bubble walls experience during baking expansion. Performing a test under only one particular set of conditions of rate, temperature, and strain will almost certainly not be applicable to another set of deformation conditions: it is necessary to define the set of deformation conditions under which a dough operates in practice and then to perform the rheological test under these conditions.

### **Recording Dough Mixers**

The farinograph and associated instruments such as the mixograph measure force or torque during mixing of doughs. These instruments are the most widely used physical dough testing machines found in

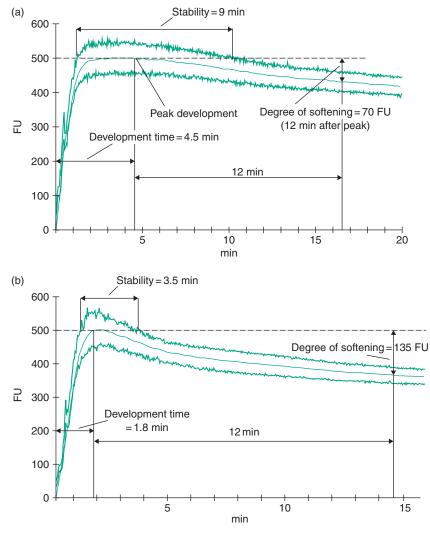


Figure 1 Analysis of farinograph mixing traces for a (a) strong flour and (b) weak flour showing commonly measured parameters. (Reproduced with permission from Brabender OHG, Duisburg, Germany.)

cereals' laboratories throughout the world. Their main uses are: (1) to predict the amount of water to add to flour to achieve a dough of fixed torque (consistency) during mixing (water absorption); (2) to measure the mixing characteristics of flour; and (3) to predict baking performance. The farinograph is a useful quality control tool for the baking and milling industries, and the mixograph provides an indication of the mixing requirements for relatively small amounts of flour (2–10 g), which is useful for wheat plant breeders in the early stages of selection of good quality traits in breeding programs.

The Brabender Farinograph, developed in the early 1930s, measures force or torque during gentle mixing of a dough at fixed speed using two counter-rotating z-blades mounted horizontally in a mixing bowl. Mixing torque is recorded as farinograph units

(FUs) against time to give a mixing curve either on standard chart paper by means of a pen attached via a series of levers to a torque-recording device or directly onto a PC (Figure 1).

To calculate the water absorption, a fixed amount of flour (normally 300 g) is mixed with water. Water is added until a required maximum consistency is reached (usually 500 FU or 600 FU in the UK) at the center of the mixing curve. The amount of water added to achieve a required consistency is known as the water absorption, which can vary from 50% for a soft biscuit flour to almost 70% for standard UK bread-making flours at 600 FU consistency. Measurement of consistency and water absorption allows one to predict the processing behavior. If too much water is added to flour, dough with low consistency will be difficult to handle

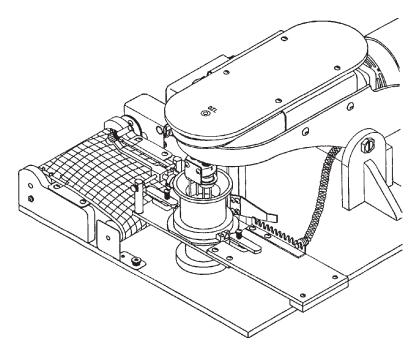


Figure 2 The mixograph. (Reproduced with permission from National Manufacturing TMCO Inc., Lincoln, Nebraska.)

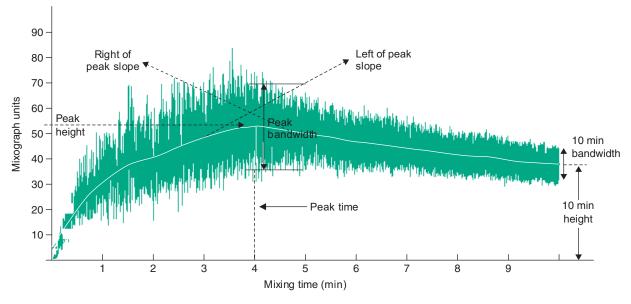
and tend to be sticky. If too little water is added to flour, the dough will tend to be stiff and difficult to process, and unit costs will be higher.

The farinograph is also used to measure the mixing characteristics of dough. Flours of varying breadmaking quality will produce different shapes of mixing curves. A poor bread-making flour produces a curve which rises rapidly to a maximum consistency (peak development), and then decreases rapidly on further mixing. A good bread-making flour takes longer to reach its maximum consistency and is much more stable, i.e., it shows little decrease in consistency (degree of softening) on further mixing (**Figure 1**). This is related to the stability of the gluten macromolecular proteins during mixing. Larger polymers, known to be related to good baking quality, are more difficult to break down than smaller ones, which is reflected in the shape and stability of the mixing curves from good and poor bread-making flours.

The mixograph, first described by Swanson and Working in 1933, is a similar recording mixer, which uses planetary rotating pins oriented vertically to mix the dough instead of blades (Figure 2). Torque during mixing is recorded either by a pen on chart paper or electronically via a torque transducer or, in more recent versions, by recording electrical output from the motor driving the pins, and mixing curves similar to those recorded by the farinograph are obtained (Figure 3). The detailed mixing curves appear quite different because of the nature of the mechanical connections between the dough mixer head and

torque recording device and also because of the different nature of mixing action between the two. The mixograph imparts a higher rate of energy input into the dough and is therefore closer to the action of the high-energy input mixers used in mechanical dough development processing in modern bakeries. In general, the mixograph uses much smaller samples: the latest model requires only 2 g of flour, whereas older models require 10 or 35 g of flour. The use of the mixograph is largely limited to North America and Australia. Both the mixograph and farinograph have been used to predict dough processing properties and baking quality, based on the assessment of the mixing curves. The major problem with this is that interpretation of the curves is highly subjective, and is based as much as on the "feel" of the operator as on any objective assessment of the curve. Quantification of a complex mixing trace, for example, obtained from torque-recording mixers is difficult, and has not been tackled to any great degree.

The most widely used mixograph parameter to discriminate dough quality characteristics has been "time to maximum torque" (peak mixing time), mainly because it was easiest to determine before computerized analysis of the mixograph curve. However, many recent publications have shown that peak mixing time is a poor discriminator of baking quality as measured by loaf volume. A major problem is that arbitrary selection of a single parameter does not fully describe the complex mixing curve, and that a selection of several parameters from the mixograph



**Figure 3** Typical mixograph curve showing commonly measured parameters.

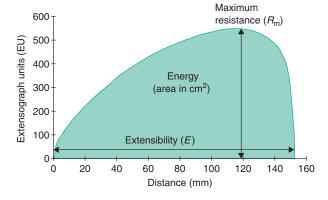
curve using multivariate statistical analysis is much more successful in predicting baking volume.

### **Extensional Instruments**

These instruments are designed to produce a large extensional or stretching deformation in the dough. Doughs often undergo large deformations in practice during processing, which often have a large extensional component. For example, extensional flows are important in mixing and sheeting of pastry and dough, converging and diverging of flow such as in extrusion and pumping, and expansion of bubbles in foams such as bread dough, cakes, and heat-extruded snacks. The Brabender Extensograph, Simon Research Extensometer, and the Chopin Alveograph are instruments used widely to obtain descriptive measurements of the extensional properties of dough.

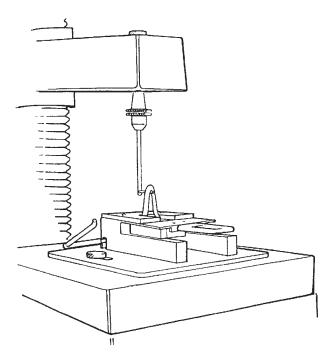
The Brabender Extensograph measures the extensibility of the dough. A flour-salt-water dough is mixed to a fixed consistency in a farinograph, and it is shaped into a cylinder and allowed to relax for various periods of time at  $30^{\circ}$ C. The dough sample is clamped in a cradle and stretched by a hook passing through the center of the sample at constant speed. A curve of force against stretching distance is recorded by a pen on chart paper. The extensibility (*E*) and maximum resistance to extension ( $R_{\rm m}$ ) are derived from the force-distance curve (Figure 4). Good bread-making performance is generally associated with high resistance to extension and good extensibility with a large curve area (energy).

The Simon Extensometer and the Stable Micro Systems Kieffer attachment to the TA-XT2 Texture

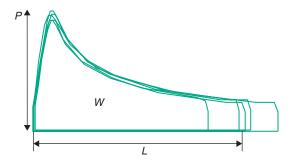


**Figure 4** Typical extensograph curve showing commonly measured parameters. (Reproduced with permission from Brabender OHG, Duisburg, Germany.)

Analyzer are the other instruments which measure the extensibility of dough and gluten. In the extensometer, a ball of dough is impaled on the two halves of a split pin. One of the pins is driven upwards at a constant speed stretching the dough into an extended ring shape. Force and time are the recorded parameters. The Kieffer system is a recently developed instrument for the measurement of extensibility of doughs and glutens (Figure 5). This uses small samples of dough  $(10\,\mathrm{g})$  or gluten  $(1-2\,\mathrm{g})$ , which is of particular value to wheat breeders in determining the processing qualities of new wheat strains at an early stage, when only limited grain quantities may be available. Small strips of material are produced in a grooved clamp arrangement, and then extended centrally by a hook passing through the sample. Force and distance up to failure are recorded on a PC.



**Figure 5** The Kieffer dough and gluten extensibility system. (Reproduced with permission from Stable Micro Systems, Godalming, UK.)



**Figure 6** Alveograph curves for dough bubble inflation showing average inflation parameters. (Reproduced with permission from Tripette & Renaud, Villeneuve-la-Garenne, France.)

The Chopin Alveograph was developed during the 1920s as a test that would simulate the deformation conditions that a dough experiences during baking. A dough is prepared from flour, salt, and water using an integral mixer to a fixed water content (and therefore variable consistency) and then extruded from the mixer following standard procedures. The dough is shaped into flat disks and then given a fixed rest period of 20 min. Each disk is clamped at its circumference and inflated by air passing through a central hole in a base plate, resulting in an expanding bubble of dough. The bubble is inflated until rupture, and the inflation pressure is recorded versus time using a pen on chart paper (Figure 6). Commonly measured



**Figure 7** *D/R* dough inflation system. (Reproduced with permission from Stable Micro Systems, Godalming, UK.)

parameters are: maximum pressure during inflation (P), which corresponds to the bubble attaining hemispherical dimensions, the maximum length or extensibility (L), which corresponds to the final volume of the bubble, and the area under the curve (W), which is proportional to the total energy used to inflate the bubble

This instrument is widely used in France, Southern Europe, and South America to assess the breadmaking potential of wheats. Good bread-making performance is usually indicated by high values of *W* and *P*. A recent development of the bubble inflation technique is the *D/R* dough inflation system by Stable Micro Systems, which measures fundamental rheological properties of the dough during inflation (stress, strain, viscosity, and strain hardening) (Figure 7).

### **Fundamental Rheological Tests**

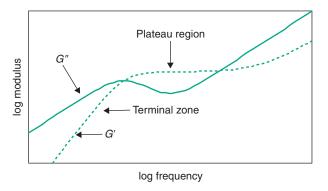
Rheological tests attempt to measure the forces required to produce controlled deformations, such as squashing (compression), bending, or pulling apart (tension), and to present them in such a way as to be independent of sample size, geometry, and mode

of testing. They measure a well-defined property, such as stress, strain, stiffness, or viscosity. A small test piece of the material is usually deformed in a controlled way, normally on a motor-driven machine, and the force is measured along with the distance moved or displacement of the object. These data are used to obtain a force—displacement curve. Normally for stiff materials we would divide the force and displacement by the "original" sample dimensions to obtain stress (force/cross-section area) and strain (displacement/original dimension), because the changes in sample dimensions are small and uniform; this allows us to remove the sample size as a variable. Many food materials are not stiff and undergo large deformations in practice, where the geometry often changes in a nonuniform and unpredictable manner, giving large and nonuniform stresses and strains along the sample. For example, dough thins out nonuniformly when stretched, in common with many polymers, giving rise to large stresses and strains not correctly calculated by the conventional method of dividing by original sample dimensions. It is then necessary to normalize by "actual" change in dimensions during deformation, in which case the sample dimensions should ideally be measured locally and independently by using contact extensometers or noncontact techniques such as laser, video, or photographic techniques. For materials which flow under normal measurement timescales (e.g., low Deborah number), stress is normally divided by strain rate (strain/time of strain application) to give viscosity.

Problems encountered with such fundamental tests are: (1) complex instrumentation which is expensive, time consuming, difficult to maintain in an industrial environment, and require high levels of technical skill, (2) often inappropriate deformation conditions, (3) difficulty in interpretation of results, and (4) slip and edge effects during testing.

The main types of fundamental rheological tests used in dough testing are: (1) dynamic oscillation, (2) creep and stress relaxation, (3) extensional measurements, and (4) flow viscometry (Table 2).

Dynamic oscillation measurements Adapted from techniques developed for measuring viscoelastic properties of polymer melts and concentrated solutions, this is one of the most popular and widely used fundamental rheological technique for measuring cereal doughs. These tests measure rheological properties such as elastic (G') and viscous (G'') moduli by the application of a small-amplitude sinusoidally oscillating (in time (t)) strain or stress and measuring the resulting response. For a viscoelastic material such as dough, the stress will be out of phase with the applied shear strain  $(\gamma_0)$ , which gives a phase angle



**Figure 8** Effect of frequency on modulus for a polymer over a wide range of frequencies.

 $\delta$ . For an elastic material the phase angle is zero: stress and strain are exactly in phase. This kind of test is normally carried out in a parallel plate geometry by rotating the upper plate with an oscillating angular velocity  $\omega$ .

In general the oscillating stress ( $\sigma_t$ ) can be represented as

$$\sigma_t = \gamma_0(G'\sin\omega t + G'\cos\omega t)$$
 [1]

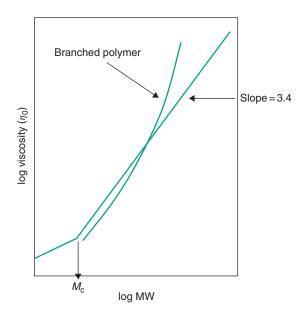
The stress is expressed in terms of an elastic component called storage modulus G' and a viscous component called loss modulus G''.

Normally the values G' and G'' are measured over a range of frequencies. Figure 8 shows the main features of the viscous (G'') and elastic (G') behavior for a polymer over a very wide range of frequencies. The lowest frequency range (called the terminal zone) describes the longest relaxation motions of the polymer molecules, and is therefore related to the relaxation of the largest molecules within the polymer. The viscosity in this region (called zero-shear viscosity) is very sensitive to MW. Beyond a critical molecular weight (M<sub>c</sub>), characteristic for each polymer, zero-shear viscosity  $(\eta_0)$  starts to increase rapidly with increasing MW, following a relationship  $\eta_0 = KMW^{3.4}$  for linear polymer melts, independent of polymer chemistry (where the constant K, depends on temperature and polymer concentration). Above this critical MW, the polymers start to entangle, giving rise to the observed rapid increase in viscosity with MW (Figure 9). If the polymers are branched, viscosity rises even more rapidly.

At intermediate frequencies, polymers show a well-defined plateau region where G' is approximately constant with frequency. For most polymers, the value of the plateau modulus is independent of MW. Figure 10 shows the effect of increasing MW on the dynamic shear modulus for a series of narrow MW linear polystyrene polymer melts. As the MW increases, a plateau in modulus begins to appear,

modulus starts to decrease rapidly into the terminal

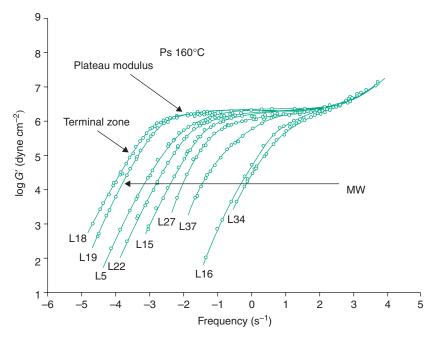
zone, where the polymer chains are free to move



**Figure 9** Effect of MW and branching on zero-shear viscosity for polymer melts.

about and act as a viscous liquid. The larger the polymer, the longer it stays entangled, and therefore the wider the frequency range over which the plateau remains. Thus, it is the width of the plateau, or the point at which it descends into the terminal zone, which is defined by the MW of the polymer. Unfortunately, most rheological measurements on dough and gluten have been performed in the plateau region, which is the region most insensitive to differences in MW. If, as is generally accepted, large MW glutenin polymers are responsible for the variations in breadmaking performance between different wheat varieties, it is to be expected that measurements of the plateau modulus will not be good indicators of baking performance.

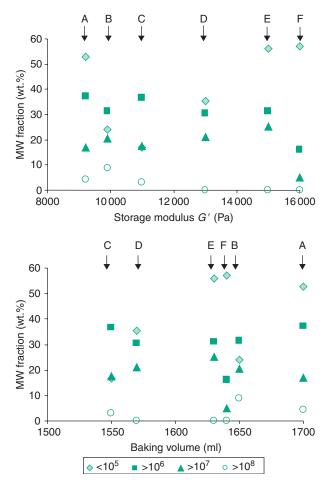
Figure 11 shows the relationship between the wt.% fraction of increasing polymer MW size fractions extracted from gluten up to values >10<sup>8</sup> (measured by field-flow fractionation and light scattering), plateau storage modulus (G' at 1 Hz) of gluten, and the corresponding baking quality from a number of wheat varieties of varying baking performance. These show that neither plateau modulus nor baking volume is related to MW up to a size of about  $5 \times 10^8$ . This confirms the observation that plateau modulus is essentially independent of MW and also that it is not the size of the soluble glutenin polymers, but the insoluble polymer fraction that is mainly responsible for baking performance. This does not support the commonly held view that the MW of the glutenin polymers is related to their small deformation



**Figure 10** Effect of increasing MW on the dynamic modulus of a series of narrow MW linear polystyrene polymer melts. (Reproduced with permission from Onogi *et al.* (1970) *Macromolecules* 3: 109, © American Chemical Society.)

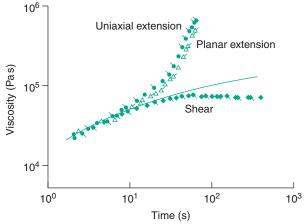
shear rheology and to baking quality. Instead, it suggests that it is more likely to be the secondary molecular structure of the insoluble glutenin that is responsible for baking performance. Recent evidence suggests that these insoluble HMW polymers are entangled with a corresponding long relaxation time, they are branched and form extensive intermolecular secondary structures held together by hydrogen bonding, and differences in these structures are likely to be strongly related to extensional rheology and baking performance.

Such dynamic measurements have the advantage of a well-developed theoretical background, readily available instrumentation, and simultaneous

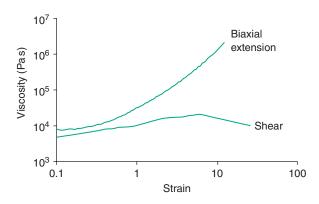


**Figure 11** Relationship between the weight of increasing MW gluten fractions, plateau storage modulus (G) measured at 1 Hz and baking volume for a number of wheat varieties. MW (obtained by light scattering) expressed as wt.% of fraction greater than a certain molecular size, calculated from the total MW distribution for glutens obtained from five UK wheat varieties and one US commercial flour: A = Hereward, B = Pillsbury, C = Riband, D = Soissons, E = Charger, F = Rialto. (Reproduced with permission from Dobraszczyk BJ and Morgenstern MP (2003) Rheology and bread-making process. *Journal of Cereal Science* 38: 229–245, © Elsevier.)

measurement of elastic and viscous moduli, while the nondestructive nature of the test enables multiple measurements to be performed as temperature, strain, or frequency are varied. Disadvantages of the dynamic oscillation method are that the deformation conditions are often inappropriate to practical processing situations, because they are carried out at rates and conditions very different from those experienced by the dough during processing or baking expansion. For example, rates of expansion during proof and oven rise in bread doughs have been calculated between  $5 \times 10^{-3}$  and  $5 \times 10^{-4}$  s<sup>-1</sup>, compared with measuring rates in rheological tests several orders of magnitude greater. Conventional oscillatory shear rheological tests usually operate in the linear region at small strains in the order of up to 1%, whereas strain in gas cell expansion during proof is known to be in the region of several hundred percent. Furthermore, most rheological tests are carried out in shear, whereas most large-strain deformations in dough (i.e., extrusion, sheeting, proof, and baking) are extensional in nature. From extensional studies on long-chain HMW polymer melts, it is known that entirely different rheological properties are obtained in shear than in tension, especially if the polymer chains are branched. For example, the elongational viscosity of low-density (branched-chain) polyethylene melts increases with both strain and strain rate (strain hardening), whereas the shear viscosity decreases with strain and strain rate (shear thinning), giving widely different values in final viscosities between elongation and shear (Figure 12). For doughs, shear and elongational viscosities at low strains are similar, with extensional viscosity slightly higher than shear viscosity, but at higher strains they



**Figure 12** Shear and extensional viscosity of LDPE (low-density polyethylene) at  $125^{\circ}$ C at constant strain rate (0.05 s<sup>-1</sup>). (Adapted from MacLeish TCB and Larson RG (1998) *Journal of Rheology* 42: 81–110.)



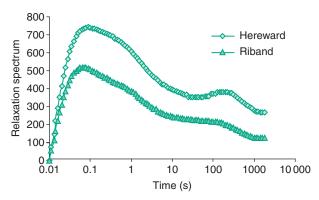
**Figure 13** Shear and biaxial extensional viscosities vs. strain for dough. (Adapted from Dobraszczyk BJ (2003) Bread-making – improving quality. In: *Measuring the Rheological Properties of Dough*, pp. 375–400. Cambridge, UK: Woodhead Publishing.)

diverge and the elongational viscosity rises steeply to give a value 2 orders of magnitude higher at failure (Figure 13). This increased strain hardening is attributed to entanglement of long-chain molecules during extensional flow, whereas in simple shear they remain coiled and can slip past each other, giving rise to observed shear thinning at higher strains. Therefore, it is considered that extensional strain hardening will be more sensitive to changes in the HMW glutenin polymers known to be responsible for baking quality.

Creep and relaxation measurements In stress relaxation measurements, deformation is held constant and the force response is measured, whereas in creep the stress is held constant and the deformation is measured. The stress of a material can be expressed in terms of its relaxation time:

$$\sigma_t = \sigma_0 \exp(-t/\tau)$$
 [2]

where  $\sigma_t$  is the stress at any time,  $\sigma_0$  the stress at unit time, t the time, and  $\tau$  the relaxation time. The relaxation time is related to the slope of a plot of log (stress or modulus) against time (Figure 14). Relaxation times are related to polymer MW and structure, with shorter relaxation times corresponding to small, rapidly relaxing molecules, and longer times corresponding to the relaxation of large polymer chains. Figure 14 shows that dough has two main relaxation processes: one with a rapid relaxation time up to 0.1 s, followed by a much longer time up to 1800 s. Relaxation results for doughs surveyed in the literature show that none of the curves show an exponential decay typical of a single relaxation time, but correspond to a decay typical of a number (spectrum) of relaxation times. This shows that



**Figure 14** Typical relaxation behavior for two doughs (Hereward – typical UK bread-making wheat and Riband – typical UK biscuit wheat) showing two main relaxation processes: (1) a rapid relaxation at shorter times ( $\sim$ 0.1 s), and (2) a slower relaxation at longer times between 100–1000 s.

a broad distribution of relaxation processes is responsible for this process within the dough, which is related to the wide MW distribution of gluten.

Many authors have shown that a slower relaxation time is associated with good baking quality, with relaxation time relatively independent of water content, mixing time, or temperature. Stress relaxation measurements on dough and gluten in shear showed that the relaxation behavior of dough could be described by two relaxation processes: a rapid relaxation process occurring over 0.1–10 s and a slower process occurring over 10-10000 s. Measurements of large-deformation creep and shear stress relaxation properties were found to be useful in discriminating between different wheat varieties of varying quality. These measurements were also found to be closely associated with baking volume and strength of durum wheat varieties. At small strain amplitudes (0.1%) doughs with different baking quality showed no differences in relaxation behavior, but at a range of large strains (up to 29%) their creep and relaxation behavior was closely correlated with the baking behavior of dough. Doughs exhibited a characteristic bimodal distribution of relaxation times (Figure 14), with the second peak clearly discriminating between cultivars with varying strength and quality, which reflects the differences in the MW distribution (MWD) of glutenin polymers. The second relaxation peak is related to the entanglement properties of HMW glutenin polymers, and has been shown to be directly related to the insoluble fraction of the HMW glutenins. Relaxation properties of doughs relate well to the MWD of their gluten and particularly to entanglements of HMW glutenin polymers, and may be used as a rapid method of discriminating variations in MWD between cultivars that vary in baking quality.

Extensional techniques There are many types of extensional flow measurements, including simple uniaxial tension, fiber wind-up or spinning, converging flow, capillary extrusion, opposed jets, lubricated compression, and bubble inflation. Several methods have been used to measure the rheological properties of dough in extension; simple uniaxial extension, where dough is stretched in one direction; and biaxial extension, where the dough is stretched in two opposing directions, which can be achieved either by compression between lubricated surfaces or by bubble.

Uniaxial extension One of the most widely used test methods to measure materials properties is the uniaxial tensile test. A strip of material is clamped at both ends and pulled apart at a fixed rate in a suitable testing machine, and the force measured at the same time as the displacement of the object. These data are used to obtain a force-extension curve. Tensile tests may produce an approximately uniform extension of a sample provided necking does not occur. Normally the force and extension are divided by the original sample dimensions to obtain stress and strain, and allow removal of the sample geometry as a variable, but for doughs undergoing large extensional deformation the actual change in dimensions must be measured or calculated. The slope of the stress-strain curve then gives the elastic modulus or stiffness. Many test methods attempt to measure the uniaxial extensional properties of doughs, such as the Simon Research Extensometer, Brabender Extensograph, Stable Micro Systems Kieffer dough, and gluten extensibility rig, but none of these gives rheological data in fundamental units of stress and strain, because the sample geometry is not defined, dimensions change extensively and nonuniformly during testing, and it is therefore impossible to define any rheological parameters such as stress, strain, strain rate, modulus, or viscosity.

Studies on the fundamental uniaxial extensional rheological properties of doughs have been carried out by many workers. Some of the earliest attempts to characterize the fundamental rheological properties of dough were in a series of uniaxial extensional measurements by Schofield and Scott Blair in the 1930s, who stretched a cylinder of dough floated on a mercury bath and measured the elongation and force. Plastic and elastic components of deformation were resolved and viscosity and elastic modulus were calculated. They showed that the rheological behavior of dough is nonlinear with strain and strain rate, i.e., elastic modulus and viscosity vary with both rate and strain. The large-extension properties of doughs have been measured by extending a ring of dough suspended in a liquid of density equivalent to

that of the dough between two hooks at constant deformation rates until rupture. The stress-strain curves showed considerable strain hardening (nonlinear increase of stiffness with increasing strain), and strain and stress at rupture were considerably lower for poor quality flours than for good quality flours.

Biaxial extension In biaxial extension, a sample is stretched at equal rates in two perpendicular directions in one plane, as in an expanding bubble. The most widely used methods for measuring biaxial extension properties of food materials are inflation techniques and compression between flat plates using lubricated surfaces, which produce purely extensional flow provided no friction occurs.

Inflation was first used as an empirical technique to measure wheat gluten and bread dough extensibility in the 1920s. This method was later developed to calculate rheological parameters, to measure the fracture and biaxial extensional rheological properties of wheat doughs and glutens during bubble inflation, and to assess the baking quality of wheat flour doughs (Figure 7). The major advantage of this test is that the deformation closely resembles practical conditions experienced by the cell walls around the expanding gas cells within the dough during proof and oven rise, i.e., large deformation biaxial extension. Extensional rheological properties can be measured at large strains up to failure and low strain rates, and the gripping problems normally associated with uniaxial tests can be minimized. Extensional rheological properties of wheat doughs have been measured using lubricated compression and bubble inflation. Differences in extensional strain hardening between varieties of different baking quality were found to relate to baking quality, with good bread-making varieties showing greater strain hardening and extensional viscosity.

### **Baking Quality and Rheology**

The link between dough rheology and baking quality is long established, mainly due to empirical evidence from manual assessments such as kneading or stretching of dough by bakers after mixing. However, the results from conventional descriptive methods and fundamental rheological studies on doughs have often given disappointing correlations with baking quality, mainly because the deformation conditions in these tests are very different than those occurring during proof and baking.

Mixing is a critical operation in dough processing where, apart from the obvious function of mixing ingredients, the structure of the dough is formed. For example, in the production of batters, pastes, and doughs, the nature of the mixing action develops

the viscoelastic properties of gluten and also incorporates air, which has a major effect on their rheology and texture. There is an intimate relationship between mixing, aeration, and rheology: the design and operation of the mixer will develop texture, aeration, and rheology to different extents, and conversely the rheology of the food will affect the time and energy input required to achieve optimal development. This is seen in the great variety of mixers used in the food industry and the fact that certain mixers are required to produce a desired texture or rheology in a food. Studies on the rheology of mixing have focused on a number of areas: (1) the effects of mixer design and operation on the development of rheology and texture; (2) empirical measurement of rheology during mixing from mixer torque or power consumption; (3) effect of rheology on mixing patterns and performance; and (4) simulation and prediction of mixing flow deformation patterns as functions of mixer geometry and rheology.

Conventionally, most industrial practice has been to record torque traces obtained during mixing of doughs using instruments such as the Brabender Farinograph or the Mixograph. However, such measurements of motor power, torque, or energy do not give any direct information about the dough rheology. These measurements only give a qualitative description of the mixing deformation of the dough. Due to the complex deformation fields within the mixer and the constantly changing sample geometry, it is not possible to easily determine any rheological parameters. There are also many factors which influence the data being recorded from such mixers, such as motor and drive losses, frictional and surface effects between the dough and mixer, dough geometry effects, varying signal damping and data acquisition rates between different mixers, effects of aeration on rheology, and rheological relaxation effects. Most of the studies on doughs have been on the relationships between mixing, rheology, and baking performance, because of the rheological changes that occur in the gluten viscoelastic network during mixing and their importance for product quality. Despite the obvious importance of mixing in the development of rheology and texture in doughs, there is very little information in the literature on the rheological changes occurring during the different stages in the mixing process. Most work has either concentrated on the empirical measurement of mixer motor torque, voltage, or power consumption during mixing as a qualitative indication of changing rheology, or on the measurement of rheological changes at some time after mixing. Since dough is a viscoelastic material that shows rapid relaxation after deformation, which varies between different flours, such measurements are far from ideal and run the risk of giving misleading information. Nevertheless, much useful information has been obtained about the effect of mixing on gluten structure, rheology, and baking performance. Numerous studies have shown that rheological measurements after mixing parallel changes in mixer torque and power consumption, especially if rheological measurements are made under large, nonlinear deformation conditions closer to those experienced in the mixer. Recent studies have suggested that qualitative elongational rheological information during mixing can be derived directly from the torque/power consumption of a mixograph.

Extensive work on dough mixing has shown that mixing speed and energy (work input) must be above a certain value to develop the gluten network and to produce satisfactory bread making, and an optimum in work input or mixing time (peak development) has been related to optimum bread-making performance, which varies depending on mixer type, flour composition, and ingredients. If a dough is undermixed or mixed well beyond its peak development, then bread of inferior quality is produced. Kilborn and Tipples in a series of papers from 1972–77 investigated factors affecting dough development. Their results indicated that:

- for a given flour, there is a minimum mixing speed and energy input (the critical mixing speed or energy) below which development could not be achieved, resulting in a loaf of poor volume, color, and texture;
- the total energy input required for peak development differs between flour types; and
- both the total energy required and the critical mixing speed for a given flour differ between mixers with different mixing actions.

For example, mixing doughs by elongational flow in sheeting to achieve optimum development required only 10–15% of the energy normally used in conventional high-speed shear mixers, suggesting that much higher rates of work input can be achieved due to the enhanced strain hardening of doughs under elongational flow.

During mixing, there is a competition between the formation of intermolecular disulfide bonds (between adjacent HMW glutenin subunits) and cleavage of these bonds: the former promotes dough development and the latter gives rise to breakdown of the gluten polymer structure and opposes development. Many authors have shown that during mixing, large increases in solubility of the HMW glutenin polymer occur which are paralleled by decreases in MW. If doughs are mixed beyond their peak development, the gluten polymers are broken down into smaller

units, reducing the viscosity and elasticity of the dough. During resting, these smaller units are repolymerized by the reformation of disulfide bonds.

### **Proof and Baking**

Fermentation (proof) is an important step in the bread-making process. During this process, the expansion of air bubbles previously incorporated during mixing provides the characteristic aerated structure of bread, which is central to its appeal. Although fermentation is clearly important in bread making, most rheological tests are performed on doughs without yeast and at room temperature and under inappropriate deformation conditions. Few studies have been made on the changing rheological properties during fermentation and baking. Direct rheological measurements have been made on yeasted bread doughs, cake batters, sour doughs, and cracker sponge and dough. Such measurements suffer from: (1) the problem of the evolving gas volume within the dough and (2) metabolites from fermentation, confounding the rheological data. The decrease in density as a result of increasing gas volume would be expected to have the effect of decreasing modulus and viscosity, but the compressibility of air counteracts this effect, especially at higher gas volumes and low densities where the moduli of the solid and gas phases converge, such as in cake batters, where shear modulus is directly related to the air content. Other approaches have been to measure the increase in height or volume of the fermenting dough using devices such as rheofermentometer or risograph, but these provide no direct information about the rheology of the material, since they do not measure force or deformation for corresponding change in unit dimensions.

Fundamental rheological studies on doughs related to baking have mostly been performed in small-deformation shear oscillations. Such dynamic rheological measurements on doughs have been investigated in many studies. Elastic (G') and viscous (G'') moduli for dough are measured over a range of frequencies. Elastic properties predominate over viscous properties, and the moduli are slightly frequency dependent, which is typical of a cross-linked polymer network. No convincing relationship has ever been established between dynamic rheological properties and baking performance. Various workers have found that flours of different baking quality have lower values of elastic (storage) modulus (G') for the higher baking quality flour. However, others have found that a higher value of G' for glutens and doughs relates to better baking performance (see Table 3). It has been shown earlier that these conflicting results arise, because most of these tests are carried out at rates and deformation

**Table 3** Correlations between rheological properties and baking performance

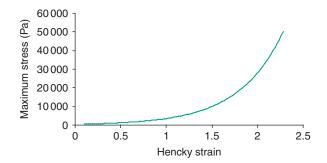
Rheological parameter	Baking parameter	Correlation
Small deformation shear of	oscillation	_
G' 1 Hz (dough) $G'$ slope	Volume	0.15 ( <i>n</i> = 48) 0.72
G' 10 Hz (wet gluten)	Volume Form ratio (W/H)	-0.85 (n = 27) 0.65
G' 1 Hz (gluten)	Volume Form ratio (H/W)	N.S. ( <i>n</i> = 20) 0.69
Tan delta	(H/W)	-0.71
<i>G</i> ′ 1 Hz	Loaf height	-0.64 (n = 8)
Tan delta		N.S.
G'	Volume	N.S. $(n = 4)$
Large deformation		
Biaxial extensional	Volume	0.89 (n = 20)
Strain hardening	Form ratio	0.80
Max. uniaxial Extensional viscosity	Loaf height	0.81
Biaxial strain hardening	Volume	0.92-0.97 ( $n=6$ )
Biaxial strain hardening	Volume	Good
Biaxial strain hardening	Volume	Good
Relaxation	Volume	Good
Creep		0.94 (n = 23)
Relaxation	Quality	Good
Shear relaxation	Quality	Good
Shear viscosity	-	

conditions very different from those experienced by the dough during baking expansion, and also because dynamic rheological parameters in the plateau region are generally insensitive to differences in MW of polymers. However, these parameters are highly sensitive to changes in starch and protein concentration and diluents such as water, which are virtually never kept constant in rheological experiments on doughs. Most dynamic rheological tests on doughs and glutens have been carried out inappropriately over a relatively narrow frequency range in the plateau zone, because the value of the plateau modulus for polymers is known to be insensitive to the changes in MW and structure that are responsible for baking quality.

During proof and baking the growth and stability of gas bubbles within the dough determines the expansion of the dough and therefore the ultimate volume and texture of the baked product. The limit of expansion of these bubbles is related directly to their stability, due to coalescence and the eventual loss of gas when the bubbles fail. The rheological properties of the expanding bubble walls will therefore be important in maintaining stability in the bubble wall and promote gas retention. The relevant rheological conditions around an expanding gas cell during proof and baking are biaxial extension, large strain, and low strain rate. Any rheological tests which seek

Extensional rheology is sensitive to polymer chain branching and entanglement interactions between HMW polymers at large deformations. The theory is simple and relatively well developed, and it generally provides good correlations with the relevant large-deformation processing-quality parameters (Table 3). Disadvantages are that there is no single well-defined and accepted method for extensional measurement, with many different methods being used depending on the type and viscosity of the material being studied; the tests often use large amounts of material and they are destructive. The measurement of extensional flow is often difficult, because the deformation is large and nonuniform; it is therefore impossible to calculate strain directly from the machine displacement, requiring the direct measurement or calculation of changes in sample dimensions, often at high speeds.

The failure of gas cell walls in doughs has been shown to be directly related to the elongational strain-hardening properties of the dough measured under large deformation biaxial extension. Strain hardening is shown as an increase in the slope of the stress—Hencky strain curve with increasing extension, giving rise to the typical J-shaped stress-strain curve observed for highly extensible materials (Figure 15). Strain hardening in doughs is expected to arise mainly from stretching of polymer chains between points of entanglement in the larger glutenin molecules, which gives rise to the increasing stiffness observed at large strains. Under extensional flow, entangled polymers exhibit strain hardening which is enhanced for polymers with a broad MW distribution, particularly a bimodal distribution and branching. It is therefore expected that the broad bimodal MW distribution and branched structure typical of gluten will result in enhanced strain hardening and a bimodal distribution of relaxation times. Recent work has shown that bread doughs exhibit strain hardening under large extensional deformations, and that these extensional rheological properties are important in baking performance. Strain hardening allows the expanding gas cell walls to resist failure



**Figure 15** Typical J-shaped stress—Hencky strain curve in biaxial extension for a dough bubble. Bubble inflation using SMS dough inflation system, maximum stress, and Hencky strain calculated for bubble wall polar region.

by locally increasing resistance to extension as the bubble walls become thinner, and provides the bubble walls greater stability against early coalescence and better gas retention. It is therefore expected that doughs with good strain hardening characteristics should result in a finer crumb texture (e.g., smaller gas cells, thinner cell walls, and an even distribution of bubble sizes) and larger baked volume than doughs with poor strain hardening properties. It has been shown that good bread-making doughs have good strain-hardening properties and inflate to larger single bubble volume before rupture, whereas poor breadmaking doughs inflate to lower volumes and have much lower strain hardening. Loaf volume for a number of commercial white flour doughs has been related directly to the failure strain and strainhardening properties of single dough bubbles measured at elevated temperatures in biaxial extension. Strain hardening and failure strain of cell walls were both seen to decrease with temperature, with cell walls in good bread-making doughs remaining stable and retaining their strain hardening properties to higher temperatures (60°C), whilst the cell walls of poor bread-making doughs became unstable at lower temperatures (45-50°C) and had lower strain hardening. Figure 16 shows that bubble wall stability (indicated by a strain hardening value of 1) is increased to progressively higher temperatures with increasing baking volume, allowing the bubbles to resist coalescence and retain gas for much longer. Bubble wall instability in poorer bread-making varieties occurs at much lower temperatures, giving earlier bubble coalescence and release of gas, resulting in lower loaf volumes and poorer texture.

### **Summary**

The use of rheological techniques for analyzing dough rheology has changed rapidly over the last few years.

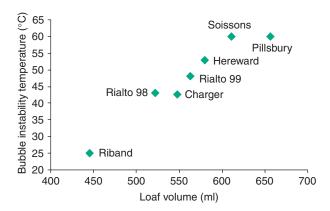


Figure 16 Temperature at which bubble wall instability occurs (as indicated by the temperature at which strain hardening drops below 1) for a number of wheat varieties vs. loaf volume.

The main aim of rheological testing of dough has been to assess gluten quality in relation to the main processes in bread making (mixing, sheeting, baking) and the final product quality. This is based on the long established practice of bakers assessing the dough quality after mixing by kneading or stretching the dough by hand and relating this to baking performance. Conventionally, such rheological tests have relied on descriptive empirical measurements of the deformation behavior of the dough during mixing, compression, or extension. However, severe limitations of these tests have been recognized because most of these tests are carried out under deformation conditions that are very different from those in the bread-making process.

New rheological tests have been developed which are based on modern polymer rheology principles, which relate the molecular size and structure of the gluten polymers to their rheology and end-use performance. Techniques such as large deformation stress relaxation, creep, and extensional strain hardening are sensitive to changes in interactions between polymers via entanglements, chain branches, and crosslinking which are seen to be the key mechanisms determining the rheology of the HMW gluten polymer network known to be responsible for baking quality.

### **Future Trends**

- Small-scale rapid rheological tests will allow detection of desirable properties at earlier stages of selection of good quality traits in wheat breeding
- Rapid advances in polymer molecular modeling, which attempts to relate changes in polymer

- structure to rheological properties, together with structural information from spectroscopic data, will soon enable prediction of the structure of the gluten polymer network.
- Development of scanning probe microscopy techniques (AFM, STM, etc.) for wet biological systems will allow direct imaging of the behavior of these polymers at a molecular level under deformation conditions similar to those of baking.

See also: Breads. Cakes, Pastries, Muffins, and Bagels. Cereals: Overview; Grain-Quality Attributes; Protein Chemistry. Extrusion Technologies. Gluten and Modified Gluten. Wheat: Breeding.

### **Further Reading**

Barnes HA, Hutton JF, and Walters K (1989) An Introduction to Rheology. UK: Elsevier.

Bloksma AH and Bushuk W (1988) Rheology and chemistry of dough. In: Pomeranz Y (ed.) Wheat Chemistry and Technology II. St. Paul, MN: AACC.

Cauvain SP (2003) Bread-Making – Improving Quality. Cambridge, UK: Woodhead Publishing.

Cauvain SP and Young LS (1998) Technology of Breadmaking. London: Blackie Academic and Professional.

Dendy DAV and Dobraszczyk BJ (2001) Cereals and Cereal Products: Technology and Chemistry. Gaithersburg: Aspen Publishers.

Dobraszczyk BJ (1999) Measurement of biaxial extensional rheological properties using bubble inflation and the stability of bubble expansion in bread doughs. In: Campbell GM, Webb C, Pandiella SS, and Niranjan K (eds.) Bubbles in Food, pp. 173-182. St. Paul, MN: American Association of Cereal Chemists.

Dobraszczyk BJ (2003) Bread Making – improving quality. In: Measuring the Rheological Properties of Dough, pp. 375-400. Cambridge, UK: Woodhead Publishing.

Dobraszczyk BJ and Morgenstern MP (2003) Rheology and the bread-making process. Journal of Cereal Science 38: 229-245.

Dobraszczyk BJ and Vincent JFV (1999) Measurement of mechanical properties of food materials in relation to texture: the materials approach. In: Rosenthal AJ (ed.) Food Texture: Measurement and Perception. Maryland: Aspen Publishers.

Faridi H and Faubion JM (1986) Fundamentals of Dough Rheology. St. Paul, MN: American Association of Cereal Chemists.

Faridi H and Faubion JM (1990) Dough Rheology and Baked Product Texture. New York: Van Nostrand.

Graessley WW (1974) The entanglement concept in polymer rheology. Advances in Polymer Science 16:

MacLeish TCB and Larson RG (1998) Journal of Rheology 42: 81-110.

Muller HG (1975) Rheology and the conventional bread and biscuit making process. *Cereal Chemistry* 52: 89–105.

Onogi et al. (1970) Macromolecules 3: 109.

Sherman P (1970) Industrial Rheology, with Particular Reference to Foods, Pharmaceuticals, and Cosmetics. London: Academic Press.

Shuey WC (1975) Practical instruments for rheological measurements on wheat products. *Cereal Chemistry* 52: 42–81.

### **Relevant Websites**

http://www.aaccnet.org - American Association of Cereal Chemists.

http://www.campden.co.uk - Campden and Chorleywood Food Research Association, UK.

http://www.icc.or.at — International Association for Cereal Science and Technology.

http://www.sciencedirect.com – Journal of Cereal Science.

# Grain Proteins and Flour Quality

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### Introduction

Wheat is unique among the edible grains because only wheat flour has the protein complex called "gluten" that can be formed into a dough with the rheological properties required for the production of leavened bread. Gluten protein is the basis of man's attraction to wheat; it is the reason for the annual cultivation of enormous numbers ( $\sim 10^{14}$ ) of wheat plants, because wheat gluten alone can sustain human desire for leavened-bread products. The rheological properties of gluten are needed not only for bread production, but also in the wider range of foods that can only be made from wheat, viz., noodles, pasta, pocket breads, pastries, cookies, and other products (Table 1). The unique properties of the gluten-producing storage proteins are formed in the white floury endosperm during grain development. Because of these unique dough-forming properties, wheat is the most important source of protein in the human diet.

Of the many species of the wheat genus (*Triticum*) (see Taxonomic Classification of Grain Species), there

Table 1 Quality attributes preferred in wheats for specific products

Product	Protein content	Grain hardness	Dough strength
Pan/sandwich breads	>13%	Hard	Strong
Flat/Arabic breads	11-13%	Hard	Medium
Steamed-Nthn China	11–13%	Hard	Medium/ strong
Steamed-Sthn China	10-12%	Soft/Medium	Medium
Alkaline noodles	11-13%	Hard	Medium
White/Udon noodles	10-12%	Medium/Soft	Medium
Instant noodles	11-12%	Medium	Medium
Biscuits/cookies/ grocery	8-10%	Very soft	Weak
Starch/gluten manufacture	>13%	Hard (soft preferred)	Strong

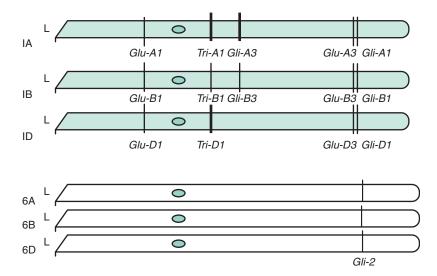
Adapted from Wrigley (1994) Developing better strategies to improve grain quality for wheat. Australian Journal of Agricultural Research 45: 1-17.

are only two major species that are grown around the world. The more popular of these two is *T. aestivum*, called "bread wheat." The other is durum wheat (*T. durum*), which is particularly suited to the production of pasta foods (*see Pasta*). Both bread and durum wheats are polyploid species containing three (AABBDD) and two (AABB) related genomes, respectively (*see Taxonomic Classification of Grain Species* and Wheat: Genetics). Many of the genes for protein synthesis are located on the group 1 and 6 chromosomes of these genomes, as illustrated in Figure 1 and Table 2.

### **Historical Perspective**

Although the term "protein," meaning primary substance, has been in use since about 1838, the terms "gluten," "gliadin," and "albumin" have even earlier origins. Gluten was one of the first few proteins to be studied, because it can be prepared so readily (by the water washing of dough) as a reasonably pure protein. This gluten-washing procedure has since become very big business internationally. This process has even been used as a rapid analytical method for determining the protein content of flour or wheatmeal samples (see Cereals: Protein Chemistry).

The importance of the proteins of flour for bread making has long been known. It is illustrated in a classic graph (Figure 2), published in 1948, relating variations in bread loaf volume to the protein content of different flour samples. The graph shows that baking quality increases with protein content for the same variety (thus illustrating the improving effect of protein quantity) and that for better varieties, increases in protein content produce greater increases (steeper slopes) in baking quality, showing the effect of protein



**Figure 1** Locations of the genes for the synthesis of wheat-flour proteins. The long arm of each chromosome (indicated "L") extends to the left, containing only the *Glu-1* loci to the left of the centromere (indicated by a circle). See Table 2 for identities of most genes. Gene locations for the triticin proteins are indicated by the *Tri-1* loci. (Adapted from Gianibelli MC, Larroque OR, MacRitchie F, and Wrigley CW (2001) Biochemical, genetic and molecular characterization of wheat glutenin and its component subunits. *Cereal Chemistry* 78: 635–646.)

Table 2 Gluten-protein classification, based on chromosomal locations of the genes and on disulfide-bond formation

Class	Gene locus	Chromosomal location of genes	Form of disulfide bonding
ω-gliadins $α$ -, $β$ -Gliadins $γ$ -Gliadins LMW subunits of glutenin HMW subunits of glutenin	Gli-1	Short arms of group 1 chromosomes	No SS bonds
	Gli-2	Short arms of group 6 chromosomes	Intrachain SS bonds
	Gli-1	Short arms of group 1 chromosomes	Intrachain SS bonds
	Glu-3	Short arms of group 1 chromosomes	Interchain and intrachain SS bonds
	Glu-1	Long arms of group 1 chromosomes	Interchain and intrachain SS bonds

quality (meaning "better loaf volume at the same protein content").

Because of the importance of gluten, much of the variation in quality between wheat samples can be explained in terms of the gluten component — its quantity and quality. Total protein content (Table 1) is generally taken as an indication of gluten quantity, although about one-fifth of the grain protein is nongluten, including the range of enzymes and structural proteins.

# **Prepare Your Own Gluten**

The historical importance of gluten in protein chemistry is due to the ease with which it can be prepared. It is a simple experiment to be performed at the kitchen sink. Mix some wheat flour with water, adding the water a little at a time, until a stiff dough is formed. Take the dough in the fingers and knead it under a gentle stream of water from the tap, placing a glass under the tap to catch the wash water. After the starch has washed out, to form a white sediment

at the bottom of the glass, a ball of gluten is left between the fingers. It may feel like a piece of chewing gum, with stronger elastic properties than the original dough, due to the removal of the starch.

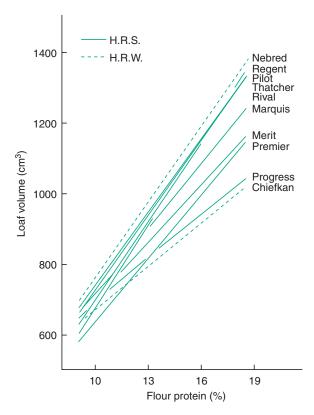
Wheat flour generally has  $\sim 9-13\%$  protein, while the remainder is mainly starch, plus 1-2% lipid and some nonstarch polysaccharides. Flour, as milled, also contains  $\sim 14\%$  moisture. About 20% of the protein in flour is water-soluble albumins and globulins; about 80% is gluten-forming proteins of which half is gliadin-type proteins and half is glutenin (Figure 3).

### Why Is Protein Important?

The protein content and type is critical to the diversity of wheat-based foods listed in **Table 1**. For each of these groups of products, there is a recommended combination of protein content, grain hardness (largely determined by protein composition), and dough properties (a function of the gluten—protein composition). Other factors come into play, particularly good milling quality and suitable starch

properties (see Starch: Chemistry and Wheat: Dry Milling).

Of greatest importance is the gluten-protein component of the grain. On the one hand, it is important to the ongoing life of the wheat plant, because the



**Figure 2** Demonstration of the distinction between protein quantity and protein quality. (Reproduced with permission from Finney KF and Barmore MA (1948) Loaf volume and protein content of hard winter and spring wheats. *Cereal Chemistry* 25: 291–312.)

gluten-forming proteins of the grain play their critical role in nature as a reserve of amino acids and nitrogen for the embryo upon germination. On the other hand, the gluten—protein component of the grain is imperative for the wheat industry because of its importance in determining processing quality.

Consideration of Table 1 indicates why wheat is segregated according to protein content after harvest and in international trade. More marketable parcels of grain can be obtained, appropriate to the processing needs of specific customers, if they suit relevant combinations of the attributes listed in Table 1. The other major factor used in segregation of wheat is variety; this factor takes into account genetic aspects of protein quality, especially grain hardness and dough properties. The genetic constitution of wheat is important because all quality traits result from the expression of genes and their interaction with the environment.

# Why Are Growth Conditions Important?

Because protein content and composition are modified by growth conditions, processing quality may not be entirely predictable, even if we know the variety and thus its genetic potential for processing into a specific type of product. The task of selecting wheat for optimum market value and processing quality is thus difficult. Nevertheless, we are learning much about what are significant aspects of protein composition and how these might be modified by growth conditions.

Growth environment is thus the "wild card" of uncertainty in using the combination of variety and protein content to select suitable wheat consignments.

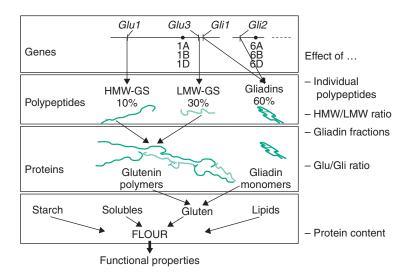


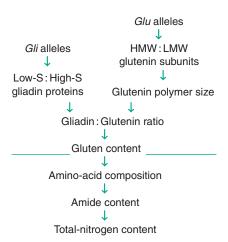
Figure 3 Relationships between the individual components of flour, especially gliadin and glutenin, the gluten-forming protein.

On occasions, for example, weaker dough properties may be obtained that are not those that would be expected from a particular protein-variety combination, leaving researchers with the job of working out the reason why.

Recent research has implicated interference from high temperatures (over 35°C) during the ripening of the grain. Evidence has been provided that these hot conditions may cause a redirection of protein synthesis, thereby changing the protein quality and thus the dough properties. Sulfur deficiency may be another environmental reason for variations in protein quality, particularly when nitrogen fertilizer is applied without sulfur. In fact, changes in dough properties have been observed for low-sulfur grain, accompanied by a decreased presence of the sulfur-rich proteins of gluten (Figure 4). Such occasional phenomena show that our tried and tested systems of selecting for grain quality still have some deficiencies to be rectified, possibly by screening harvest samples for protein quality, as well as for protein content.

# **Wheat Protein as Total Nitrogen** Content

"Wheat protein" to many in the trade (the agronomist, the grain-elevator operator, the grain salesman, the miller) means no more than a single number, the result of analysis of total nitrogen content (see Cereals: Protein Chemistry). The digestion step in nitrogen analysis (lower half of Figure 4) generally involves: (1) the use of an oxidizing acid to transform all nitrogen-containing compounds (mainly protein) to ammonia for subsequent quantitation by titration in the Kjeldahl method, or (2) pyrolysis of



**Figure 4** The degrees of formation (down to "gluten content") and disruption of gluten, illustrating the levels at which studies may be conducted on gluten-protein structure and function.

nitrogen-containing compounds to nitrogen gas in the Dumas method.

Currently, near infrared spectroscopy is routinely used to determine grain protein content, especially as a basis for segregation of wheat at the silo or elevator. This can be applied either to milled grain, as near infrared reflectance (NIR), or to whole grain, as near infrared transmission (NIT). This "instant" method involves scanning the sample in the infrared part of the spectrum, determining absorbance at certain wavelengths and computing protein content by comparison with a pretested set of calibration samples. The method is therefore comparative and it still relies on the basic reference methods for determining total nitrogen content.

It is feasible to use nitrogen content as a quantitative indicator of protein content since other nitrogencontaining compounds represent very minor components of the wheat grain. The factor 5.7 is generally used to convert the result of nitrogen analysis to protein content; this factor is much lower than the normal factor of 6.25 for other grains, reflecting the elevated level in gluten of nitrogen-rich amino acids, such as glutamine.

# **Determination of Protein Quality** versus Quantity

The concept of characterizing protein by the protein content alone indicates loss of important information about protein composition. All such information is lost in direct (Kjeldahl, Dumas) analysis of total nitrogen content or in correlative methods (NIR, NIT) based on them. If amino-acid composition is known in addition to protein content, valuable additional information is provided about essential amino-acid levels, important in nutrition (Figure 4). However, even the analysis of amino-acid composition destroys information about the amino-acid sequences of proteins and these are essential to an ultimate understanding of protein quality.

Likewise, there is significant loss of information about protein-protein interactions as a result of extracting protein into dilute solution from flour or dough, especially if in doing so, we have broken disulfide bonds. Most methods of analyzing protein structure and composition involve extraction, but we need to remember that the resulting picture may still be different from what actually occurs in the water-poor medium of dough, where interactions with other groups of compounds (lipids and carbohydrates) are likely to assume greater importance.

In addition to information about protein content, the feed formulator needs to know about amino-acid

composition, particularly the content of essential amino acids. The general deficiency in the amino-acid lysine limits the nutritional value of wheat in feed for animals and in food for humans if protein is restricted in the diet. Knowledge about the content and sequence of amino acids in the many proteins of the wheat grain is fundamental to our understanding of their functional properties. This information has been accumulating over many years, but recent advances in molecular biology are providing this information at an increasing rate as nucleotide sequences are being provided, from which amino-acid sequences can be derived for the corresponding proteins.

# The Diversity of Structure and Function of Wheat Proteins

Modern methods of protein fractionation have indicated that the protein of the wheat grain is made up of thousands of distinct protein species — these are needed to catalyze and regulate the synthetic processes of making a wheat grain, the host of enzymes needed in the germ, the scutellum and the endosperm to carry the process of life on into a new plant, and the reserve proteins stored away in the embryo and the endosperm to provide the building blocks for this ongoing process. This storage protein, gluten, is the plant's contribution to making another plant, even though for man, it is essential for bread making.

The structural requirements of a protein to meet these natural and industrial functions are similar in a key area, namely, solubility. Both functions require a protein of low aqueous solubility, first, so that these nutrients are not washed away from the imbibing seed, and second, for bread making so that the protein network of dough is not highly soluble. The nutritional need of the embryo is met by having storage proteins of low solubility, due to the high level of glutamine residues, together with the consequent efficient storage of nitrogen (two N atoms in the aminoacid glutamine).

The hydrolytic enzymes (particularly those with amylase and protease activities) that appear on germination are essential contributors to the ongoing plant-to-plant process, but they interfere with man's plans for bread making. Wheat breeders have sought means of reducing or delaying their production prior to the induction of the germination process, but there is the dilemma in this task of meeting the needs of both farmer and baker. Wheat grains that produce amylases and proteases before germination are described as pregerminated.

## **Diversity, Even for Storage of Proteins**

Given the apparently simple requirements of a storage protein, it may thus have seemed reasonable at the dawn of cereal chemistry for cereal chemists to think of gluten as one protein, or even of gliadin and glutenin as single, homogeneous entities. We now know that even these storage proteins are many different proteins (polypeptides), each synthesized under the control of the appropriate gene (*see Proteomics*).

As the first products of gene expression, proteins are synthesized more faithfully, with less influence from environmental factors, than other groups of compounds. The DNA of genes in the nucleus is transcribed to produce messenger RNA, which in turn is the basis for translation to produce the newly synthesized polypeptide in the ribosome, with transfer RNA presenting the appropriate amino acids in the prescribed sequence as the polymer chain grows. The high degree of fidelity to genotype suits proteins well to the task of variety identification, the most common laboratory method for which, is the analysis of protein composition by gel electrophoresis or high-performance liquid chromatography (see Variety Identification of Cereal Grains).

#### **The Gliadin Proteins**

The gliadin proteins are used routinely for the identification of wheat varieties. Figure 5 shows the results of cathodic gel electrophoresis of gliadins proteins, which are readily extracted from flour with 6% urea solution or even with ethylene glycol (antifreeze). The water-soluble albumins and globulins are also extracted with the gliadins, but they migrate ahead of the gliadins under the electrophoresis conditions used, and they have been lost from the lower end of the gel.

The genes controlling the synthesis of the gliadin proteins are located on the short arms of chromosomes 1A, 1B, 1D, 6A, 6B, and 6D (Figures 1 and 3, and Table 2). At each of these six genetic loci, there is a group of genes responsible for a part of the spectrum of gliadin proteins. It is possible to separate out the gliadins associated with the each locus. This is demonstrated in Figure 6, with the respective "blocks" of gliadins drawn in separate columns beside the photograph of the original electrophoresis gel. The whole picture of all the gliadins in this case is obtained by superimposing all the drawn blocks of bands on top of one another.

The gliadin proteins contribute to the viscosity and extensibility of dough, due to their modest molecular size range (under 100 000 Da). The omega-gliadins are the largest in this molecular size range; they lack sulfur-containing amino acids (particularly

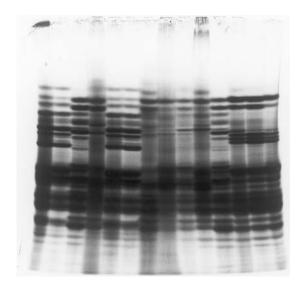


Figure 5 Gliadin proteins, fractionated by gel electrophoresis at pH 3 by the method used for routine variety identification in a precast polyacrylamide gel. Gliadin proteins, extracted into solution from flour samples, have been applied at the top of each vertical lane, and the respective protein zones have moved down the gel, under the electric field, until the current was stopped. The protein zones (horizontal bars) have been revealed in the gel with a stain. The eleven lanes on the left were all obtained from different samples of the Canadian variety Marquis, the remaining three lanes (at right) are samples of the variety Chinese Spring. The diversity of gliadin patterns for Marquis is unusual, presumably reflecting the age of this traditional variety, with such polymorphism arising from original heterogeneity, plus admixture and mis-labeling over many

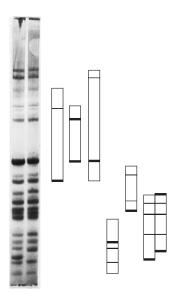


Figure 6 Gliadin proteins, fractionated by gel electrophoresis at pH 3 by a research method (photo of two lanes at left) from two biotypes of the Australian variety Suneca. The drawn block patterns to the right of the photo show the groups of gliadin proteins that are controlled by sets of genes on chromosomes (left to right) 1A, 1B, 1D, 6A, 6B, and 6D. The pair of blocks at the extreme right shows that the difference between the two biotypes arises with genes on chromosome 6D. (Illustration provided by Dr. E. Metakovsky.)

cysteine), so that there is no possibility of covalent bonding between the omega-gliadins via disulfide links (Figures 3 and 4, and Table 2). Although the other groups of gliadins (the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins) have sulfhydryl groups, the disulfide cross-links are mainly intra-chain, with no significant disulfide bonding between the gliadin polypeptides, so that they are monomeric (single polypeptide chains (Figure 3)).

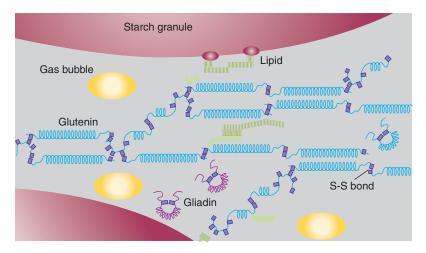
# The Glutenin Proteins – Polymers of **Polypeptides**

The lack of inter-chain disulfide cross-links for the gliadins distinguishes them from the glutenin polypeptides, which are extensively cross-linked to form polymers of glutenin subunits with molecular weights well over  $\sim 10^5 - 10^7 \,\mathrm{Da}$  (Figure 3). Native glutenin, with disulfide bonds intact, contributes the resistance to the extension of dough, due to the sizes of these long polymers. They are depicted in Figure 7 as strings of glutenin polypeptides (appearing as coils) joined by disulfide bonds.

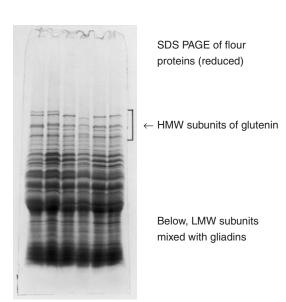
These large glutenin molecules are balanced by the smaller gliadin proteins in providing the appropriate molecular combination for dough quality and good baking properties. Thus, the ratio of glutenin to gliadin is a major determinant of protein quality. In addition, the size distribution of glutenin polymers is significant. Excessive dough strength and a long mixing time are associated with a high proportion of very large glutenin polymers. On the other hand, a lower size distribution provides a weaker dough, which is preferable for biscuit/cookie manufacture (Table 1).

The size distribution of the glutenin polymers appears to be partly determined by the composition of the glutenin subunits that form the polymeric structure. These subunit polypeptides are characterized by extracting them from flour in the presence of a reagent that breaks disulfide bonds, plus the detergent sodium dodecyl sulfate (SDS), followed by SDS gel electrophoresis (Figure 8). The high-molecular-weight (HMW) subunits appear at the top of the electrophoretic pattern (bracketed in Figure 8), whereas the low-molecular-weight (LMW) subunits appear further down the gel pattern, mixed with gliadin polypeptides.

The genes for the synthesis of the HMW subunits are located at the Glu-1 loci on the long arms of the group 1 chromosomes (Figures 1 and 3, and Tables 2 and 3). These HMW subunits have been numbered 1, 2, 2, 3, etc., down the SDS gel pattern. They often appear in pairs (e.g., 2 with 12, 5 with 10, 17 with 18) because their synthesis is controlled together in these pairs by



**Figure 7** Diagrammatic representation of the molecules involved in dough formation (not to scale). The surfaces of two starch granules appear with some lipid coating. Gliadin molecules appear as single-chain molecules, contrasting with the polymers of glutenin, made up of subunits joined by disulfide bonds, either long or short coils (HMW or LMW subunits, respectively). Also present are gas bubbles and lipids between the starch granules. (Reproduced with permission from Wrigley CW (1996) Giant proteins with flour power. *Nature* 381: 738–739.)



**Figure 8** Polypeptides of flour, extracted from six varieties of wheat in the presence of the detergent sodium dodecyl sulfate (SDS) after breaking disulfide bonds, followed by SDS gel electrophoresis. The HMW subunits of glutenin are bracketed at the top of the pattern. (Adapted from Wrigley CW, Autran JC, and Bushuk W (1982) Identification of cereal varieties by gel electrophoresis of the grain proteins. *Advances in Cereal Science and Technology* 5: 211–259.)

genes at the *Glu-1* loci. For example, the *Glu-D1* locus may have either the *Glu-D1d* allele, corresponding to the subunit 5 + 10 combination, or the *Glu-D1a* allele, corresponding to subunits 2 + 12 (Table 3).

**Table 3** The *Glu-1* scoring system to predict dough strength, based on the composition of HMW subunits of glutenin in a flour or grain sample

Score	GI	u-A1	GI	u-B1	GI	u-D1
	Allele	Subunit	Allele	Subunit	Allele	Subunit
4					d	5+10
3	а	1	i	17 + 18		
3	b	2*	b	7 + 8		
3			f	13 + 16		
2			С	7 + 9	а	2 + 12
2					b	3 + 12
1	С	Null	а	7	С	4 + 12
1			d	6 + 8		
1			e	20		

A score of 1, 2, 3, or 4 is allocated to each set of subunits for each locus (*Glu-A1*, *Glu-B1*, or *Glu-D1*), and these scores are summed to give a total score out of ten. A high score indicates a prediction of strong dough properties. This system is being used to help wheat breeders to select parents and progeny of suitable dough quality.

The HMW subunits of glutenin are more effective than the LMW subunits in contributing to the functional properties of glutenin, even though the LMW subunits are present at  $\sim$ 3 times the level of the HMW subunits. Even within each of these subclasses of subunits, some are more effective than others. In particular, within the HMW subunits, polypeptides such as those numbered 5 and 10 (the *d* allele, coded by the *Glu-D1* locus) are more effective than the allelic subunits 2 and 12 (the *a* allele; Table 3).

It has thus been possible to produce a *Glu-D1* scoring system (Table 3) by which to predict the dough

properties of a specific flour or grain sample, depending on the composition of the HMW subunits. This scoring system has proved valuable for breeders, who can use the system to predict quality by analyzing the protein composition of individual seeds, thus avoiding the much greater task of producing large grain quantities for milling and dough testing. Nevertheless, the prediction based on glutenin-subunit composition at best indicates genetic potential, not taking into account the effects of growth conditions on dough quality. The glutenin score is often used in the early stages of a breeding program, when there are thousands of lines to be screened, but small-scale milling and dough testing are used to determine protein quality in the later stages when the range of promising lines has been greatly reduced.

## **Future Prospects**

Considerable research attention has been focused on the study of wheat-gluten proteins in recent decades, resulting in large numbers of research papers, review articles, and books on the general subject. A major impediment to research progress has been the difficulty in extracting the very large glutenin polymers into solution to permit more detailed study by current biochemical methods. In fact, the act of extraction into solution risks damaging the information that is being sought. Possibly, the future will provide novel methodology for the study of these unique proteins.

The application of the proteome approach (see Proteomics) offers a fresh new look to the problems of gluten functionality at the polypeptide level, opening the opportunity of identifying proteins that might not have been previously taken into account as wheatquality modifiers during protein synthesis and processing in the immature endosperm. This approach offers better opportunities than before for the integration of DNA with protein studies, and thus for renewed approaches to the genetic improvement of wheat quality, including a better understanding of the complex interactions of gene expression with growth conditions.

See also: Cereals: Protein Chemistry. Gluten and Modified Gluten. Nitrogen Metabolism. Protein Synthesis and Deposition. Proteomics. Variety Identification of Cereal Grains.

## **Further Reading**

Fabriana G and Lintas C (eds.) (1988) Durum Wheat: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.

Finney KF and Barmore MA (1948) Loaf volume and protein content of hard winter and spring wheats. Cereal Chemistry 25: 291-312.

Gianibelli MC, Larroque OR, MacRitchie F, and Wrigley CW (2001) Biochemical, genetic and molecular characterization of wheat glutenin and its component subunits. Cereal Chemistry 78: 635-646.

Henry RJ and Kettlewell PS (eds.) (1996) Cereal Grain Quality. London: Chapman and Hall.

Ng PKW and Wrigley CW (eds.) (2002) Wheat Quality Elucidation: The Bushuk Legacy. St. Paul, MN: American Association of Cereal Chemists.

Pomeranz Y (ed.) (1988) Wheat: Chemistry and Technology, 3rd edn. St. Paul, MN: American Association of Cereal Chemists.

Shewry PR and Casey R (eds.) (1999) Seed Proteins. The Netherlands: Kluwer Academic.

Shewry PR and Lookhart GL (2003) Wheat Gluten Protein Analysis. St. Paul, MN: American Association of Cereal Chemists.

Shewry PR and Miflin BO (1985) Seed storage proteins. In: Pomeranz Y (ed.) Advances in Cereal Science and Technology, vol. 7, pp. 1–83. St. Paul, MN: American Association of Cereal Chemists.

Sikorski ZE (ed.) The Chemical and Functional Properties of Food Proteins. Lancaster, PA: Technomic Publishing.

Wrigley (1994) Developing better strategies to improve grain quality for wheat. Australian Journal of Agricultural Research 45: 1-17.

Wrigley CW (1996) Giant proteins with flour power. Nature 381: 738-739.

#### **Relevant Websites**

www.wheat.pw.usda.gov - Graingenes.

www.icc.or.at - International Association for Cereal Science and Technology.

www.crop.cri.nz - New Zealand Institute of Crop and Food Research.

www.usda.gov - United States Department of Agriculture.

www.aaccnet.org - American Association of Cereal

www.campden.co.uk - Campden and Chorleywood Food Research Association.

www.cgc.ca - Canadian Grain Commission, Winnipeg.

Whole-Plant Utilization see Plants: Whole-Plant Utilization.

# WHOLE-GRAIN VERSUS REFINED PRODUCTS

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#### Introduction

The nutritional guidelines developed by many countries recommend that the greatest intake of food types should be from the range of plant sources, especially grains. Within that broad category, the consumption of whole-grain foods is strongly recommended. These guidelines have been depicted in various pictorial forms, especially as a pyramid with the main ingredients of the diet shown as the base, with progressively less of other food groups shown as the smaller segments towards the apex of the pyramid (see Nutrition: Guidelines for Grain-Based Foods).

A recent re-evaluation of the nutrition pyramid of the USA recommends significant changes, namely, that the primary accent at the base should be for whole-grain foods, and that the consumption of refined-grain products should be moved towards the top of the pyramid. This accentuates the apparent dietary benefits of whole-grain products (e.g., wholegrain bread, brown rice) in preference to foods made from refined-grain products (e.g., white bread, white pasta, polished rice) (see Nutrition: Mineral Composition; Vitamin Composition).

Recently, such recommendations have been backed up by an explicit health claim authorized by the Food and Drug Administration (FDA) of the USA, namely "Diets rich in whole grains and other plant foods but low in total fat, saturated fat, and cholesterol, may reduce the risk of heart disease and cancers." Despite these clear indications of the health benefits of wholegrain foods, there has continued to be a degree of confusion in the public generally, as well as some controversy about the extent of the advantages of whole-grain foods. In addition, although there has been a modest swing from refined grain-based

foods to those made from whole-grain ingredients, this has not been overwhelming in many countries. It is thus valuable to consider the range of factors that have been the basis of the whole-grain recommendations, to consider the extent that whole-grain advantages are still provided in other types of grain-based foods, and to examine these factors as they relate to the diversity of grain species (Table 1).

At its extremes, public comment has extended to the ridiculous with remarks such as "The nutritional value for a breakfast cereal made from refined grains is less than that of its cardboard packet." The confusion caused by such extremist comments has led to apathy for many people. Accordingly, government authorities and regulators have taken educational initiatives in many countries, with balanced campaigns to promote public awareness of the benefits of grainbased foods generally, and of whole-grain products in particular.

#### **Historical Perspective**

Whole-grain foods have long been basic to mankind's diet. Prehistorically, the cereal grains were an important component of the diet even when man, the "hunter-gatherer", included various types of grain in the daily gathering. Inevitably, these grains were consumed as the whole grain, after various forms of primitive processing, such as crushing, soaking, boiling, or baking (see Cereals: Overview). Then followed the development of technology of sieving to remove the coarse outer layers, to produce a flour of fine uniform particle size, which was deemed to be of better quality. This refined product was thus assumed to be better, perhaps because of the extra work involved in its production, and that its consumption conferred some prestige, suiting it to the upper classes. This sociological prejudice against whole-grain foods has continued right through to the present day, so that there is some disdain for foods made from wholegrain meal.

In contrast to these sociological considerations, there are classical stories of nutritional deficiencies

Cereal species	Grain, as harvested	Initial processing	Further processing	Final product as usually consumed
Maize	Grain on the cob, with outer bracts	Remove bracts and grain from cob	Wet or dry milling to isolate endosperm	Refined flour, as crushed endosperm
Sweet corn	Grain on the cob, with outer bracts	Remove bracts	Consume "as is" after boiling	Whole grain on the cob
Popcorn	Grain on the cob, with outer bracts	Remove bracts and grain from cob	Consume "as is" after heating	Popped whole grains
Rice	Grain with hulls	Remove hulls	Remove germ and bran layers	Refined grain, as whole endosperm
Wheat, rye, triticale	Naked grain	Dry milling to isolate endosperm	Baking of refined flour (endosperm)	Baked and extruded foods
Malting barley	Grain with lemma and palea	Submit to malting, "as is"	Brewing	Beer
Food barley	Grain with lemma and palea	Remove lemma and palea	Pearl off bran layer, leaving endosperm	Soup, from boiling whole endosperm
Oats	Grain with lemma and palea	Remove lemma and palea, leaving groat	Heat treatment of whole grain	Porridge, from whole grain

Table 1 The common cereal grains, common forms of processing, and the concepts of "whole-grain" and "refined" products

associated with the consumption of foods that are solely based on refined grains. These include nutritional problems associated with the consumption of milled rice versus brown rice containing the bran layer (Table 1) (see Nutrition: Beriberi, A Deficiency Related to Grains).

On the other hand, during the past century, attempts have been made to compensate for the loss of vital nutrients in refined products by adding back purified preparations of specific nutrients, such as vitamins and minerals in a process known as "fortification" (see Fortification of Grain-Based Foods). This process has been successful to the extent that it has probably enhanced the nutritional status of communities for whom fortified foods have been available, but it appears to be an expensive and inefficient means of enhancing public nutrition when the same advantages and more could be provided merely by avoiding the refinement process in the first place.

# International Health Claims for Whole-Grain Foods

Quality cereal-food products start with appropriate selection for these qualities in plant breeding, and continue via appropriate farm management, harvesting and processing. However, acceptance at the consumer level is essential to the advancement of public health. Appropriate attitudes must be present in the mind of the consumer, encouraged by public knowledge about the components in cereals and improved food-processing techniques. The ultimate gain is achieved by providing quality grain for consumer food products. However, consumer acceptance is also based on quality taste, so there is a vital interplay

between hedonic acceptance and "what is supposed to be good for you."

Education in health information is thus essential to motivate greater acceptance of nutritionally suitable foods in general, and of whole-grain foods in particular. In general, whole grains are promoted and perceived as being healthy compared to nonwhole-grain foods. Although nutrition information (nutrient content claims) is important for promotion of increased grain consumption, increased consumer interest lies in health concerns.

#### **Health Claims in the USA**

Consumers are interested in health claims and health messages. Information about foods and health is best received when the message is kept simple. Consumers receive health claims and messages as a positive input, while information about disease prevention and death statistics are perceived more negatively. In the USA, the health claim, with specific wording, must be displayed on a processed-food package. This is defined by FDA guidelines in the USA. For most health claims, lengthy scientific and clinical information is required. A common health message that may appear in bold letters on the front panel of cereal or food containers is "Helps lower bad cholesterol while maintaining good cholesterol." This wording may be best interpreted as making a health claim friendlier to consumers. Health claims are based on science, and science will continue to expand the use of health information about grains to benefit the consumer, the grain and food industries, and agriculture.

Although Sweden approved its first health claim recently, health claims have been unique to the United States. There are 14 FDA-approved health claims in the USA. Twelve of these claims are based on

a standard of scientific validity, which consists of two parts:

- that the totality of evidence supports the substancedisease relationship, and
- that there is significant scientific agreement among qualified experts that the relationship is valid.

The necessary scientific information to petition for a health claim is well-described by the FDA. This reference is highly recommended for anyone interested in understanding the various types and designs of research experiments necessary to draft and receive an approved health claim. Not all studies submitted in a health claim petition may statistically or adequately support the substance-disease relationship, but it is the totality of evidence that is considered. For the FDA-approved oat-bran health claim, 37 of 41 submitted clinical trails were accepted by the FDA. Seventeen of these studies demonstrated a cholesterol-lowering effect by oat/oat bran, four were inadequate in duration, five were equivocal, and 11 showed no effect.

#### Statement by US National Academy of Science

Two of the 14 FDA-approved health claims are based on the existence of an authoritative statement by an appropriate scientific agency of the United States. These two claims do not need the specific depth of scientific data required of the other 12 claims. These two authoritative statement-based health claims are for potassium and whole grains. Specifically, the whole-grain claim was based on the authoritative statement that appeared in a US National Academy of Science (NAS) Report, entitled "Diet and Health: Implications for Reducing Chronic Disease Risk (1989)."

The statement appearing in the NAS report reads: "Diets high in plants foods (i.e., fruits, vegetables, legumes, and whole-grain cereal) are associated with a lower occurrence of coronary heart disease and cancers of the lung, colon, esophagus, and stomach." Although this authoritative statement pertains to all plant foods, the petition and authorized health claim is specific only for whole-grain foods. At issue, and the purpose of this article, is the question of the inclusion of all grains in this claim. To be more specific, the current whole-grain foods health claim appears most applicable for whole wheat. If the whole-grain foods claim could be strengthened to include all grains, consumer awareness would be heightened and consumption of a greater variety of grains could be achieved. This issue is based on the belief that a greater variety of grains in the diet is more beneficial than a single-grain diet.

Every sector would have greater opportunities for gain if consumers knew more about the health benefits of all grains. The inclusion of all cereals or grains under one claim might induce food manufacturers to investigate and develop new whole-grain foods. Consumers still want foods which taste good and many grains may not acquire consumer acceptance because of undesirable taste characteristics. Some grains have only regional importance and significance in consumption. The whole-grain claim should be amended to allow for a wider variety of grain-based food products and those having a mixture of whole grains.

## The Diversity of Grain Morphology

The cereal grains that are common in our diet are listed in Table 1, together with indications of how their diverse morphologies relate to the concept of "whole grain" and "refined" in our diet. There is considerable diversity in the structure and morphology of the many grains. This is evident even within the cereal grains that are commonly consumed (Table 1). For these reasons, the two key terms "whole grain" and "refined" are not easily defined. For cereal grains, however, "refined" can generally be regarded as material that consists mainly of the endosperm, with the outer layers of bran and glumes removed. Explanations of these aspects of grain morphology are provided in other articles, particularly Cereals: Overview. Grain, Morphology of Internal Structure; Grain and Plants, Morphology and Oilseeds, Overview.

Table 1 summarizes the processing steps for the various cereals, leading to the form in which each is normally consumed. These considerations provide the implications for possible loss of nonendosperm materials, e.g., loss of bran layers for milled rice and even the value of the outer husks of barley for filtration in the brewing process (see Barley: Milling and Processing; Malting). The summary in Table 1 is confined to the most common uses of these grains, omitting less usual uses and also omitting the uncommon types of these grains, such as the existence of hull-less genotypes in some cases, such as hull-less barley, which threshes free of the lemma and palea glumes like wheat.

# **Compositional Differences between** the Grain Components

The basis of health claims for whole grains relates to the distinctive compositional differences between the outer layers of the grains and the endosperm, which is the inner floury tissue that constitutes the anatomical "refined" product. Table 2 lists the fiber contents of a limited range of cereal grains. Details of the full range of nutritionally significant components are listed for a wide range of foods and raw materials in Appendix: Grain Composition Tables. It thus provides a ready means of comparing compositional details for whole and refined forms of the full range of grain species and for a diversity of food products made from them. It must be realized, however, that these values are indicative, and that the actual composition of a specific food may vary above or below these figures depending on the genotype (variety), growth environment, and processing conditions.

Examination of these tables indicates that there are considerable differences in various nutrients depending on whether foods are made from whole or refined materials, especially in nutritionally important components such as fiber, vitamins, and minerals. Examples of these contrasts are provided for wheat and rice in Table 3. The higher content of protein and fat in the

Table 2 The dietary fiber content of cereal grains

Cereal species	Form consumed	Dietary fiber content (g/100 g)
Maize	Whole yellow corn	11.0
Rice	White, milled	1.3
Rice	Brown	3.5
Wheat	Whole grain	12.2
Rye	Whole grain	11.7
Triticale	Whole grain	14.6
Barley	Whole grain	10.3
Oats	Whole grain (groats)	9.5
Sorghum	Whole grain (25 pearled)	7.5
Millet	Whole grain	8.5

whole-grain materials (whole-meal wheat and brown rice) especially reflect the presence of the germ (embryo) in the whole-grain materials, which are also richer in most of the vitamins and minerals. Nevertheless, despite these differences, it must be appreciated that there are very good levels of all these nutrients in the white wheat flour and in the white rice. Information on the significance of these many nutrients is provided in Nutrition: Effects of Food Processing; Mineral Composition; Vitamin Composition. Wheat: Ultrastructure of the Grain, Flour and Dough. The values given in Tables 2 and 3 are indicative, but actual values for a specific sample are likely to differ from these values, due to variations in growth conditions and genetic differences between varieties. For example, different sources of information have been used for the values for rice fiber in Tables 2 and 3 but the differences between brown and white rice are evident in either case.

## Strengths of the Whole-Grain Health Claims

Based on scientific evidence, including epidemiological studies and experimental clinical studies, the nutritional importance and health benefits of whole grains as part of a varied diet, is well documented, and further evidence is accumulating. Many health professionals adhere to this advice and attribute various health benefits to whole grains. The exact mechanisms of the efficacious effects brought about by the consumption of whole grains are less well understood. However, dietary fiber and phenolic compounds are frequently cited as the major functional-food

**Table 3** Nutrient composition of whole-grain and refined wheat and rice, expressed as the mass of the nutrient per 100 g of grain at its normal moisture content

Nutrient	Wheat wholemeal	Wheat flour	Brown rice flour	White rice flour
Protein (g)	13.6	10.2	7.2	6.0
Fat (g)	1.8	1.0	2.7	1.4
Dietary fiber (g)	12.1	2.7	4.6	2.4
Ash (g)	1.2	0.5	1.5	0.6
Thiamin (mg)	0.45	0.12	0.44	0.14
Riboflavin (mg)	0.22	0.04	0.08	0.02
Niacin (mg)	6.3	1.2	6.3	2.6
Pantothenic acid (mg)	1.0	0.44	1.6	0.8
Vitamin B <sub>6</sub> (mg)	0.33	0.04	0.73	0.44
Potassium (mg)	403	106	287	76
Calcium (mg)	33	17	10	10
Iron (mg)	4	1	1.9	0.4
Phosphorus (mg)	343	106	333	96
Zinc (mg)	3	1	2.4	0.8
Magnesium (mg)	135	23	112	36

Values (taken from **Appendix**: Grain Composition Tables) are indicative, varying above or below these figures depending on the genotype and growth environment for the grains.

ingredients that are most likely to be responsible. Based on the volume of consumption and dietary fiber content, grains are among the foods that provide the highest amounts of dietary fiber.

There is a long history of dietary fiber as a healthy food ingredient. The proposed health benefits of dietary fiber probably exceed that of other functional-food ingredients. The NAS recently established dietary reference intakes for dietary fiber, giving figures of  $38 \,\mathrm{g} \,\mathrm{day}^{-1}$  for men and  $25 \,\mathrm{g} \,\mathrm{day}^{-1}$  for women. Median intakes of dietary fiber in the US diet for men and women are currently 17 and 13 g day<sup>-1</sup>, respectively. Since whole-grain-based foods are among the most significant food sources of dietary fiber, increased consumption of whole grains has the greatest potential to help consumers meet the recommended dietary reference intakes. However, a heavy diet of whole-grain foods could become monotonous. The existing whole-grain foods health claim has merit because it is an important first step in public-health policy to inform the consumer and promote the consumption of whole-grain foods.

# Limitations of the Whole-Grain Health Claims

Limitations on claims for whole-grain benefits mainly focus on regulatory aspects of the topic. They center on:

- the lack of an overall definition for whole grain;
- the lack of whole-grain identification for cereals and other grains;
- the specification that a whole-grain food must contain 51% or more of one whole-grain ingredient by weight per reference amount customarily consumed; and
- that a whole grain must contain 11% dietary fiber.

The original authoritative statement of the NAS covered a broad array of plant foods, not simply whole cereals or whole wheat. To authorize the whole-grain health claim, the FDA used dietary fiber as the marker or index for a standard of compositional identity. However, the FDA has not established a definition for a whole grain. A clear standard of identity for a whole-grain status is not known for cereals listed in **Table 1**.

Maize, rice, and wheat are the three most abundant cereals produced in the world and their annual production is approximately equivalent. Whole wheat is a common term and relatively well-perceived among scientists and consumers as being the kernel with its bran covering, complete with germ, aleurone layer, and endosperm. Ground and/or cracked whole wheat

can be used to make many foods with high consumer acceptance. Wheat flour of normal extraction rate ( $\sim$ 76–80%) contains a low level of bran and aleurone layer. Flour of 72% extraction rate would be considered to be relatively low in flour yield commercially; it would be considered to contain virtually no bran and a minimum amount of aleurone layer. One commercial method of reconstituting "whole-grain flour" is to add wheat bran back to low-extraction flour, but this does not provide the full range of tissues that constitute whole wheat.

Whole-grain forms of corn (maize) are common in the diet of many cultures. In western diets, corn-onthe-cob and canned whole kernel corn ("sweet corn") contains ~11% total dietary fiber, but is generally classified as a vegetable and not a cereal. In the original petition to the FDA, popcorn was mentioned as a potential source of whole grain. Popcorn contains an average 12% dietary fiber. However, these forms of whole grain do not make major contributions to the western diet. Possibly more significant are breakfast cereals, such as corn flakes (see Cereals: Breakfast Cereals), but in this case, the raw material is primarily the endosperm of the corn kernel. Nevertheless, corn flakes do contain a significant level of dietary fiber, primarily in the form of arabinoxylans and resistant starch (see Cereals: Chemistry of Nonstarch Polysaccharides).

What constitutes whole rice and what fractions are included? Rice is usually steamed, but nutritional considerations depend on whether it is white rice or brown rice that is consumed, the latter being perceived as "healthier." White rice cannot be described as a whole grain, because the outer bran and aleurone layers have been removed.

For the purposes of the whole-grain foods health claim, "whole-grain foods" are those that contain 51% or more of whole-grain ingredients. The current whole-grain health claim is applied most often to wheat. Although food manufacturers may not rush to produce whole-grain foods from corn, sorghum, millet or rye cereals, there should be a method to allow mixtures of different grains, which could use the whole-grains health claim as an incentive for increased sales and greater variety among components in a whole-grain food. Once the identity of individual, whole cereals is established, some compromise can be used to set the level (percent) of a mixture that can constitute a whole-grain food product.

#### Conclusions

The health benefits of all foods are replacing the importance of nutrient content in the eyes of the consumer. Nutrient content claims, health claims, and

health messages (i.e., structure/function claims and logos) help the consumer seek and compare the nutritional quality and health benefits of foods. Nutrient-content claim descriptors ("high," "low," "no") help the consumer select which foods are a good source of some nutrient. Alternatively, these messages help them to refrain from eating too much of some food components (sodium, saturated fat, cholesterol). Scientific evidence for the health benefits of whole grains is accumulating. The chemical composition of whole-grain cereals indicates that they are high in dietary fiber, some vitamins and minerals, with the further advantage of having an abundance of phenolic compounds compared to other foods. To obtain these functional-food ingredients will require a varied diet and a diet that includes various grains. Nevertheless, it must be emphasized that foods made predominantly from refined grains also provide good nutrition, compared to many competing foods.

Consumers can expect to see increased efforts by food processors to provide a greater variety of whole-grain products. The allowances for the current health claim for whole-grain foods, as defined by the FDA, are discriminatory as it promotes the consumption of one cereal over other grains. The limitations of the current whole-grain foods health claim must provide opportunities to change the claim and make everyone more aware of whole grains and their important to health. There can be a health gain from the consumption of all grains. While whole grains are preferable in the diet, this does not discount the nutritional value and health benefits of all ingredients in grain-based foods (including white rice, noodles, pasta, and white bread).

See also: Celiac Disease. Grain, Morphology of Internal Structure. Nutraceuticals from Grains. Nutrition: Guidelines for Grain-Based Foods; Effects of Food Processing; Mineral Composition; Soy-Based Foods; Vitamin Composition. Wheat: Ultrastructure of the Grain, Flour and Dough. Appendix: Grain Composition Tables.

#### **Further Reading**

Food and Drug Administration (1999) Whole Grain Foods and Risk of Heart Disease and Certain Cancers, Docket

No. 99P-2209. FDA-Rockville, MD: Food and Drug Administration.

Kritchevsky D and Bonfield C (eds.) (1994) *Dietary Fiber* in *Health and Disease*. St. Paul, MN: American Association of Cereal Chemists.

NAS (1989) Diet and Health: Implications for Reducing Chronic Disease Risk (1989). US National Academy of Science (NAS) Report.

Henry RJ and Kettlewell PS (eds.) (1996) Cereal Grain Quality. London: Chapman and Hall.

Juliano BO (ed.) (1985) Rice: Chemistry and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Marquet L, Salvin JL, and Fulcher RG (eds.) (2002) Whole-Grain Foods in Health and Disease. St. Paul, MN: American Association of Cereal Chemists.

Nestle M (2002) *Food Politics*. Berkeley, CA: University of California Press.

Pomeranz Y (ed.) (1988) Wheat: Chemistry and Technology, 3rd edn. St. Paul, MN: American Association of Cereal Chemists.

United States Department of Agriculture and United States Department of Health and Human Services (2000) Nutrition and Your Health: Dietary Guidelines for Americans, 5th edn. Home and Garden Bulletin No. 232. Washington, DC.

Webster FH (ed.) (1985) Oats: Chemistry and Technology.St. Paul, MN: American Association of Cereal Chemists.

Willett WC and Stampfer MJ (2003) Rebuilding the food pyramid. *Scientific American* 288(1): 64–71.

White PJ and Johnson AJ (eds.) (2003) Corn: Chemistry and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.

Wood PJ (ed.) (1993) Oat Bran. St. Paul, MN: American Association of Cereal Chemists.

#### **Relevant Websites**

www.aaccnet.org – American Association of Cereal Chemists.

www.campden.co.uk - Campden and Chorleywood Food Research Association.

www.usda.gov – United States Department of Agriculture.

www.wheatfoods.org — Wheat Foods Council. www.gograins.grdc.com.au — GoGrains.

# **APPENDIX 1**

# **Grain Composition Table**

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The grain nutrient composition table developed by the Nutrition Coordinating Center (NCC) at the University of Minnesota contains values compiled from scientific literature, the USDA Nutrient Database for Standard Reference, manufacturer's information, and estimated data. The data in the table include both analytic and estimated values. (For further information regarding specific values, contact The Nutrition Coordinating Center, Division of Epidemiology, School of Public Health, 1300 South Second Street, Suite 300, Minneapolis Minnesota 55454-1015.) Estimated values were derived from (1) a different

form of the same food (e.g., raw to cooked), (2) a similar food, or (3) calculation of recipes or formulations.

See also: Amaranth. Beans. Carbohydrate Metabolism. Fortification of Grain-Based Foods. Labeling of Grain-Based Foods. Nutraceuticals from Grains. Nutrition: Soy-Based Foods. Peanuts. Rice: Chinese Food Uses. Rye. Snack Foods, Processing. Soybean: Soymilk, Tofu, and Okara. Sunflower. Teff. Tortillas. Triticale. Whole-Grain Versus Refined Products.

#### **Relevant Websites**

http://www.ncc.umn.edu - Nutrition Coordinating Center.

http://www.nal.usda.gov – US Department of Agriculture, Agriculture Research Service. USDA National Nutrient Database for Standard Reference, Nutrient Data Laboratory Home page.

Note: Turn overleaf for Table.

Description	Serving size	Weight (g)	kcal	Protein (g)	Total carbohydrate	Fat (g)	Ash (g)	Dietary fiber (g)	K (mg)	Na (mg)	Ca (mg)	Fe (mg)	P (mg)
					(g)								
Grains, flours, and cooked cereals (enriched or for	ified nutrients	s)											
Amaranth, dry	0.22 CP	45	168	6.50	29.78	2.93	1.37	6.84	165	9	69	3.42	205
Barley bran flour	0.30 CP	45	54	8.03	30.44	2.95	2.00	30.40	50	7	254	7.38	348
Barley flour	0.30 CP	45	155	4.73	33.53	0.72	0.58	4.55	139	2	14	1.21	133
Barley malt flour	0.28 CP	45	162	4.63	35.24	0.83	0.62	3.20	101	5	17	2.12	136
Barley, cooked	0.89 CP	140	172	4.83	37.90	0.56	0.71	7.60	137	71	16	1.22	108
Barley, dry	0.23 CP	45	158	4.46	34.97	0.52	0.50	7.02	126	4	13	1.13	99
Bread flour	0.33 CP	45	164	4.65	34.34	0.44	0.21	1.22	48	1	7	2.09	49
Buckwheat flour	0.38 CP	45	151	5.68	31.77	1.40	1.14	4.50	260	5	18	1.83	152
Buchwheat groats, dry	0.24 CP	40	138	4.69	29.98	1.08	0.88	4.12	128	4	7	0.99	128
Bulgur, cooked	0.77 CP	140	114	4.09	25.26	0.45	0.50	6.09	136	6	14	0.81	100
Bulgur, dry	0.32 CP	45	154	5.53	34.14	0.60	0.68	8.24	185	8	16	1.11	135
Cake flour	0.33 CP	45	163	3.69	35.11	0.39	0.18	0.77	47	1	6	3.29	38
Chickpea flour	0.50 CP	45	166	10.08	26.01	3.01	1.27	4.86	381	29	20	2.19	143
Corn bran, dry	3 TB	15	34	1.25	12.85	0.14	0.05	12.83	7	1	6	0.42	11
Corn flour	0.39 CP	45	164	4.20	34.32	1.70	0.71	4.32	134	2	63	3.24	100
Corn grits, white, cooked	1 CP	242	147	3.49	31.46	0.48	1.62	0.63	54	572	5	0.48	29
Corn grits, white, dry	0.26 CP	40	148	3.52	31.84	0.48	0.16	0.64	55	0	1	0.45	29
Corn grits, write, dry Corn grits, yellow, dry	0.26 CP	40	148	3.52	31.84	0.48	0.16	0.64	55 55	0	1	0.40	29
Cornmeal, cooked	1 CP	240	130	3.02	27.60	0.48	0.10	2.64	58	306	6	0.40	30
	0.39 CP	45	164	4.20	34.32	1.70	0.98	4.32	134	2	63	3.24	100
Cornmeal, masa harina, dry	0.39 CP 0.33 CP	45 45	156	3.59	33.59	0.70	2.87	4.32 3.14	69	607	158	0.45	280
Cornmeal, self-rising, dry		-					-	-					
Cornmeal, white, dry	0.33 CP	45	165	3.82	34.96	0.74	0.27	3.33	73	1	2	0.50	38
Cornmeal, yellow, dry	0.33 CP	45	165	3.82	34.96	0.74	0.27	3.33	73	1	2	0.50	38
Cornstarch	1.25 TB	10	38	0.03	9.13	0.01	0.01	0.09	0	1	0	0.05	1
Couscous, cooked	0.89 CP	140	180	6.09	36.99	0.31	1.22	2.39	79	363	14	0.52	81
Couscous, dry	0.26 CP	45	169	5.74	34.84	0.29	0.28	2.25	75	5	11	0.49	77
Cracked wheat, cooked	1 CP	242	178	7.19	38.07	0.99	1.31	6.39	213	185	22	2.03	182
Farina (creamed wheat), instant cooking, cooked	1 CP	241	121	3.50	24.92	0.46	1.42	1.06	38	289	105	0.05	34
Farina (creamed wheat), quick cooking, cooked	1 CP	239	119	3.37	24.76	0.43	1.79	0.93	43	370	106	0.05	93
Farina (creamed wheat), regular cooking, cooked	1 CP	251	122	3.46	25.25	0.50	0.93	1.26	40	287	106	0.05	38
Farina (creamed wheat), dry	0.23 CP	40	148	4.20	30.60	0.60	0.24	1.52	48	3	6	0.60	46
Hominy, canned	1 CP	165	119	2.44	23.53	1.45	1.42	4.13	15	347	17	1.02	58
Kamut flour	0.25 CP	45	153	6.17	32.66	0.84	0.72	5.49	182	2	15	1.75	195
Kasha, cooked	1 CP	168	178	6.03	38.56	1.39	1.13	5.29	165	7	12	1.28	164
Kasha, dry	0.24 CP	40	138	4.69	29.98	1.08	0.88	4.12	128	4	7	0.99	128
Millet, cooked	0.58 CP	140	169	4.94	32.63	1.89	1.46	3.81	87	3	5	1.34	128
Millet, dry	0.23 CP	45	170	4.96	32.78	1.90	1.46	3.83	88	2	4	1.35	128
Oat bran, cooked	1 CP	219	91	6.42	24.53	2.61	1.73	5.72	210	261	25	2.02	272
Oat bran, dry	0.43 CP	40	98	6.92	26.49	2.81	1.16	6.16	226	2	23	2.16	294
Oatmeal (fortified), flavored, dry	0.49 CP	55	202	5.17	42.33	2.30	1.67	4.07	171	267	166	6.28	183
Oatmeal (fortified), plain, dry	0.49 CP	40	148	6.20	25.60	2.44	2.00	4.36	150	114	143	11.57	187

Oatmeal, dry	0.49 CP	40	154	6.40	26.80	2.52	0.76	4.24	140	2	21	1.68	190
Oatmeal, instant cooking, flavored, cooked	1 CP	234	156	3.98	32.67	1.78	1.29	3.14	132	207	132	4.84	141
Oatmeal, instant cooking, plain, cooked	1 CP	234	130	5.45	22.53	2.15	1.76	3.84	132	102	130	10.20	165
Oatmeal, regular cooking, cooked	1 CP	234	145	6.04	25.32	2.39	1.71	4.00	132	389	24	1.59	179
Potato flour	0.28 CP	45	161	3.11	37.39	0.15	1.41	2.66	450	25	29	0.62	76
Quinoa, cooked	0.89 CP	140	141	4.93	25.94	2.18	1.09	2.23	279	9	26	3.49	154
Quinoa, dry	0.26 CP	45	168	5.90	31.01	2.61	1.31	2.66	333	9	27	4.16	185
Rice (creamed rice), dry	0.22 CP	40	148	2.52	32.96	0.20	0.16	0.28	57	2	10	1.52	52
Rice bran, dry	2 TB	15	47	2.00	7.45	3.13	1.50	3.15	223	1	9	2.78	252
Rice flour, brown	0.28 CP	45	163	3.25	34.42	1.25	0.69	2.07	130	4	5	0.89	152
Rice flour, white	0.28 CP	45	165	2.68	36.06	0.64	0.27	1.08	34	0	5	0.16	44
Rolled wheat, cooked	1 CP	242	138	4.53	30.40	0.80	2.11	3.85	157	568	20	1.38	153
Rolled wheat, dry	0.43 CP	40	137	4.48	30.08	0.80	0.64	3.80	156	1	16	1.36	152
Rye flour, medium	0.44 CP	45	159	4.23	34.87	0.80	0.68	6.57	153	1	11	0.95	93
Rye, whole grain, dry	0.27 CP	45	151	6.64	31.39	1.13	0.91	6.57	119	3	15	1.20	168
Self-rising flour	0.36 CP	45	155	4.36	33.06	0.41	2.91	1.15	46	571	189	2.30	114
Sorghum	0.24 CP	45	153	5.09	33.58	1.49	0.71	6.22	158	3	13	1.98	129
Soy flour, defatted	0.51 CP	45	148	21.15	17.27	0.55	2.77	7.88	1073	9	108	4.16	303
Soy flour, full fat	0.51 CP	45	196	15.54	15.84	9.29	2.01	4.32	1132	6	93	2.87	222
Soy flour, low fat	0.51 CP	45	167	20.94	17.09	3.02	2.74	4.59	1157	8	85	2.70	267
Spelt	0.38 CP	45	143	5.76	33.08	0.90	0.81	5.13	185	0	12	1.98	189
Teff, dry	0.23 CP	45	166	4.01	34.61	0.90	1.26	6.08	252	6	86	2.26	197
Triticale, whole grain	0.34 CP	45	152	5.93	32.91	0.81	0.83	6.57	210	1	16	1.17	144
Wheat bran (unprocessed)	0.25 CP	15	32	2.33	9.68	0.64	0.87	6.42	177	0	11	1.59	152
Wheat, cracked whole wheat	0.33 CP	45	153	6.17	32.66	0.84	0.72	5.49	182	2	15	1.75	156
Wheat, germ	2 TB	15	57	4.37	7.44	1.61	0.75	1.94	142	1	7	1.36	172
Wheat, hard red spring	0.23 CP	45	148	6.93	30.61	0.86	0.85	5.49	153	1	11	1.62	149
Wheat, hard red winter	0.23 CP	45	147	5.67	32.03	0.69	0.71	5.49	163	1	13	1.44	130
Wheat, hard white	0.23 CP	45	154	5.09	34.16	0.77	0.47	5.49	194	1	14	2.05	160
Wheat, soft red winter	0.27 CP	45	149	4.66	33.41	0.70	0.76	5.63	179	1	12	1.44	222
Wheat, soft, white	0.27 CP	45	153	4.81	33.91	0.90	0.69	5.72	196	1	15	2.42	181
Wheat, sprouted	0.42 CP	45	89	3.37	19.14	0.57	0.43	0.50	76	7	13	0.96	90
White flour, wheat, all-purpose (enriched)	0.36 CP	45	164	4.65	34.34	0.44	0.21	1.22	48	1	7	2.09	49
White flour, wheat, all-purpose (unenriched)	0.36 CP	45	164	4.65	34.34	0.44	0.21	1.22	48	1	7	0.53	49
Whole wheat flour, red wheat	0.38 CP	45	153	6.17	32.66	0.84	0.72	5.49	182	2	15	1.75	156
Posts and vice (envished nutvicents)													
Pasta and rice (enriched nutrients) Chow mein noodles, crisp type	0.55 CP	25	132	2.10	14.39	7.69	0.65	0.98	30	110	5	1.18	40
Egg noodles, cooked	0.55 CF 0.9 CP	140	186	6.65	34.78	2.06	0.83	1.54	39	10	17	0.84	40 97
	0.9 CP 0.8 CP	140	197		39.68	0.94	0.34	1.82	43	10	10	0.84	97 76
Lo mein noodles, soft type, cooked				6.68									
Macaroni/spaghetti noodles, white cooked	1 CP	140	197	6.68	39.68	0.94	0.34	1.82	43	1	10	0.70	76
Macaroni/spaghetti noodles, whole wheat, cooked	1 CP	140	174 155	7.46	37.16	0.76	0.62	3.92	62	4	21	1.48	125
Rice, brown, cooked	0.7 CP	140	155	3.61	32.14	1.26	0.64	2.52	60 65	7	14	0.59	116
Rice, brown, uncooked	0.25 CP	45	167	3.89	34.61	1.36	0.69	2.71	65	8	15	0.64	125
Rice, white, cooked	0.9 CP	140	182	3.77	39.44	0.39	0.57	0.56	49	1	14	0.28	60 50
Rice, white, uncooked	0.25 CP	45	180	3.72	38.92	0.39	0.57	0.55	48	1	14	0.36	59

Table Continued

Description	Serving size	Weight (g)	kcal	Protein (g)	Total carbohydrate (g)	Fat (g)	Ash (g)	Dietary fiber (g)	K (mg)	Na (mg)	Ca (mg)	Fe (mg)	P (mg)
Rice/cellophane noodles, cooked	0.8 CP	140	153	1.27	34.86	0.28	0.25	1.40	6	27	6	0.20	28
Spaetzle or spatzen, cooked	0.9 CP	140	118	5.07	18.31	2.39	0.95	0.62	65	247	28	1.30	67
Wild rice, cooked	0.85 CP	140	141	5.59	29.88	0.48	0.56	2.52	141	4	4	0.84	115
Wild rice, uncooked	0.3 CP	45	160	6.30	33.71	0.54	0.63	2.84	160	5	5	0.95	130
Cereal, ready-to-eat (fortified nutrients)													
Bran flakes without raisins	0.75 CP	29	92	2.90	22.91	0.58	1.74	5.08	171	207	15	8.12	157
Bran nuggets, unsweetened	0.33 CP	30	75	2.10	24.00	0.65	2.36	12.90	300	203	19	4.50	150
Corn flakes, unsweetened	1 CP	28	101	1.96	24.08	0.22	0.90	0.98	25	203	2	8.40	14
Corn nuggets, unsweetened	1 CP	30	112	2.10	25.80	0.27	0.98	0.60	25	288	100	9.00	22
Corn, puffed	1.33 CP	30	113	1.80	25.80	0.60	1.14	0.90	35	267	150	8.10	40
Oat flakes	0.75 CP	30	96	4.05	23.31	1.17	1.13	3.61	127	210	12	8.10	141
Oat rings, unsweetened	1 CP	30	111	3.30	22.20	1.80	1.35	2.70	96	273	100	8.10	100
Rice flakes	1 CP	31	117	6.98	22.01	0.48	0.61	0.74	61	224	9	8.37	68
Rice nuggets, unsweetened	1.25 CP	33	124	2.08	28.55	0.36	1.02	0.36	42	354	3	1.98	44
Rice, puffed	1 CP	14	54	0.98	12.29	0.13	0.06	0.20	16	1	1	0.40	17
Wheat and barley flakes	0.75 CP	29	106	2.90	23.64	0.84	0.70	2.55	99	125	11	8.10	88
Wheat and barley nuggets	0.5 CP	58	208	6.26	47.15	1.10	1.45	5.05	178	354	20	16.20	139
Wheat flakes without raisins	1 CP	30	110	3.24	23.79	0.93	1.01	2.10	104	222	55	8.10	95
Wheat, puffed	1.25 CP	15	55	2.44	11.46	0.32	0.23	1.41	55	1	4	0.66	50
Wheat, shredded, unsweetened	2 biscuits	46	156	4.78	38.13	0.55	0.74	5.29	196	3	20	1.44	168
Baby food cereals (fortified nutrients)													
Barley, instant, dry	6.25 TB	15	55	1.67	11.30	0.51	0.51	1.23	59	7	90	6.75	75
Oatmeal, instant, dry	6 TB	15	60	2.04	10.38	1.17	0.48	0.99	71	5	90	6.75	75
Rice, instant, dry	6 TB	15	61	1.31	11.99	0.63	0.38	0.15	115	3	90	6.75	50
Breads and other related products (enriched nutri	ents)												
Bagel, egg, plain or with seasoning	1 each	55	153	5.83	29.15	1.16	0.88	1.27	37	278	7	2.19	46
Bagel, oat bran, plain or with seasoning	1 each	55	141	4.52	30.88	0.74	1.19	1.98	81	321	9	1.86	86
Bagel, rye, plain or with seasoning	1 each	55	157	4.14	33.95	0.50	0.79	2.52	79	176	8	1.62	60
Bagel, white flour, plain or with seasoning	1 each	55	151	5.78	29.37	0.88	0.99	1.27	56	294	41	1.96	53
Bagel, whole wheat, plain or with seasoning	1 each	55	163	4.91	34.84	0.55	0.83	2.49	94	177	10	1.90	83
Biscuit, baking powder or buttermilk	1 each	55	222	3.58	24.21	12.36	1.57	0.78	88	245	120	1.52	83
Boston brown bread	2 slices	50	98	2.60	21.65	0.75	1.40	2.35	159	316	35	1.05	56
Bread crumbs, plain	1/4 CP	30	119	3.75	21.75	1.62	1.02	0.72	66	259	68	1.84	44
Bread or rolls, French	2 slices	50	136	4.40	25.00	1.75	0.95	1.35	55	292	39	1.47	52
Bread or rolls, rye	2 slices	50	130	4.25	24.15	1.65	1.25	2.90	83	330	37	1.42	63
Bread or rolls, sourdough	2 slices	50	136	4.40	25.00	1.75	0.95	1.35	55	292	39	1.47	52
Bread or rolls, white	2 slices	50	134	4.10	24.75	1.80	0.95	1.15	60	269	54	1.52	47

Bread or rolls, whole wheat	2 slices	50	123	4.85	23.05	2.10	1.15	3.45	126	264	36	1.65	115
Bread, barley	2 slices	50	142	3.91	28.11	1.49	0.78	2.91	83	176	19	1.34	66
Bread, bran	2 slices	50	129	4.40	24.07	1.92	1.03	2.07	86	267	47	1.57	74
Bread, egg	2 slices	50	147	4.38	25.68	2.77	0.52	0.88	70	91	27	1.52	60
Bread, English muffin	2 slices	55	130	4.26	25.43	1.00	1.22	1.49	72	257	96	1.38	74
Bread, focaccia	1 slice	50	151	3.39	23.49	4.63	1.27	0.96	45	419	6	1.54	41
Bread, gluten-free	2 slices	50	110	5.05	20.37	1.48	0.96	2.22	98	205	12	1.40	91
Bread, hovis	2 slices	50	131	4.80	24.15	1.45	1.05	1.04	127	277	45	1.73	61
Bread, Irish soda	2 slices	50	138	3.16	26.13	2.59	0.88	1.03	129	186	36	1.22	54
Bread, Italian	2 slices	50	136	4.40	25.00	1.75	0.95	1.35	55	292	39	1.47	52
Bread, oatmeal	2 slices	50	135	4.20	24.25	2.20	1.00	2.00	71	300	33	1.35	63
Bread, pumpernickel	2 slices	50	130	4.25	24.15	1.65	1.25	2.90	83	330	37	1.42	63
Bread, raisin	2 slices	50	137	3.95	26.15	2.20	0.90	2.15	114	195	33	1.45	55
Bread, wheat, reduced calorie (light, high		50	99	4.55	21.80	1.15	0.95	6.00	61	256	40	1.48	51
Breadsticks, bread type	2 each	55	149	4.84	27.50	1.93	1.05	1.49	61	321	43	1.62	57
Breadsticks, cracker type	1.5 each	15	68	1.46	10.83	2.08	0.35	0.55	21	104	2	0.61	20
Crepe, plain	2 each	110	224	8.10	30.27	7.46	1.79	0.85	183	344	136	1.74	150
Croissant, plain	1 each	55	250	4.43	25.78	14.44	0.48	0.86	72	73	30	1.51	63
Croutons, plain	2/3 CP	7	28	0.83	5.15	0.46	0.18	0.36	9	49	5	0.29	8
Eggroll wrapper	1 each	32	92	2.83	18.05	0.64	0.19	0.62	29	21	5	1.10	31
English muffin, oat bran	1 each	55	120	4.78	24.64	1.76	0.96	2.53	120	158	53	1.92	123
English muffin, rye	1 each	55	130	4.02	25.72	1.25	0.83	2.45	99	158	50	1.60	72
English muffin, white	1 each	55	129	4.24	25.30	0.99	1.21	1.49	72	255	96	1.38	73
English muffin, whole wheat	1 each	55	112	4.84	22.22	1.16	1.54	3.69	116	350	146	1.35	155
Pancake, white flour, plain, from mix - no	fat added 3 each	110	213	5.72	40.38	2.75	1.80	1.54	88	689	33	2.21	75
Pancake, white flour, plain, from recipe	3 each	110	291	8.14	38.93	11.47	4.76	1.09	155	900	347	2.59	224
Pancake, whole wheat, plain	3 each	110	269	9.01	35.51	11.46	5.08	4.59	276	877	347	2.23	306
Pita, white	1 each	50	138	4.55	27.85	0.60	0.95	1.10	60	268	43	0.70	49
Pita, whole wheat	1 each	50	133	4.90	27.50	1.30	1.00	3.70	85	266	8	1.53	90
Popover	1 each	55	107	4.90	15.77	2.41	0.95	0.49	93	208	53	1.04	79
Rolls, crescent (refrigerated dough)	2 each	50	173	3.35	23.75	7.35	1.65	0.80	79	601	10	1.30	193
Rolls, hamburger, white	1 each	50	143	4.25	25.15	2.55	1.00	1.35	71	280	70	1.59	44
Rolls, hamburger, whole wheat	1 each	50	123	4.85	23.05	2.10	1.15	3.45	126	264	36	1.65	115
Rolls, hard	1 each	50	147	4.95	26.35	2.15	1.00	1.15	54	272	48	1.64	50
Rolls, hot dog, white	1 each	50	143	4.25	25.15	2.55	1.00	1.35	71	280	70	1.59	44
Rolls, hot dog, whole wheat	1 each	50	123	4.85	23.05	2.10	1.15	3.45	126	264	36	1.65	115
Rolls, kaiser	1 each	50	147	4.95	26.35	2.15	1.00	1.15	54	272	48	1.64	50
Rolls, submarine or hoagie	1/2 each	50	137	4.40	25.95	1.50	1.00	1.50	57	305	38	1.27	53
Scone	1 each	55	215	4.43	29.89	8.95	1.78	0.99	117	357	98	1.56	90
Taco shell	2 each	30	140	2.16	18.72	6.78	0.54	2.25	54	110	48	0.75	74
Tortilla, corn	2 each	55	122	3.14	25.63	1.38	0.66	2.86	85	89	96	0.77	173
Tortilla, white flour	1 each	55	179	4.79	30.58	3.91	0.99	1.82	72	263	21	1.82	68
Tortilla, whole wheat flour	1 each	55	107	3.99	21.15	1.45	1.05	3.56	118	225	11	1.13	101
Waffles, bran, plain	1 each	85	244	7.46	36.73	9.28	4.25	5.12	256	851	220	2.59	257
Waffles, white flour, plain, from recipe	1 each	85	286	6.72	29.39	15.82	2.66	0.87	135	444	216	1.92	160
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Table Continued

Description	Serving size	Weight (g)	kcal	Protein (g)	Total carbohydrate (g)	Fat (g)	Ash (g)	Dietary fiber (g)	K (mg)	Na (mg)	Ca (mg)	Fe (mg)	P (mg)
Waffles, white flour, plain, frozen	1 each	85	226	5.52	34.60	7.00	2.09	1.17	69	672	71	2.18	84
Wonton wrapper	1 each	8	23	0.71	4.51	0.16	0.05	0.15	7	5	1	0.27	8
Crackers (enriched nutrients)													
Butter crackers	10 each	30	152	2.09	16.95	8.45	0.75	0.55	22	254	3	0.94	22
Cheese crackers	10 each	30	151	3.03	17.46	7.59	0.99	0.72	44	299	45	1.43	65
Cheese-filled sandwich crackers	4 each	30	143	2.79	18.51	6.33	1.20	0.56	129	420	77	0.72	122
Cheese-filled sandwich crackers, whole wheat	4 each	30	149	2.94	17.46	7.50	1.14	0.92	92	274	61	0.79	115
Matzo or matzoh crackers, egg	1 each	30	112	3.08	23.13	0.59	0.21	0.81	52	6	8	1.29	41
Matzo or matzoh crackers, plain	1 each	30	119	3.00	25.11	0.42	0.18	0.90	34	1	4	0.95	27
Matzo or matzoh crackers, whole wheat	1 each	30	105	3.93	23.67	0.45	0.48	3.54	95	1	7	1.40	92
Melba toast	3 each	15	59	1.89	11.49	0.48	0.45	0.94	30	124	14	0.56	29
Peanut butter-filled sandwich crackers	4 each	30	146	3.33	17.61	7.17	0.96	0.84	67	283	29	0.91	72
Rye wafer, plain	3 each	30	100	2.88	24.12	0.27	1.23	6.87	148	238	12	1.78	100
Saltine or soda crackers	10 each	30	130	2.76	21.45	3.54	0.99	0.90	38	391	36	1.62	32
Whole wheat crackers	10 each	30	133	2.64	20.58	5.16	0.81	3.15	89	198	15	0.92	88
Zwieback	2 each	15	68	1.87	10.89	1.88	0.15	0.17	8	19	1	0.30	7
Cookies (enriched nutrients)													
Animal cracker	12 each	30	129	1.94	21.31	4.36	0.59	1.17	43	122	26	0.84	42
Biscotti, with nuts	1 each	30	130	2.99	14.47	7.10	0.49	1.16	76	51	25	0.86	52
Brownie, butterscotch, without nuts	1 each	40	170	1.57	28.34	5.66	0.90	0.25	92	179	49	0.95	33
Brownie, chocolate, without nuts	1 each	40	188	2.16	22.68	10.83	0.30	1.17	71	10	9	0.78	46
Cookies and bars, arrowroot	3 each	30	129	1.94	21.31	4.36	0.59	1.17	43	122	26	0.84	42
Cookies and bars, butterscotch chip	3 each	30	157	1.67	19.20	8.36	0.55	0.34	50	180	12	0.57	24
Cookies and bars, chocolate chip	3 each	30	142	1.67	19.16	6.67	0.34	0.54	30	107	7	0.78	21
Cookies and bars, chocolate, wafer	7 each	30	130	1.98	21.72	4.26	0.60	1.02	63	174	9	1.20	40
Cookies and bars, date bar	1 each	30	98	1.01	16.84	3.30	0.39	1.05	87	62	9	0.49	21
Cookies and bars, fig bar	2 each	30	104	1.11	21.27	2.19	0.48	1.38	62	105	19	0.87	19
Cookies and bars, fortune cookie	4 each	30	100	1.58	22.50	0.95	0.41	0.45	21	55	29	0.50	24
Cookies and bars, gingerbread	3 each	30	144	1.90	22.28	5.43	0.57	0.47	139	61	34	1.18	28
Cookies and Bars, gingersnap	3 each	30	159	1.53	23.09	6.95	0.36	0.40	102	62	20	0.98	20
Cookies and bars, graham cracker, plain	4 squares	30	118	1.09	22.43	2.81	0.57	0.29	51	167	7	0.62	12
Cookies and bars, granola	3 each	30	145	2.50	18.40	7.61	0.38	1.25	92	16	15	0.78	58
Cookies and bars, lemon bar	2 each	30	131	1.50	18.55	5.80	0.40	0.20	20	115	14	0.42	22
Cookies and bars, macaroon (coconut)	1 each	30	108	1.16	17.35	4.10	0.29	0.52	60	65	2	0.23	14
Cookies and bars, molasses	3 each	30	127	1.51	23.49	3.15	0.70	0.42	157	143	22	1.14	19
Cookies and bars, oatmeal	3 each	30	175	2.50	26.30	6.85	0.60	1.02	76	114	29	0.99	57
Cookies and bars, peanut butter	3 each	30	156	2.82	17.93	8.62	0.61	0.60	76	154	18	0.64	42
Cookies and bars, pfeffernuesse	2 each	30	120	2.05	20.53	3.37	0.50	0.50	136	90	22	1.17	26
Cookies and bars, pizzelle	2 each	30	183	1.64	7.79	16.35	0.50	0.22	17	159	5	0.45	20

Cookies and bars, raisin	3 each	30	134	1.35	20.36	5.69	0.32	0.57	87	43	11	0.61	22
Cookies and bars, Rice Krispie bar	2 each	30	112	0.96	22.76	2.26	0.41	0.13	15	138	2	0.63	15
Cookies and bars, rosette	2 each	30	140	1.25	5.96	12.50	0.38	0.17	13	122	4	0.35	15
Cookies and bars, shortbread	3 each	30	179	2.11	18.69	10.76	0.35	0.52	26	124	7	0.90	24
Cookies and bars, sugar	3 each	30	161	1.64	17.93	9.10	0.21	0.36	39	57	3	0.65	18
Cookies and bars, sugar wafer with creme filling	4 each	30	147	1.07	20.38	7.40	0.39	0.97	40	93	8	0.50	22
Cookies and bars, vanilla sandwich	3 each	30	142	1.67	19.16	6.67	0.34	0.54	30	107	7	0.78	21
Cookies and bars, vanilla wafer	7 each	30	139	1.88	21.00	5.63	0.44	0.79	40	140	5	0.93	25
Krumkake	2 each	30	158	2.49	20.87	7.23	0.31	0.41	27	51	8	0.80	30
Cakes, pastries, and other desserts (enriched nutrients)													
Angel food cake, white or flavored, not frosted or glazed	1 piece	55	164	3.96	36.58	0.10	0.47	0.17	110	93	4	0.78	13
Anisette (mandelbrodt toast), plain	3 slices	30	131	2.31	17.70	5.69	0.33	0.41	26	37	22	0.80	33
Cake, apple, not frosted or glazed	1 piece	80	269	2.73	34.89	13.41	0.55	1.48	66	156	12	0.94	34
Cake, banana, not frosted or glazed	1 piece	80	274	4.32	47.54	7.54	1.14	0.68	78	191	94	1.38	84
Cake, butter, not frosted or glazed	1 piece	80	289	3.58	40.95	12.55	1.60	0.30	48	478	57	0.83	197
Cake, carrot, without nuts, not frosted or glazed	1 piece	80	298	3.27	35.82	16.11	0.70	1.27	80	192	17	1.14	43
Cake, chiffon, not frosted or glazed	1 piece	55	184	3.73	25.46	7.48	1.06	0.35	53	219	52	0.89	60
Cake, chocolate, not frosted or glazed	1 piece	80	279	3.48	41.84	11.99	0.79	1.46	82	160	32	1.39	67
Cake, fruitcake	1 piece	125	404	5.46	63.98	15.25	1.63	2.61	347	244	85	2.61	102
Cake, German chocolate, not frosted or glazed	1 piece	80	279	3.48	41.84	11.99	0.79	1.46	82	160	32	1.39	67
Cake, gingerbread, not frosted or glazed	1 piece	80	263	3.01	42.46	9.12	1.57	0.77	428	264	64	2.52	42
Cake, jelly roll, yellow cake	1 piece	80	195	4.78	37.20	2.98	1.02	0.32	70	174	68	0.92	83
Cake, oatmeal, not frosted or glazed	1 piece	80	254	3.18	43.44	7.90	0.90	1.06	103	270	27	1.27	59
Cake, pound, chocolate, not frosted or glazed	1 piece	80	385	4.98	42.02	21.75	0.66	0.77	66	76	40	1.54	77
Cake, pound, white, not frosted or glazed	1 piece	80	374	4.95	38.31	22.20	0.62	0.66	52	72	40	1.42	70
Cake, spice, not frosted or glazed	1 piece	80	274	4.32	47.54	7.54	1.14	0.68	78	191	94	1.38	84
Cake, sponge, white or yellow, not frosted or glazed	1 piece	55	158	3.80	30.38	2.33	0.35	0.31	73	27	13	0.81	49
Cake, white, not frosted or glazed	1 piece	80	288	4.33	43.40	11.12	1.64	0.58	78	343	105	1.16	74
Cake, yellow, not frosted or glazed	1 piece	80	274	4.32	47.54	7.54	1.14	0.68	78	191	94	1.38	84
Cream puff shell	2 each	80	293	7.24	18.27	21.06	0.93	0.62	80	245	29	1.51	94
Cupcake, chocolate, commercial packaged	1.5 each	80	283	3.23	48.58	9.58	1.58	1.94	92	470	14	1.62	58
Dumplings with fruit, apple	1/2 each	125	304	2.44	44.95	13.23	0.41	2.05	79	69	10	1.18	29
Fritter, apple	1 CP	55	209	4.83	18.25	13.00	1.93	0.70	82	493	80	1.25	86
Phyllo pastry	$1\frac{1}{3}$ sheet	28.35	84	2.19	17.93	0.21	0.35	0.59	23	98	3	0.99	23
Pie crust or shell, chocolate cookie type	1/8 pie	29.17	153	1.44	15.81	9.76	0.44	0.74	46	127	7	0.88	29
Pie crust or shell, egg yolk pastry, single layer	1/8 pie	29.77	171	2.05	15.85	10.82	0.52	0.45	22	155	6	0.84	28
Pie crust or shell, graham cracker, regular	1/8 pie	30.51	161	0.59	17.12	10.33	0.31	0.16	28	91	4	0.35	7
Pie crust or shell, puff pastry, frozen	1/2 shell	20	113	1.50	9.13	7.69	0.20	0.20	18	50	4	0.93	19
Pie crust or shell, regular pastry, single layer, frozen	1/8 pie	16.38	85	1.10	8.12	5.38	0.32	0.29	11	106	2	0.50	11
Pie crust or shell, regular pastry, single layer, prepared from mix	1/8 pie	20.8	104	1.40	10.32	6.33	0.45	0.37	14	152	2	0.63	15
Pie crust or shell, regular pastry, single layer, prepared from recipe	1/8 pie	28.12	154	1.68	12.40	10.71	0.47	0.44	17	154	3	0.76	18
Pie crust or shell, vanilla wafer type	1/8 pie	36.97	190	1.92	20.82	11.49	0.58	0.78	43	208	7	0.92	27
Pies, apple, double pastry crust	1/8 pie	125	302	2.66	41.35	14.30	0.78	1.78	92	206	8	1.28	33
Pies, chocolate cream	1/8 pie	125	341	6.01	40.45	18.39	1.38	1.76	216	181	112	1.49	160
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Table Continued

Description	Serving size	Weight (g)	kcal	Protein (g)	Total carbohydrate (g)	Fat (g)	Ash (g)	Dietary fiber (g)	K (mg)	Na (mg)	Ca (mg)	Fe (mg)	P (mg)
Pies, pudding filling, chocolate	1/8 pie	85	194	3.35	22.43	10.22	1.28	0.63	118	310	65	0.89	165
Pies, pudding filling, vanilla	1/8 pie	85	195	3.09	22.99	10.08	1.20	0.38	94	312	65	0.68	139
Pies, pumpkin	1/8 pie	125	255	5.54	31.79	12.09	1.60	1.78	233	286	119	1.49	122
Shortcake, biscuit type	2 each	80	254	4.50	35.68	10.46	2.06	0.95	78	372	151	1.86	105
Shortcake, sponge type	2 each	55	158	3.80	30.38	2.33	0.35	0.31	73	27	13	0.81	49
Granola and cereal bars (fortified nutrients)													
Breakfast bar	1.5 each	40	156	2.16	24.79	5.60	0.42	1.36	47	61	<i>555</i>	5.00	278
Cereal bar	1 each	40	153	2.15	29.39	3.24	0.39	1.08	49	65	13	1.95	46
Snacks and chips (enriched nutrients)													
Bagel chips	0.5 CP	30	132	2.91	21.29	3.87	0.59	1.12	50	155	10	1.40	35
Cheese balls, puffs or twists	1 CP	30	165	1.76	15.87	10.58	0.98	1.46	50	308	17	0.83	27
Corn chips	1 CP	30	164	1.65	15.15	10.82	0.56	1.44	32	170	1	0.81	16
Corn nuts	0.35 CP	30	132	2.55	21.99	4.23	0.81	2.07	83	165	3	0.50	83
Popcorn, commercially popped (prepopped), not "buttered"	2.75 CP	30	151	2.64	17.16	8.43	1.08	3.33	66	265	2	0.59	66
Popcorn, hot-air popped	3.75 CP	30	115	3.60	23.37	1.26	0.54	4.53	90	1	3	0.80	90
Popcorn, microwave popped from package, with salt	3.33 CP	30	151	2.18	14.17	10.00	0.95	2.75	55	243	2	0.49	55
Popcorn, popped in fat	2.75 CP	30	166	2.25	14.61	11.43	0.34	2.83	56	1	2	0.50	56
Pretzels, hard type	1.33 CP	30	114	2.73	23.76	1.05	1.47	0.96	44	515	11	1.30	34
Pretzels, soft type	1/4 medium	30	109	3.10	22.89	0.29	1.58	0.81	32	561	5	1.40	32
Rice cake	3 each	30	116	2.46	24.45	0.84	0.74	1.26	87	98	3	0.45	108
Taco or tortilla chips	1 CP	30	152	2.10	19.04	7.40	0.65	1.78	49	181	11	1.00	26
Wheat nuts	0.35 CP	30	214	4.24	5.30	20.11	1.03	1.06	78	201	4	0.75	94
Legumes													
Adzuki beans, cooked	0.4 CP	90	115	6.77	22.29	0.09	1.20	6.57	479	7	25	1.80	151
Bayo beans, cooked	0.5 CP	90	35	2.70	9.06	0.17	1.33	3.02	147	1	25	0.70	84
Black beans, cooked	0.5 CP	90	128	7.83	23.68	0.51	1.11	5.76	331	1	63	2.23	141
Broad beans, cooked	0.5 CP	90	128	7.83	23.68	0.51	1.11	5.76	331	1	63	2.23	141
Brown beans, cooked	0.5 CP	90	125	8.76	22.59	0.32	1.58	5.67	505	5	81	3.33	102
Cowpeas, cooked	0.5 CP	90	104	6.96	18.69	0.48	0.85	5.85	250	4	22	2.26	140
Fava beans	0.5 CP	90	90	5.46	16.92	0.31	1.17	5.22	367	48	20	1.22	57
Garbanzo beans, cooked	0.55 CP	90	148	7.97	24.67	2.33	0.83	6.84	262	6	44	2.60	151
Kidney beans, cooked	0.5 CP	90	114	7.80	20.53	0.45	0.98	5.76	363	2	25	2.65	128
Lentils, cooked	0.45 CP	90	104	8.12	18.13	0.34	0.75	7.11	332	2	17	3.00	162
Lima beans, cooked	0.5 CP	90	104	7.02	18.80	0.34	1.04	6.30	457	2	15	2.15	100
Mung beans, cooked	0.45 CP	90	125	8.76	22.59	0.32	1.58	5.67	505	5	81	3.33	102
Navy beans, cooked	0.55 CP	90	128	7.83	23.68	0.52	1.11	5.76	331	1	63	2.23	141
Northern beans, cooked	0.5 CP	90	125	8.76	22.59	0.31	1.58	5.67	505	5	81	3.33	102
Pigeonpeas, cooked	0.6 CP	90	106	7.51	19.00	0.35	0.61	7.47	326	2	13	1.16	89
i igeoripeas, cookea	0.0 OF	90	100	7.51	19.00	0.33	0.01	1.41	320	_	13	1.10	OS

Distribusion analysis	0.5.00	00	400	7.00	00.00	0.47	4 00	774	404	0	40	0.05	444
Pinto beans, cooked	0.5 CP	90	123	7.39	23.09	0.47	1.22	7.74	421	2	43	2.35	144
Soybeans, cooked	0.5 CP	90	156	14.98	8.93	8.07	1.72	5.40	464	1	92	4.63	221
Soybeans, dry roasted, salted	0.3 CP	30	117	12.01	7.86	5.21	1.84	3.88	449	162	67	3.13	170
Soybeans, oil roasted, salted	0.3 CP	30	141	10.57	10.07	7.62	1.16	5.31	441	49	41	1.17	109
Split peas, yellow or green, cooked	0.45 CP	90	106	7.51	19.00	0.35	0.61	7.47	326	2	13	1.16	89
Tepary beans	0.35 CP	90	58	4.23	10.77	0.14	0.80	4.59	212	6	35	1.03	5
Meat substitutes													
Miso	1 TB	17	35	2.01	4.75	1.03	2.16	0.92	28	620	11	0.47	26
Tempeh	0.5 CP	85	164	15.76	7.98	9.18	1.38	2.87	350	8	94	2.30	226
Tofu (soybean curd), extra firm	0.35 CP cubes	85	82	8.85	1.67	5.28	0.70	0.34	113	9	88	1.45	138
Tofu (soybean curd), firm	0.35 CP cubes	85	78	8.41	2.31	4.74	0.90	0.26	154	10	142	1.43	118
Tofu (soybean curd), silken	1 slice (1" thick)	85	53	5.87	2.04	2.30	0.51	0.09	165	31	27	0.88	77
Tofu (soybean curd), soft	0.35 CP cubes	85	52	5.57	1.53	3.14	0.60	0.17	102	7	94	0.94	78
Tota (soybean sara), sort	0.00 01 00000	00	02	0.07	1.00	0.14	0.00	0.17	102	,	04	0.04	70
Alcoholic beverages													
Beer, light, low calorie	8 FO	236	66	0.47	3.07	0.00	0.24	0.00	42	7	12	0.09	28
Beer, low alcohol	8 FO	240	74	0.60	10.56	0.00	0.24	1.20	12	7	12	0.00	26
Beer, regular	8 FO	237.6	97	0.71	8.79	0.00	0.24	1.19	59	12	12	0.07	29
Scotch, plain	1.5 FO	41.7	96	0.00	0.00	0.00	0.00	0.00	1	0	0	0.02	2
Whiskey, plain	1.5 FO	41.7	96	0.00	0.00	0.00	0.00	0.00	1	0	0	0.02	2
Ingredients used in grain products													
Almonds, raw	0.2 CP	30	173	6.38	5.92	15.19	0.93	3.54	218	0	74	1.29	142
Arrowroot flour	0.2 CP	30	107	0.09	26.45	0.03	0.02	1.02	3	1	12	0.10	2
Beer nuts, peanuts	0.2 CP	30	180	7.41	7.44	14.92	1.20	1.94	192	85	25	0.52	145
Cashews, oil roasted	0.2 CP	30	173	4.85	8.56	14.46	0.96	1.14	159	5	12	1.23	128
Coconut, dried, unsweetened	0.2 CP	15	99	1.03	3.66	9.68	0.29	2.45	81	6	4	0.50	31
Filberts, raw	0.2 CP	30	188	4.49	5.01	18.23	0.69	2.91	204	0	34	1.41	87
Flax seeds	0.25 CP	30	148	5.85	10.28	10.20	1.05	8.37	204	10	60	1.87	149
Flour, peanut, defatted	0.5 CP	30	98	15.66	10.41	0.17	1.43	4.74	387	54	42	0.63	228
Flour, peanut, low fat	0.5 CP	30	128	10.14	9.38	6.57	1.57	4.74	407	0	39	1.42	152
Macadamia nuts, raw	0.2 CP	30	215	2.37	4.15	22.73	0.34	2.58	110	2	26	1.11	56
Peanut butter, with salt	2 TB	32.25	191	8.13	6.22	16.46	1.05	1.90	216	151	12	0.59	119
Peanuts, raw	0.2 CP	30	174	7.91	5.68	14.79	1.04	2.07	205	2	26	0.55	155
Peanuts, roasted, dry roasted, salted	0.2 CP	30	174	7.91	5.68	14.79	1.04	2.76	205	244	26	0.55	155
Peanuts, roasted, oil roasted, salted	0.2 CP	30	174	7.91	5.68	14.79	1.04	2.76	205	130	26	0.55	155
Pecans, raw	0.3 CP	30	207	2.75	4.16	21.59	0.45	2.88	123	0	21	0.76	83
Poppy seeds	0.2 CP	30	160	5.41	7.11	13.41	2.03	3.00	210	6	435	2.82	255
Sesame seeds, kernels (hulled), dried	0.2 CP	30	176	7.91	2.82	16.43	1.39	3.48	122	12	39	2.34	233
Sesame seeds, kernels (hulled), toasted	0.2 CP	30	170	5.09	7.81	14.40	1.20	5.07	122	12	39	2.33	232
Sunflower seeds, dry roasted, salted	0.2 CP	30	175	5.80	7.01	14.94	1.68	2.70	255	234	21	1.14	347
Sunflower seeds, oil roasted, salted	0.2 CP	30	185	6.41	4.42	17.24	1.16	2.70	145	181	17	2.01	342
Sunflower seeds, on roasted, saited	0.2 CP	30	171	6.83	5.63	14.87	1.06	3.15	207	1	35	2.03	212
Tapioca, dry	1 TB	10	36	0.03	8.87	0.00	0.01	0.09	1	0	2	0.16	1
Walnuts, raw	0.25 CP	30	196	4.57	4.11	19.56	0.53	2.01	132	1	29	0.10	104
vvailuto, iavv	0.23 01	30	130	4.57	4.11	19.50	0.55	2.01	102	'	23	0.07	,

Table Continued

Description	Serving size	Weight (g)	Zn (mg)	Cu (mg)	Mg (mg)	Vit. A (IU)	Vit. Α (μg RE)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Folate (mcg)	Pantothenic acid (mg)	Vit. B <sub>6</sub> (mg)
Grains, flours, and cooked cereals (enriched or for	tified nutrier	its)											
Amaranth, dry	0.22 CP	45	1.43	0.35	120	4	0	0.036	0.095	0.581	22	0.473	0.099
Barley bran flour	0.30 CP	45	4.05	0.81	122	0	0	0.032	0.050	2.070	7	0.144	0.059
Barley flour	0.30 CP	45	0.90	0.15	43	0	0	0.167	0.050	2.822	4	0.063	0.180
Barley malt flour	0.28 CP	45	0.93	0.12	44	17	2	0.140	0.140	2.538	17	0.261	0.297
Barley, cooked	0.89 CP	140	1.04	0.21	39	0	0	0.098	0.056	2.240	11	0.140	0.126
Barley, dry	0.23 CP	45	0.96	0.19	36	0	0	0.086	0.050	2.070	10	0.126	0.117
Bread flour	0.33 CP	45	0.32	0.06	10	0	0	0.351	0.221	2.655	49	0.198	0.018
Buckwheat flour	0.38 CP	45	1.40	0.23	113	4	0	0.189	0.086	2.768	24	0.198	0.261
Buckwheat groats, dry	0.24 CP	40	0.97	0.25	88	3	0	0.088	0.108	2.056	17	0.492	0.140
Bulgur, cooked	0.77 CP	140	0.64	0.11	55	2	0	0.070	0.042	1.694	9	0.350	0.112
Bulgur, dry	0.32 CP	45	0.87	0.15	74	3	0	0.104	0.054	2.300	12	0.468	0.153
Cake flour	0.33 CP	45	0.28	0.06	7	0	0	0.401	0.194	3.056	49	0.207	0.014
Chickpea flour	0.50 CP	45	1.26	0.41	75	0	0	0.221	0.050	0.792	197	0.275	0.221
Corn bran, dry	3 TB	15	0.23	0.04	10	11	1	0.002	0.015	0.411	1	0.096	0.023
Corn flour	0.36 CP	45	0.80	0.08	50	99	10	0.644	0.338	4.428	59	0.297	0.167
Corn grits, white, cooked	1 CP	242	0.17	0.02	12	0	0	0.048	0.024	0.484	2	0.194	0.048
Corn grits, white, dry	0.26 CP	40	0.16	0.03	11	0	0	0.058	0.018	0.540	2	0.192	0.060
Corn grits, yellow, dry	0.26 CP	40	0.16	0.03	11	86	9	0.052	0.016	0.480	2	0.192	0.060
Cornmeal, cooked	1 CP	240	0.26	0.02	15	76	8	0.048	0.024	0.480	2	0.120	0.096
Cornmeal, masa harina, dry	0.39 CP	45	0.80	0.08	50	99	10	0.644	0.338	4.428	59	0.297	0.167
Cornmeal, self-rising, dry	0.33 CP	45	0.31	0.04	18	91	9	0.063	0.022	0.450	22	0.135	0.108
Cornmeanl, white, dry	0.33 CP	45	0.32	0.04	18	0	0	0.063	0.022	0.450	22	0.140	0.117
Cornmeal, yellow, dry	0.33 CP	45	0.32	0.04	18	96	10	0.063	0.022	0.450	22	0.140	0.117
Cornstarch	1.25 TB	10	0.01	0.01	0	0	0	0.000	0.000	0.000	0	0.000	0.000
Couscous, cooked	0.89 CP	140	0.39	0.13	22	1	0	0.070	0.042	1.666	10	0.588	0.056
Couscous, dry	0.26 CP	45	0.37	0.11	20	1	0	0.072	0.036	1.571	9	0.558	0.050
Cracked wheat, cooked	1 CP	242	1.55	0.19	73	4	0	0.242	0.121	3.340	23	0.532	0.169
Farina (creamed wheat), instant cooking, cooked	1 CP	241	0.31	0.07	12	0	0	0.024	0.024	0.241	5	0.145	0.024
Farina (creamed wheat), quick cooking, cooked	1 CP	239	0.31	0.05	12	0	0	0.024	0.024	0.239	5	0.167	0.024
Farina (creamed wheat), regular cooking, cooked	1 CP	251	0.30	0.08	10	1	0	0.025	0.025	0.251	5	0.176	0.025
Farina (creamed wheat), dry	0.23 CP	40	0.35	0.08	11	1	0	0.024	0.040	0.280	10	0.208	0.044
Hominy, canned	1 CP	165	1.73	0.05	26	69	7	0.000	0.017	0.050	2	0.248	0.000
Kamut flour	0.25 CP	45	1.32	0.17	62	4	0	0.203	0.099	0.950	20	0.455	0.153
Kasha, cooked	1 CP	168	1.24	0.32	114	4	0	0.118	0.134	2.638	22	0.638	0.185
Kasha, dry	0.24 CP	40	0.97	0.25	88	3	0	0.088	0.108	2.056	17	0.492	0.140
Millet, cooked	0.58 CP	140	0.76	0.34	52	19	2	0.182	0.126	2.14	38	0.378	0.168
Millet, dry	0.23 CP	45	0.76	0.34	51	19	2	0.189	0.130	2.124	38	0.383	0.171
Oat bran, cooked	1 CP	219	1.16	0.15	88	1	0	0.438	0.088	0.350	19	0.548	0.066
Oat bran, dry	0.43 CP	40	1.24	0.16	94	1	0	0.468	0.088	0.372	21	0.596	0.064
Oatmeal (fortified), flavored, dry	0.49 CP	55	1.08	0.13	47	1650	493	0.490	0.561	6.600	132	0.237	0.660
Oatmeal (fortified), plain, dry	0.49 CP	40	1.23	0.14	59	1428	428	0.428	0.484	5.716	114	0.500	0.572

Oatmeal, dry	0.49 CP	40	1.23	0.14	59	1	0	0.292	0.056	0.312	13	0.496	0.048
Oatmeal, instant cooking, flavored, cooked	1 CP	234	0.84	0.09	37	1273	381	0.374	0.421	5.101	102	0.187	0.515
Oatmeal, instant cooking, plain, cooked	1 CP	234	1.08	0.12	53	1258	377	0.374	0.421	5.031	101	0.445	0.515
Oatmeal, regular cooking, cooked	1 CP	234	1.17	0.14	57	1	0	0.281	0.047	0.304	12	0.468	0.047
Potato flour	0.28 CP	45	0.24	0.09	29	9	1	0.104	0.023	1.580	11	0.212	0.347
Quinoa, cooked	0.89 CP	140	1.25	0.31	80	3	0	0.070	0.154	1.106	18	0.392	0.084
Quinoa, dry	0.26 CP	45	1.49	0.37	95	4	0	0.090	0.180	1.319	22	0.473	0.099
Rice (creamed rice), dry	0.22 CP	40	0.45	0.10	9	0	0	0.168	0.048	2.256	12	0.220	0.080
Rice bran, dry	2 TB	15	0.91	0.11	117	0	0	0.413	0.042	5.100	9	1.109	0.611
Rice flour, brown	0.28 CP	45	1.10	0.10	50	0	0	0.198	0.036	2.853	7	0.716	0.333
Rice flour, white	0.28 CP	45	0.36	0.06	16	0	0	0.063	0.009	1.166	2	0.369	0.198
Rolled wheat, cooked	1 CP	242	1.07	0.19	50	3	0	0.169	0.121	1.984	32	0.363	0.169
Rolled wheat, dry	0.43 CP	40	1.06	0.18	49	3	0	0.160	0.120	1.960	31	0.368	0.156
Rye flour, medium	0.44 CP	45	0.90	0.13	34	5	0	0.131	0.050	0.779	9	0.221	0.122
Rye, whole grain, dry	0.44 CP	45	1.68	0.20	54	5	0	0.144	0.113	1.921	27	0.657	0.130
Self-rising flour	0.36 CP	45	0.30	0.06	10	0	0	0.329	0.207	2.489	45	0.189	0.018
Sorghum	0.24 CP	45	0.63	0.29	59	15	2	0.108	0.063	1.319	38	0.563	0.077
Soy flour, defatted	0.51 CP	45	1.11	1.83	131	3	0	0.315	0.113	1.175	137	0.900	0.257
Soy flour, full fat	0.51 CP	45	1.76	1.31	193	54	5	0.261	0.522	1.944	155	0.716	0.207
Soy flour, low fat	0.51 CP	45	0.53	2.29	103	17	2	0.171	0.126	0.972	185	0.819	0.234
Spelt	0.31 CP	45	1.67	0.18	60	4	0	0.171	0.068	2.952	20	0.455	0.153
Teff, dry	0.33 CP	45	1.68	0.10	63	19	2	0.140	0.063	0.810	38	0.433	0.171
Triticale, whole grain	0.23 CI 0.34 CP	45	1.20	0.44	69	5	0	0.240	0.059	1.287	33	0.977	0.180
Wheat bran (unprocessed)	0.34 CI 0.25 CP	15	1.09	0.25	92	2	0	0.171	0.033	2.037	12	0.327	0.195
Wheat, cracked whole wheat	0.23 CP	45	1.32	0.13	62	4	0	0.076	0.007	2.862	20	0.456	0.153
Wheat, germ	0.33 CF 2 TB	15	2.50	0.17	48	15	2	0.250	0.099	0.838	92	0.430	0.147
Wheat, hard red spring	0.23 CP	45	1.25	0.09	56	0	0	0.230	0.123	2.568	19	0.209	0.151
Wheat, hard red spring Wheat, hard red winter	0.23 CP	45 45	1.19	0.19	57	0	0	0.227	0.049	2.459	17	0.421	0.135
Wheat, hard white	0.23 CP	45 45	1.19	0.20	42	0	0	0.172	0.052	1.972	17	0.429	0.166
,	0.23 CP 0.27 CP		1.18	0.10	42 57	0	0	0.174	0.049	2.160	18	0.429	0.166
Wheat, soft red winter	0.27 CP 0.27 CP	45 45	1.16	0.20	40	0	0	0.177	0.043	2.160	18	0.383	0.122
Wheat arrested	0.27 CP 0.42 CP						3						
Wheat, sprouted		45 45	0.74	0.12	37 10	30 0	0	0.104	0.072	1.390	17 40	0.428	0.122
White flour, wheat, all-purpose (enriched)	0.36 CP 0.36 CP	45 45	0.32	0.06			0	<b>0.351</b>	0.221	2.655	<b>49</b>	0.198	0.018
White flour, wheat, all-purpose (unenriched)		45	0.32	0.06	10	0	0	0.054	0.018	0.563	12	0.198	0.018
Whole wheat flour, red wheat	0.38 CP	45	1.32	0.17	62	4	U	0.203	0.099	2.862	20	0.455	0.153
Pasta and rice (enriched nutrients)													
Chow mein noodles, crisp type	0.55 CP	25	0.35	0.04	13	0	0	0.145	0.105	1.488	23	0.133	0.028
Egg noodles, cooked	0.9 CP	140	0.87	0.13	27	69	19	0.042	0.028	0.560	10	0.196	0.056
Lo mein noodles, soft type, cooked	0.8 CP	140	0.74	0.14	25	10	1	0.028	0.140	0.560	10	0.154	0.056
Macaroni/spaghetti noodles, white, cooked	1 CP	140	0.74	0.14	25	10	1	0.028	0.028	0.560	10	0.154	0.056
Macaroni/spaghetti noodles, whole wheat, cooked	1 CP	140	1.13	0.24	42	4	0	0.154	0.056	0.994	7	0.588	0.112
Rice, brown, cooked	0.7 CP	140	0.88	0.14	60	0	0	0.140	0.028	2.142	6	0.392	0.196
Rice, brown, uncooked	0.25 CP	45	0.95	0.15	65	0	0	0.153	0.032	2.309	6	0.423	0.212
Rice, white, cooked	0.23 CI	140	0.69	0.10	17	0	0	0.133	0.022	0.560	3	0.546	0.126
Rice, white, uncooked	0.25 CP	45	0.68	0.09	17	0	0	0.032	0.022	0.720	4	0.540	0.126
, mino, anoonoa	0.20 0.	10	3.00	0.00	.,	Ü	Ü	0.002	0.022	0.720	7	0.040	continued

Table Continued

Description	Serving	Weight	Zn	Cu	Mg	Vit. A	Vit. A	Thiamin	Riboflavin	Niacin	Folate	Pantothenic	Vit. B <sub>6</sub>
	size	(g)	(mg)	(mg)	(mg)	(IU)	(μg RE)	(mg)	(mg)	(mg)	(mcg)	acid (mg)	(mg)
Rice/cellophane noodles, cooked	0.8 CP	140	0.35	0.06	4	0	0	0.028	0.000	0.098	4	0.014	0.014
Spaetze or spatzen, cooked	0.9 CP	140	0.39	0.04	9	126	38	0.196	0.224	1.386	34	0.392	0.042
Wild rice, cooked	0.85 CP	140	1.88	0.17	45	0	0	0.070	0.126	1.806	36	0.210	0.196
Wild rice, uncooked	0.3 CP	45	2.12	0.19	51	0	0	0.081	0.144	2.039	41	0.239	0.221
Cereal, ready-to-eat (fortified nutrients)													
Bran flakes without raisins	0.75 CP	29	3.77	0.15	41	1250	<i>375</i>	0.377	0.435	4.988	403	0.274	2.030
Bran nuggets, unsweetened	0.33 CP	30	1.50	0.14	62	510	151	0.360	0.420	5.100	404	0.446	2.010
Corn flakes, unsweetened	1 CP	28	0.08	0.02	3	501	141	0.364	0.428	5.012	102	0.920	0.504
Corn nuggets, unsweetened	1 CP	30	3.75	0.23	8	<i>500</i>	140	0.375	0.426	5.010	200	0.108	0.501
Corn, puffed	1.33 CP	30	3.75	0.03	8	<i>529</i>	155	0.375	0.426	5.010	200	0.121	0.501
Oat flakes	0.75 CP	30	3.75	0.10	49	<i>750</i>	225	0.375	0.425	5.000	400	0.328	2.000
Oat rings, unsweetened	1 CP	30	3.75	0.04	40	<i>500</i>	150	0.375	0.426	5.010	200	0.023	0.501
Rice flakes	1 CP	31	0.90	0.06	19	767	230	0.527	0.589	7.130	400	0.490	1.984
Rice nuggets, unsweetened	1.25 CP	33	0.60	0.07	16	750	225	0.429	0.462	5.511	116	0.323	0.561
Rice, puffed	1 CP	14	0.15	0.13	4	0	0	0.057	0.007	0.875	22	0.048	0.000
Wheat and barley flakes	0.75 CP	29	1.20	0.15	30	<i>750</i>	224	0.374	0.426	5.000	100	0.198	0.502
Wheat and barley nuggets	0.5 CP	58	1.20	0.21	58	<i>750</i>	224	0.377	0.423	5.000	100	0.501	0.499
Wheat flakes without raisins	1 CP	30	0.71	0.10	32	1250	<i>375</i>	0.375	0.426	5.001	100	0.216	0.501
Wheat, puffed	1.25 CP	15	0.46	0.09	20	1	0	0.095	0.059	0.792	23	0.072	0.020
Wheat, shredded, unsweetened	2 biscuits	46	1.26	0.14	54	3	0	0.124	0.051	2.562	20	0.423	0.184
Baby food cereals (fortified nutrients)													
Barley, instant, dry	6.25 TB	15	0.47	0.07	17	0	0	0.225	0.270	1.999	4	0.080	0.056
Oatmeal, instant, dry	6 TB	15	0.55	0.08	22	0	0	0.225	0.270	1.999	5	0.229	0.023
Rice, instant, dry	6 TB	15	0.23	0.04	21	5	0	0.225	0.270	1.999	2	0.128	0.104
Breads and other related products (enriched nutri	ients)												
Bagel, egg, plain or with seasoning	1 each	55	0.42	0.05	14	18	5	0.297	0.132	1.892	48	0.369	0.050
Bagel, oat bran, plain or with seasoning	1 each	55	0.47	0.08	22	0	0	0.319	0.193	2.162	49	0.297	0.033
Bagel, rye, plain or with seasoning	1 each	55	0.46	0.08	15	1	0	0.264	0.176	2.046	44	0.237	0.050
Bagel, white flour, plain or with seasoning	1 each	55	0.48	0.09	16	0	0	0.297	0.176	2.508	48	0.198	0.028
Bagel, whole wheat, plain or with seasoning	1 each	55	0.62	0.09	25	1	0	0.292	0.193	2.701	48	0.319	0.066
Biscuit, baking powder or buttermilk	1 each	55	0.28	0.04	9	38	11	0.231	0.171	1.705	32	0.187	0.017
Boston brown bread	2 slices	50	0.25	0.04	32	19	4	0.005	0.060	0.560	6	0.285	0.040
Bread crumbs, plain	1/4 CP	30	0.37	0.05	14	0	0	0.228	0.132	2.055	33	0.090	0.030
Bread or rolls, French	2 slices	50	0.43	0.10	14	0	0	0.235	0.145	2.190	48	0.190	0.025
Bread or rolls, rye	2 slices	50	0.57	0.10	20	1	0	0.215	0.170	1.900	43	0.220	0.040
Bread or rolls, sourdough	2 slices	50	0.43	0.10	14	0	0	0.235	0.145	2.190	48	0.190	0.025
Bread or rolls, white	2 slices	50	0.31	0.07	12	0	0	0.235	0.170	1.985	48	0.195	0.030
Bread or rolls, whole wheat	2 slices	50	0.97	0.14	43	2	0	0.175	0.100	1.920	25	0.275	0.090
Bread, barley	2 slices	50	0.61	0.09	18	7	2	0.210	0.140	2.010	<i>35</i>	0.220	0.055

Bread bran	2 slices	50	0.58	0.10	24	1	0	0.210	0.140	1.960	39	0.225	0.055
Bread, egg	2 slices	50	0.35	0.05	10	143	39	0.250	0.215	1.925	43	0.290	0.030
Bread, English muffin	2 slices	55	0.39	0.07	12	0	0	0.242	0.154	2.145	45	0.248	0.022
Bread, focaccia	1 slice	50	0.26	0.05	7	6	1	0.250	0.185	2.045	47	0.205	0.020
Bread, gluten-free	2 slices	50	0.63	0.11	31	1	0	0.205	0.125	1.325	33	0.280	0.050
Bread, hovis	2 slices	50	0.49	0.10	14	1	0	0.185	0.190	2.250	47	0.260	0.040
Bread, Irish soda	2 slices	50	0.30	0.07	11	105	28	0.175	0.135	1.260	23	0.160	0.045
Bread, Italian	2 slices	50	0.43	0.10	14	0	0	0.235	0.145	2.190	48	0.190	0.025
Bread, oatmeal	2 slices	50	0.51	0.11	19	2	1	0.200	0.120	1.570	31	0.170	0.035
Bread, pumpernickel	2 slices	50	0.57	0.10	20	1	0	0.215	0.170	1.900	43	0.220	0.040
Bread, raisin	2 slices	50	0.36	0.10	13	0	0	0.170	0.200	1.735	44	0.195	0.035
Bread, wheat, reduced calorie (light high fiber)	2 slices	50	0.56	0.07	20	1	0	0.210	0.150	1.940	36	0.315	0.065
Breadsticks, bread type	2 each	55	0.47	0.11	15	0	0	0.258	0.160	2.409	52	0.209	0.028
Breadsticks, cracker type	1.5 each	15	0.14	0.02	5	0	0	0.099	0.063	0.812	14	0.074	0.012
Crepe, plain	2 each	110	0.73	0.06	20	455	128	0.286	0.374	1.936	45	0.638	0.066
Croissant, plain	1 each	55	0.35	0.05	10	561	159	0.248	0.220	1.892	43	0.303	0.028
Croutons, plain	2/3 CP	7	0.06	0.01	2	1	0	0.043	0.019	0.381	9	0.030	0.002
Eggroll wrapper	1 each	32	0.20	0.03	5	22	7	0.179	0.131	1.334	26	0.154	0.013
English muffin, oat bran	1 each	55	0.59	0.08	30	0	0	0.335	0.248	2.244	<i>78</i>	0.479	0.055
English muffin, rye	1 each	55	0.48	0.07	14	1	0	0.253	0.237	2.321	<i>75</i>	0.379	0.066
English muffin, white	1 each	55	0.39	0.07	12	0	0	0.242	0.154	2.134	45	0.248	0.022
English muffin, whole wheat	1 each	55	0.88	0.12	39	4	0	0.165	0.077	1.876	27	0.385	0.088
Pancake, white flour, plain, from mix - no fat added	3 each	110	0.46	0.08	14	31	7	0.374	0.264	2.794	54	0.319	0.044
Pancake, white flour, plain, from recipe	3 each	110	0.68	0.07	20	211	62	0.352	0.374	2.431	<i>53</i>	0.594	0.055
Pancake, whole wheat, plain	3 each	110	1.50	0.17	64	208	61	0.198	0.253	2.453	26	0.792	0.165
Pita, white	1 each	50	0.42	0.09	13	0	0	0.134	0.048	1.070	12	0.200	0.015
Pita, whole wheat	1 each	50	0.76	0.15	35	3	0	0.170	0.040	1.420	18	0.415	0.130
Popover	1 each	55	0.42	0.03	10	155	46	0.165	0.220	1.116	28	0.402	0.039
Rolls, crescent (refrigerated dough)	2 each	50	0.19	0.04	7	0	0	0.175	0.110	1.535	22	0.180	0.020
Rolls, hamburger, white	1 each	50	0.31	0.06	10	0	0	0.240	0.155	1.965	48	0.265	0.020
Rolls, hamburger, whole wheat	1 each	50	0.97	0.14	43	2	0	0.175	0.100	1.920	25	0.275	0.090
Rolls, hard	1 each	50	0.47	0.08	14	0	0	0.240	0.170	2.120	48	0.205	0.020
Rolls, hot dog, white	1 each	50	0.31	0.06	10	0	0	0.240	0.155	1.965	48	0.265	0.020
Rolls, hot dog, whole wheat	1 each	50	0.97	0.14	43	2	0	0.175	0.100	1.920	25	0.275	0.090
Rolls, kaiser	1 each	50	0.47	0.08	14	0	0	0.240	0.170	2.120	48	0.205	0.020
Rolls, submarine or hoagie	1/2 each	50	0.44	0.10	14	0	0	0.260	0.165	2.375	48	0.195	0.020
Scone	1 each	55	0.36	0.07	11	433	116	0.209	0.198	1.491	31	0.292	0.050
Taco shell	2 each	30	0.42	0.04	32	52	5	0.069	0.015	0.405	32	0.141	0.090
Tortilla, corn	2 each	55	0.52	0.08	36	70	7	0.061	0.039	0.825	63	0.105	0.121
Tortilla, white flour	1 each	55	0.39	0.15	14	0	0	0.292	0.160	1.964	68	0.319	0.028
Tortilla, whole/wheat flour	1 each	55	0.85	0.11	40	2	0	0.132	0.066	1.854	13	0.297	0.099
Waffles, bran, plain	1 each	85	1.28	0.15	79	190	56	0.264	0.331	2.831	41	0.689	0.187
Waffles, white flour, from recipe	1 each	85	0.57	0.05	16	183	54	0.281	0.306	1.938	43	0.493	0.051
Waffles, white flour, plain, frozen	1 each	85	0.42	0.09	11	34	10	0.349	0.255	2.559	50	0.306	0.026
Wonton wrapper	1 each	8	0.05	0.01	1	6	2	0.045	0.033	0.334	7	0.038	0.003

Table Continued

Description	Serving size	Weight (g)	Zn (mg)	Cu (mg)	Mg (mg)	Vit. A (IU)	Vit. Α (μg RE)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Folate (mcg)	Pantothenic acid (mg)	Vit. B <sub>6</sub> (mg)
	5126	(9)	(IIIg)	(IIIg)	(IIIg)	(10)	(μy nL)	(IIIg)	(IIIg)	(IIIg)	(IIICG)	aciu (my)	(IIIg)
Crackers (enriched nutrients)													
Butter crackers	10 each	30	0.14	0.03	4	0	0	0.159	0.099	1.194	22	0.090	0.009
Cheese crackers	10 each	30	0.34	0.06	11	20	5	0.171	0.129	1.401	24	0.159	0.165
Cheese-filled sandwich crackers	4 each	30	0.19	0.02	11	31	9	0.135	0.204	1.131	25	0.153	0.015
Cheese-filled sandwich crackers, whole wheat	4 each	30	0.26	0.05	16	32	9	0.108	0.129	0.954	19	0.186	0.078
Matzo or matzoh crackers, egg	1 each	30	0.24	0.05	8	22	6	0.222	0.141	1.575	32	0.171	0.033
Matzo or matzoh crackers, plain	1 each	30	0.20	0.02	8	0	0	0.177	0.087	1.167	<i>35</i>	0.132	0.036
Matzo or matzoh crackers, whole wheat	1 each	30	0.78	0.10	40	0	0	0.110	0.081	1.623	3	0.370	0.048
Melba toast	3 each	15	0.30	0.04	9	0	0	0.041	0.617	0.104	19	0.104	0.015
Peanut butter-filled sandwich crackers	4 each	30	0.32	0.08	16	0	0	0.128	0.101	1.767	25	0.136	0.035
Rye wafer, plain	3 each	30	0.84	0.14	36	0	0	0.128	0.087	0.474	14	0.171	0.081
Saltine or soda crackers	10 each	30	0.23	0.06	8	0	0	0.170	0.139	1.575	37	0.137	0.011
Whole wheat crackers	10 each	30	0.64	0.13	30	0	0	0.060	0.030	1.356	8	0.243	0.054
Zwieback	2 each	15		0.01	1	30	9	0.048	0.033	0.377	7	0.033	0.003
Cookies (enriched nutrients)													
Animal cracker	12 each	30	0.28	0.04	12	1	0	0.108	0.069	0.999	14	0.123	0.027
Biscotti. with nuts	1 each	30	0.36	0.14	21	95	27	0.108	0.105	0.774	17	0.150	0.036
Brownie, butterscotch, without nuts	1 each	40	0.16	0.08	9	28	8	0.076	0.072	0.568	12	0.136	0.016
Brownie, chocolate, without nuts	1 each	40	0.38		22	46	12	0.056	0.080	0.420	10	0.136	0.016
Cookies and bars, arrowroot	3 each	30	0.28	0.04	12	1	0	0.108	0.069	0.999	14	0.123	0.027
Cookies and bars, butterscotch chip	3 each	30	0.17	0.06	7	291	76	0.078	0.063	0.537	12	0.108	0.018
Cookies and bars, chocolate chip	3 each	30	0.15	0.04	6	0	0	0.120	0.078	0.900	16	0.078	0.009
Cookies and bars, chocolate, wafer	7 each	30	0.33	0.14	16	2	1	0.060	0.081	0.858	15	0.144	0.015
Cookies and bars, date bar	1 each	30	0.14	0.05	9	5	0	0.060	0.033	0.477	7	0.126	0.024
Cookies and bars, fig bar	2 each	30	0.12		8	2	0	0.048	0.066	0.561	8	0.120	0.024
Cookies and bars, fortune cookie	4 each	30	0.12	0.05	6	0	0	0.040	0.042	0.444	8	0.039	0.024
Cookies and bars, fortune cookie  Cookies and bars, gingerbread	3 each	30	0.11	0.06	24	14	4	0.037	0.042	0.444	18	0.039	0.063
Cookies and bars, gingersnap	3 each	30	0.10		14	13	4	0.123	0.007	0.759	14	0.103	0.036
		30	0.14	0.08	9	0	0	0.099	0.072	0.759	14	0.123	0.036
Cookies and bars, graham cracker, plain	4 squares				21		24				20		
Cookies and bars, granola	3 each	30	0.69	0.13	2	83		0.111	0.084	0.798	20 10	0.162	0.093
Cookies and bars, lemon bar	2 each	30	0.13	0.02	7	294	78	0.060	0.069	0.423		0.117	0.012
Cookies and bars, macaroon (coconut)	1 each	30	0.22	0.04		0	0	0.003	0.039	0.063	1	0.093	0.030
Cookies and bars, molasses	3 each	30	0.14	0.07	27	0	0	0.117	0.072	0.948	16	0.141	0.072
Cookies and bars, oatmeal	3 each	30	0.35	0.07	16	323	85	0.132	0.075	0.639	14	0.180	0.018
Cookies and bars, peanut butter	3 each	30	0.29	0.04	14	133	35	0.069	0.060	1.353	15	0.132	0.039
Cookies and bars, pfeffernuesse	2 each	30	0.18	0.06	23	164	43	0.129	0.096	0.999	18	0.177	0.063
Cookies and bars, pizzelle	2 each	30	0.12	0.01	2	36	11	0.066	0.072	0.477	11	0.126	0.012
Cookies and bars, raisin	3 each	30	0.11	0.05	6	142	38	0.069	0.057	0.477	9	0.078	0.030
Cookies and bars, Rice Krispie bar	2 each	30	0.19	0.04	5	331	95	0.129	0.138	1.650	<i>35</i>	0.099	0.168
Cookies and bars, rosette	2 each	30	0.10	0.01	2	27	8	0.051	0.054	0.363	9	0.096	0.009
Cookies and bars, shortbread	3 each	30	0.16	0.03	5	540	141	0.153	0.102	1.140	21	0.096	0.009
Cookies and bars, sugar	3 each	30	0.12	0.02	3	12	4	0.105	0.075	0.789	15	0.090	0.009

Cookies and bars, sugar wafer with creme filling	4 each	30	0.17	0.06	7	1	0	0.057	0.045	0.426	8	0.081	0.018
Cookies and bars, vanilla sandwich	3 each	30	0.15	0.04	6	0	0	0.120	0.078	0.900	16	0.078	0.009
Cookies and bars, vanilla wafer	7 each	30	0.21	0.07	11	1	0	0.126	0.084	0.948	17	0.075	0.009
Krumkake	2 each	30	0.19	0.03	4	278	79	0.123	0.114	0.900	20	0.171	0.015
Cakes, pastries, and other desserts (enriched nutrients)													
Angel food cake, white or flavored, not frosted or glazed	1 piece	55	0.08	0.03	5	0	0	0.094	0.182	0.720	12	0.083	0.006
Anisette (mandelbrodt toast), plain	3 slices	30	0.17	0.02	4	34	10	0.123	0.105	0.891	19	0.153	0.015
Cake, apple, not frosted or glazed	1 piece	80	0.22	0.04	6	89	19	0.128	0.128	0.912	21	0.224	0.032
Cake, banana, not frosted or glazed	1 piece	80	0.37	0.06	10	400	107	0.208	0.208	1.512	31	0.288	0.032
Cake, butter, not frosted or glazed	1 piece	80	0.28	0.03	5	350	100	0.136	0.160	1.040	34	0.320	0.024
Cake, carrot, without nuts, not frosted or glazed	1 piece	80	0.30	0.06	8	4286	440	0.144	0.152	1.120	26	0.280	0.072
Cake, chiffon, not frosted or glazed	1 piece	55	0.28	0.03	5	115	34	0.110	0.165	0.764	23	0.297	0.028
Cake, chocolate, not frosted or glazed	1 piece	80	0.46	0.18	24	47	13	0.152	0.144	1.160	24	0.200	0.024
Cake, fruitcake	1 piece	125	0.79	0.29	47	503	129	0.287	0.213	1.738	39	0.488	0.138
Cake, German chocolate, not frosted or glazed	1 piece	80	0.46	0.18	24	47	13	0.152	0.144	1.160	24	0.200	0.024
Cake, gingerbread, not frosted or glazed	1 piece	80	0.30	0.17		29	8	0.200	0.136	1.672	28	0.376	0.200
Cake, jelly roll, yellow cake	1 piece	80	0.41	0.03	8	168	50	0.112	0.216	0.728	24	0.440	0.040
Cake, oatmeal, not frosted or glazed	1 piece	80	0.39	0.10		379	100	0.144	0.112	0.776	18	0.248	0.024
Cake, pound, chocolate, not frosted or glazed	1 piece	80	0.42	0.08	12	119	33	0.200	0.224	1.432	34	0.368	0.032
Cake, pound, white, not frosted or glazed	1 piece	80	0.38	0.05	7	122	34	0.200	0.224	1.448	35	0.376	0.032
Cake, spice, not frosted or glazed	1 piece	80	0.37	0.06	10	400	107	0.208	0.208	1.512	31	0.288	0.032
Cake, sponge, white or yellow, not frosted or glazed	1 piece	55	0.31	0.03	6	125	36	0.105	0.165	0.682	23	0.352	0.039
Cake, white, not frosted or glazed	1 piece	80	0.25	0.05	10	48	14	0.176	0.208	1.296	25	0.184	0.016
Cake, yellow, not frosted or glazed	1 piece	80	0.37	0.06	10	400	107	0.208	0.208	1.512	31	0.288	0.032
Cake, puff shell	2 each	80	0.59	0.05	10	1072	288	0.208	0.312	1.392	41	0.640	0.056
Cupcake, chocolate, commercial packaged	1.5 each	80	0.46	0.19	26	12	1	0.184	0.128	1.416	26	0.128	0.016
Dumplings with fruit, apple	1/2 each	125	0.19	0.06	8	136	29	0.188	0.125	1.363	25	0.138	0.038
Fritter, apple	1 CP	55	0.40	0.04	9	137	40	0.182	0.125	1.249	30	0.396	0.039
Phyllo pastry	1 3 sheet	28.35	0.15	0.03	5	0	0	0.164	0.102	1.245	23	0.094	0.009
Pie crust or shell, chocolate cookie type	1/8 pie	29.17	0.13	0.10	12	2	0	0.044	0.058	0.624	11	0.082	0.012
Pie crust or shell, egg yolk pastry, single layer	1/8 pie	29.77	0.19	0.03	4	42	13	0.131	0.095	0.965	21	0.158	0.015
Pie crust or shell, graham cracker, regular	1/8 pie	30.51	0.15	0.02	5	0	0	0.046	0.033	0.351	6	0.130	0.013
Pie crust or shell, puff pastry, frozen	1/2 shell	20	0.03	0.02	2	10	3	0.106	0.031	0.331	14	0.037	0.012
Pie crust or shell, regular pastry, single layer, frozen	1/2 shell	16.38	0.13	0.02	2	0	0	0.100	0.052	0.732	11	0.122	0.005
Pie crust or shell, regular pastry, single layer, prepared from mix	1/8 pie	20.8	0.10	0.02	3	0	0	0.106	0.052	0.799	15	0.040	0.006
Pie crust or shell, regular pastry, single layer prepared from recipe	1/8 pie	28.12	0.10	0.02	4	0	0	0.100	0.007	0.755	18	0.000	0.006
Pie crust or shell, vanilla wafer type	1/8 pie	36.97	0.12	0.02	11	305	79	0.127	0.075	0.939	17	0.073	0.000
Pies, apple, double pastry crust	1/8 pie	125		0.07	8	40	79 4	0.120	0.085 0.138	1.600	27	0.001	0.011
	•		0.20		-			0.200 0.150	0.136 0.250	0.950	21 29	0.200	0.036
Pies, chocolate cream	1/8 pie	125		0.24	42 15	363	103 30	0.130 0.128			29 18	0.588	
Pies, pudding filling, chocolate	1/8 pie	85 85	0.37		10	104			0.153	0.876			0.026
Pies, pudding filling, vanilla	1/8 pie	85 105	0.30	0.03		103	30 735	0.128 0.125	0.153 0.250	0.858 0.975	17 26	0.230 0.613	0.026
Pies, pumpkin	1/8 pie	125	0.56	0.09	23	7078							0.063
Shortcake, biscuit type	2 each	80 55	0.35	0.06	12	53	15	0.288	0.216	2.104	39	0.240	0.024
Shortcake, sponge type	2 each	55	0.31	0.03	6	125	36	0.105	0.165	0.682	23	0.352	0.039

Table Continued

Description	Serving size	Weight (g)	Zn (mg)	Cu (mg)	Mg (mg)	Vit. A (IU)	Vit. Α (μg RE)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Folate (mcg)	Pantothenic acid (mg)	Vit. B <sub>6</sub> (mg)
Granola and cereal bars (fortified nutrients)													
Breakfast bar	1.5 each	40	4.15	0.54	111	1389	417	0.416	0.476	5.564	111	2.780	0.556
Cereal bar	1 each	40	1.63	0.06	14	810	243	0.404	0.464	5.420	108	0.140	0.540
Snacks and chips (enriched nutrients)													
Bagel chips	0.5 CP	30	0.24	0.07	8	2	0	0.204	0.144	1.623	<i>37</i>	0.162	0.021
Cheese balls, puffs or twists	1 CP	30	0.20	0.03	9	42	4	0.147	0.096	0.999	26	0.108	0.057
Corn chips	1 CP	30	0.15	0.02	8	42	4	0.141	0.081	0.981	26	0.060	0.051
Corn nuts	0.35 CP	30	0.53	0.04	34	61	6	0.012	0.039	0.507	0	0.111	0.069
Popcorn commercially popped (prepopped), not "buttered"	2.75 CP	30	0.77	0.12	29	23	2	0.045	0.063	0.426	5	0.093	0.054
Popcorn, hot-air popped	3.75 CP	30	1.03	0.13	39	32	3	0.060	0.084	0.582	7	0.126	0.072
Popcorn, microwave popped from package, with salt	3.33 CP	30	0.63	0.08	24	19	2	0.036	0.051	0.354	4	0.078	0.045
Popcorn, popped in fat	2.75 CP	30	0.65	0.08	25	20	2	0.039	0.054	0.363	4	0.078	0.045
Pretzels, hard type	1.33 CP	30	0.26	0.08	11	0	0	0.138	0.186	1.575	51	0.087	0.036
Pretzels, soft type	1/4 medium	30	0.21	0.04	7	0	0	0.234	0.147	1.770	32	0.135	0.012
Rice cake	3 each	30	0.90	0.13	39	0	0	0.018	0.048	2.343	6	0.300	0.045
Taco or tortilla chips	1 CP	30	0.21	0.20	11	52	5	0.177	0.108	1.218	32	0.102	0.066
Wheat nuts	0.35 CP	30	1.41	0.13	26	8	1	0.138	0.069	0.462	51	0.117	0.081
Legumes													
Adzuki beans, cooked	0.4 CP	90	1.59	0.27	47	0	0	0.108	0.054	0.648	109	0.387	0.090
Bayo beans, cooked	0.5 CP	90	0.38	0.14	13	0	0	0.063	0.018	0.198	6	0.153	0.081
Black beans, cooked	0.5 CP	90	0.95	0.27	53	0	0	0.180	0.054	0.477	126	0.234	0.144
Broad beans, cooked	0.5 CP	90	0.95	0.27	53	0	0	0.180	0.054	0.477	126	0.234	0.144
Brown beans, cooked	0.5 CP	90	1.24	0.26	57	0	0	0.108	0.045	0.126	73	0.207	0.081
Cowpeas, cooked	0.5 CP	90	1.16	0.24	48	0	0	0.180	0.054	0.450	187	0.369	0.090
Fava beans	0.5 CP	90	0.40	0.50	31	292	29	0.063	0.054	0.963	19	0.144	0.108
Garbanzo beans, cooked	0.55 CP	90	1.38	0.32	43	0	0	0.108	0.054	0.477	155	0.261	0.126
Kidney beans, cooked	0.5 CP	90	0.96	0.22	41	0	0	0.144	0.054	0.522	117	0.198	0.108
Lentils, cooked	0.45 CP	90	1.14	0.23	32	18	2	0.153	0.063	0.954	163	0.576	0.162
Lima beans, cooked	0.5 CP	90	0.86	0.22	39	0	0	0.144	0.054	0.378	75	0.378	0.144
Mung beans, cooked	0.45 CP	90	1.24	0.26	57	0	0	0.108	0.045	0.126	73	0.207	0.081
Navy beans, cooked	0.55 CP	90	0.95	0.27	53	0	0	0.180	0.054	0.477	126	0.234	0.144
Northern beans, cooked	0.5 CP	90	1.24	0.26	57	0	0	0.108	0.045	0.126	73	0.207	0.081
Pigeonpeas, cooked	0.6 CP	90	0.90	0.16	32	96	10	0.171	0.054	0.801	58	0.540	0.045
Pinto beans, cooked	0.5 CP	90	0.97	0.23	50	15	2	0.171	0.081	0.360	155	0.252	0.144
Soybeans, cooked	0.5 CP	90	1.04	0.37	77	9	1	0.144	0.252	0.360	48	0.162	0.207
Soybeans, dry roasted, salted	0.3 CP	30	0.69	0.57	61	8	1	0.111	0.168	0.360	57	0.222	0.156
Soybeans, oil roasted, salted	0.3 CP	30	0.94	0.25	44	8	1	0.030	0.042	0.423	63	0.135	0.063
Split peas, yellow or green, cooked	0.45 CP	90	0.90	0.16	32	96	10	0.171	0.054	0.801	58	0.540	0.045

Tepary beans	0.35 CP	90	0.54	0.16	25	0	0	0.036	0.045	0.522	29	0.198	0.108
Meat substitutes													
Miso	1 TB	17	0.56	0.07	7	15	1	0.016	0.042	0.146	6	0.044	0.037
Tempeh	0.5 C	85	0.97	0.48	69	43	4	0.068	0.306	2.244	20	0.238	0.187
Tofu (soybean curd), extra firm	0.35 CP cubes	85	0.95	0.15	60	9	1	0.043	0.034	0.281	22	0.051	0.034
Tofu (soybean curd), firm	0.35 CP cubes	85	0.83	0.20	35	0	0	0.068	0.051	0.697	56	0.068	0.068
Tofu (soybean curd), silken	1 slice (1" thick)	85	0.52	0.17	23	0	0	0.085	0.034	0.213	39	0.043	0.094
Tofu (soybean curd), soft	0.35 CP cubes	85	0.54	0.14	23	0	0	0.043	0.034	0.459	37	0.043	0.043
Alcoholic beverages													
Beer, light, low calorie	8 FO	236	0.07	0.05	12	0	0	0.024	0.071	0.920	10	0.094	0.071
Beer, low alcohol	8 FO	240	0.00	0.00	13	0	0	0.000	0.000	0.000	0	0.168	0.120
Beer, regular	8 FO	237.6	0.05	0.02	14	0	0	0.024	0.071	1.609	14	0.143	0.119
Scotch, plain	1.5 FO	41.7	0.02	0.01	0	0	0	0.004	0.000	0.004	0	0.000	0.000
Whiskey, plain	1.5 FO	41.7	0.02	0.01	0	0	0	0.004	0.000	0.004	0	0.000	0.000
Ingredients used in grain products													
Almonds, raw	0.2 CP	30	1.01	0.33	83	3	0	0.072	0.243	1.179	9	0.105	0.039
Arrowroot flour	0.2 CP	30	0.02	0.01	1	26	3	0.000	0.000	0.000	2	0.039	0.003
Beer nuts, peanuts	0.2 CP	30	1.87	0.37	52	1	0	0.072	0.030	4.014	35	0.393	0.072
Cashews, oil roasted	0.2 CP	30	1.43	0.65	77	0	0	0.126	0.054	0.540	20	0.357	0.075
Coconut, dried, unsweetened	0.2 CP	15	0.30	0.12	14	0	0	0.009	0.015	0.090	1	0.120	0.045
Filberts, raw	0.2 CP	30	0.74	0.52	49	12	1	0.192	0.033	0.540	34	0.276	0.168
Flax seeds	0.25 CP	30	1.25	0.31	109	0	0	0.051	0.048	0.420	83	0.459	0.279
Flour, peanut, defatted	0.5 CP	30	1.53	0.54	111	1	0	0.210	0.144	8.100	74	0.822	0.150
Flour, peanut, low fat	0.5 CP	30	1.80	0.61	14	1	0	0.138	0.051	3.450	40	0.462	0.090
Macadamia nuts, raw	0.2 CP	30	0.39	0.23	39	0	0	0.360	0.048	0.741	3	0.228	0.084
Peanut butter, with salt	2 TB	32.25	0.94	0.05	51	1	0	0.026	0.032	4.322	24	0.261	0.145
Peanuts, raw	0.2 CP	30	1.99	0.39	56	1	0	0.075	0.033	4.284	38	0.417	0.078
Peanuts, roasted, dry roasted, salted	0.2 CP	30	1.99	0.39	56	1	0	0.075	0.033	4.284	38	0.417	0.078
Peanuts, roasted, oil roasted, salted	0.2 CP	30	1.99	0.39	56	1	0	0.075	0.033	4.284	38	0.417	0.078
Pecans, raw	0.3 CP	30	1.36	0.36	36	23	2	0.198	0.039	0.351	7	0.258	0.063
Poppy seeds	0.2 CP	30	3.07	0.49	99	0	0	0.255	0.051	0.294	17	0.239	0.132
Sesame seeds, kernels (hulled), dried	0.2 CP	30	3.08	0.44	104	20	2	0.216	0.024	1.404	29	0.204	0.045
Sesame seed, kernels (hulled), toasted	0.2 CP	30	3.07	0.44	104	20	2	0.363	0.141	1.632	29	0.204	0.045
Sunflower seeds, dry roasted, salted	0.2 CP	30	1.59	0.55	39	0	0	0.033	0.075	2.112	71	2.112	0.240
Sunflower seeds, oil roasted, salted	0.2 CP	30	1.56	0.54	38	15	2	0.096	0.084	1.239	70	2.082	0.237
Sunflower seeds, raw	0.2 CP	30	1.52	0.53	106	0	0	0.687	0.075	1.350	68	2.025	0.231
Tapioca, dry	1 TB	10	0.01	0.00	0	0	0	0.000	0.000	0.000	0	0.014	0.001
Walnuts, raw	0.25 CP	30	0.93	0.48	47	12	1	0.102	0.045	0.597	29	0.171	0.162

Grams = g; kilocalories = kcal; milligrams = mg; international units = IU; retinol equivalents = RE; micrograms =  $\mu$ g; milliliters = ml; K = potassium; Na = sodium; Ca = calcium; Fe = iron; P = phosphorus; Zn = zinc; Cu = copper; Mg = magnesium; Vit. A = vitamin A; CP = cup; TB = tablespoon; FO = fluid ounces; and micrograms of retinol equivalents =  $\mu$ g RE. Nutrition Data System for Research Food and Nutrient Database Version 33, © 2002 Regents of the University of Minnesota, Nutrition Coordinating Center. Values in bold italics indicate fortification or enrichment.

# **APPENDIX 2**

## **Foods for Celiac Diets**

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#### Introduction

Adherence to a gluten-free diet is the primary management strategy for anyone with celiac disease, dermatitis herpetiformis, or with any other form of gluten intolerance (*see* Celiac Disease). However, it is very difficult to be sure which foods are gluten free, except for certain products that are specifically labeled as being free of gluten, as is indicated by the gluten-free symbol (Figure 1).

This appendix provides a list of food ingredients, indicating which of them fall into one of the four categories indicated. The use of this list should thus assist in formulating a gluten-free diet through the use of ingredient labels on food products. These labels are listed and explained in Table 1.

Throughout this appendix, the term "gluten" refers to those proteins that cause celiac disease, the storage proteins of the grains of wheat, and of related cereal species, namely, rye, triticale, barley, and oats (*see* Cereals: Overview; Protein Chemistry. Gluten and Modified Gluten).

#### **Use of this List**

This list has been compiled by the Coeliac Society of Australia Inc., and it is reproduced here with



Figure 1 The gluten-free symbol, an ear of wheat crossed out.

permission. (The list does not come with any recommendations from this society on what is a "safe level" of gluten in the diet for celiacs. This list of food ingredients has been compiled in good faith from information available to the Coeliac Society of Australia Inc., which is a voluntary organization and is not a medical or professional body. The Society makes no recommendation as to its use and does not accept any responsibility for its use or misuse.)

The Coeliac Society of Australia Inc. believes that ideally, gluten should be entirely removed from the celiac diet, but that individual celiacs should consult their dietitian or doctor. To be labeled "gluten free" in Australia and New Zealand, a food must contain "no detectable gluten" by the most sensitive universally accepted test method. Current testing can achieve a detection level of 0.001-0.002% (10-20 ppm). Below this detection level, a food can be labeled "gluten free." The gluten content should then be included in the nutrition information panel on the product and be indicated as zero or not detected. Sometimes, foods labeled "gluten free" may contain ingredients that would otherwise be of concern, e.g., maltodextrin from wheat. In these circumstances, the gluten-free label overrides the ingredient listing on the food because either the individual ingredient or the final product is gluten free.

Many food additives are indicated by an international system of numbers. These may include colors, flavors, or flavorings, and they can include other substances as carriers, e.g., maltodextrin, starch, and dextrose in dry products. In Australia and New Zealand, they are identified if coming from a glutencontaining grain. Flavors used in candies, drinks, and ice cream are almost certainly gluten free. The additives maltitol — hydrogenated glucose syrup (965), caramel color (150), and amylase (1100) may possibly originate from gluten-containing grains, but they have been tested to contain "no detectable gluten." Monosodium glutamate (621) and other glutamates (620, 622, 623, 624, 625) may be derived from glucose syrup.

With respect to alcoholic beverages, advice to celiacs in Canada and the USA is to avoid all alcohol and alcoholic beverages derived from wheat or rye. This includes distilled products such as whisky, some vodka, and gin. As distillation is a recognized method of purification, clear distilled liquids (e.g., white vinegar, ethyl alcohol, and spirits) are regarded as gluten free in the following list. Alcoholic beverages are in

#### Table 1 Ingredients list

The following ingredient labels are used on food products: gluten free, X contains gluten, can sometimes be manufactured from or contain ingredients derived from a gluten-containing grain, and **7** no detectable gluten even if derived from a gluten-containing grain. Beans (lima) Beans (navy) Acacia gum (gum arabic) Beans (pinto) Acetic acid Beans (red kidney) Agar agar Beans (wax yellow) Albumen Beans sprouts Ale Beef Alfalfa sprouts Beef extract Algin (alginic acid) Beef fat Allspice Beef stock **Almonds** Beef tallow Alpha tocopherol acetate Beer Amaranth Beeswax Amylase Beetroot **Anchovies** Besan Anchovy extract Beta-carotene Anti-caking agents\* Beverage whitener  $^{\star}$  "anti-caking agents" do not normally contain gluten. On occasions a Bicarbonate of soda modified starch may be used. The term anti-caking agent should then be **Biotin** followed in brackets by a number in the 1400 series or the name "modified starch." **Bitters** Antioxidants Black bean Apple Black currant Apple juice Blackberry Apple puree Boysenberry Apricot **Brains** √ X Arrowroot Bran (wheat, oat, barley) Artichokes Bran (rice) Artificial sweetener Brandy\* Artificial sweetening substances \* May contain caramel color. Ascorbic acid Brazil nuts Asparagus Brewers yeast Aspartame Broccoli Aspic  $\mathbf{x}^{\vee}$ Bromelain Brussel sprouts √ X Aubergine (eggplant) Bucatini Buckwheat (kasha) В Buckwheat flour √ X Bacon\* Burghul (bulgar, bulgur, bulghur) Bacon (smoked)\* Butter \* Bacon may contain glucose syrup/dextrose - check ingredients. Butter oil Buttermilk solids Baking powder Bamboo shoots C Banana Banana extract Cabbage √X X X X Barley Caffeine Barley flakes Calamari (no flour or batter) Barley (malt) Calcium carbonate Barley (pearl) Calcium caseinate Basil Calcium cyclamate Batter (unless labeled "gluten free") Calcium hydrogen phosphate Bay leaf Calcium pantothenate Beans Canola oil Beans (borlotti) Capers Beans (broad) Capsicum Beans (butter) Caramel color Beans (garbanzo) Caraway Beans (kidney) Carbonated water

,	0 1 (010)	,	
$\sqrt{}$	Carboxymethyl cellulose (CMC)		Clams
	Cardamom		Cloves
$\sqrt{}$	Carmine	$\sqrt{}$	Cocoa
$\sqrt{}$	Carnauba wax	$\sqrt{}$	Cocoa butter
$\sqrt{}$	Carob	$\sqrt{}$	Cocoa fat
	Carob bean gum		Cocoa liquid
	Carob powder	$\checkmark$	Cocoa mass
	Carrageenan		Cocoa powder
V	Carrots	$\sqrt{}$	Coconut
V	Carrot juice	$\sqrt{}$	Coconut (desiccated)
\$/	Casein	$\sqrt{}$	Coconut cream
1/	Cashews	$\sqrt{}$	Coconut milk
./	Cassava (manioc)	$\sqrt{}$	Coconut oil
./	Cassia (oil)	$\sqrt{}$	Cod liver oil
·/	Cayenne pepper	/	Coffee
<b>v</b> /	Celery	√ <b>☑</b>	Cognac*
<b>v</b> /	Celery root powder		•
<b>v</b> <sub>/</sub>	· ·	,	* May contain caramel color.
$\sqrt{}$	Celery juice		Cointreau
$\checkmark$	Cellulose	$\sqrt{}$	Colors*
X	Cereal (wheat, rye, barley, and oats)		* "Colors" may contain certain diluents including starch and dextrose.
$\sqrt{}$	Champignons		However, this is not common and colors are used at very low levels.
$\sqrt{}$	Channa	$\checkmark$	Compounded chocolate
$\sqrt{}$	Cheese	$\sqrt{}$	Condensed milk
	Cheese (blue)	Ò	Confectionery
	Cheese (cheddar)		Confectionery sprinkles
1	Cheese (cottage)	. /	Copha
1/	Cheese (fetta)	<b>\</b> /	Copper sulfate
./	Cheese (mozzarella)	$\checkmark$	Coriander
./	Cheese (neufchatel)	$\sqrt{}$	Corn
<b>v</b> /	Cheese (parmesan)	$\sqrt{}$	
<b>v</b> /	Cheese (romano)	$\sqrt{}$	Cornstarch
V	· ·		Corn gluten (term used in the USA)
	Cheese flavor	$\sqrt{}$	Corn grits (maize)
$\sqrt{}$	Cheese powder		Corn solids
$\sqrt{}$	Cherries		Corn syrup
$\sqrt{}$	Cherries (black)		Corn syrup (solids)
	Cherries (glace)*	$\bigvee$	Corn flakes
	Cherries (imitation)*		Corn flour (maize)
	* May contain glucose syrup.	X	Corn flour (wheat)
./	Cherry paste	$\checkmark$	Corn meal (maize)
./	Chickpea	/	Cottonseed oil
<b>v</b> /	Chicken	×	Couscous
<b>v</b> /	Chicken fat	/	Crab
$\stackrel{\checkmark}{\Box}$	Chicken flavor	$\sqrt{}$	
		√ <sub>,</sub>	Cream (powder, light, sour, thickened)
	Chicken powder	$\sqrt{}$	Cream of tartar
Ц	Chicken stock	$\sqrt{}$	Cucumber
$\sqrt{}$	Chicory		Culture (e.g., acidophillus)
	Chilli		Cumin
$\sqrt{}$	Chilli powder		Currants
	Chives		Curry powder
	Chlorophyll	$\checkmark$	Cyanocobalamin
	Chocolate		Cyclamate
\$/	Chocolate (milk)	•	
ý	Chocolate (dark)	D	
1/	Chocolate bits	,	Dal (dhal)
<b>v</b> /	Cholecalciferol	√ <sub>/</sub>	Dasheen (eddo)
	Chutney	√ <sub>/</sub>	
<u></u>	Cider*	$\checkmark$	Dates
		X	Dextrin (wheat)
,	* May contain caramel color.	$\sqrt{}$	Dextrin (maize)
$\sqrt{}$	Cinnamon	<b>7</b>	Dextrose
$\sqrt{}$	Citric acid		Dextrose monohydrate
$\sqrt{}$	Citrus juices	$\sqrt{}$	Dibasic calcium phosphate
$\checkmark$	Citrus peel*		Dill
	* May contain glucose syrup.		Dill pickle

The following ingredient labels are used on food products: gluten free, X contains gluten, can sometimes be manufactured from or contain ingredients derived from a gluten-containing grain, and **7** no detectable gluten even if derived from a gluten-containing grain. Fruit (glace)\* Dill seeds √ X \* May contain glucose syrup. Dinkel (spelt) Χ Fruit juice concentrate Durum wheat Fruit pulp Fruit sugar syrup Ε Egg G Eggplant Galangal root Egg albumin Garlic Egg liquid Garlic powder Egg powder Gelatine Egg white  $\Box$ Gherkin Egg white powder Gherkin relish Egg yolk Gin\* Egg yolk powder \* Distilled from fermented grains. Elderberry juice 7 **Emulsifiers** Ginger 7 Glucono delta lactone Enzyme amylase ✓ Glucose Ethyl alcohol\* ✓ Glucose powder \* Ethyl alcohol may be produced from wheat but is distilled. **7** Glucose syrup Ethyl maltol **7** Glucose syrup (dried) Ethyl vanillin Х Gluten Glycerin F Glycerol x \ \ \ \ \ \ \ \ \ Farina Glycine Fat (animal, vegetable) Golden syrup Fenugreek Graham flour Ferrous sulphate Gram (chickpea) Figs Grapefruit (juice) Fish Grape juice Flavors\* Guar gum\*  $^{\star}$  "Flavor" and "natural flavor" may include carriers; in dry products, \* May have laxative effect (unrelated to gluten). maltodextrin, starch or dextrose can be used. In savory products, flavors Guava may be derived in part from the hydrolysis of cereals. Sweet flavors used in ice cream, drinks, candies, etc., should be gluten free. Guava juice 1 Flavor enhancers (see monosodium glutamate) Gum arabic (acacia gum) Flax Н Flax seeds  $\sqrt{}$ Ham Flour (barley) Ham (manufactured) Flour (oat) Hazelnuts Flour (plain) Herbs Flour (rye) Flour (self raising) Herring Hominy (hominy grits) Flour (spelt) Homous (hummus, hommous) Flour (wheat) Flour (wholemeal) Honey (powder) **レイイイイイ** Flour (amaranth) Honey crumble pieces Flour (buckwheat) Hops Flour (potato) Horseradish (extract) Hydrolyzed protein (maize, soy) Flour (rice) Hydrolyzed grain protein (maize, soy) Flour (soy) Hydrolyzed plant protein (maize, soy) Flour (tapioca) Hydrolyzed vegetable protein (maize, soy) Flour treatment agents Hydrolyzed wheat protein Fondant √ **☑** Food acids Fructose I **7** Fructose syrup Icing sugar mixture (from maize starch) X Fruit (dried mixed)\* Icing sugar mixture (from wheat starch)

/	Icing sugar (pure)	Χ	Malt
Ĭ	Inulin	X	Malt extract
/	Invert sugar	Х	Malt flavoring
/	Invert syrup	X	Malt sugar/syrup
/,	Invertase	X	Malt vinegar
//	Iron	X	Malted milk
√,			
√,	Iron pyrophosphate	$\sqrt{}$	Maltodextrin (maize, potato, tapioca)
$\checkmark$	Isomalt	X	Maltodextrin (wheat)*
			* The gluten level in wheat maltodextrin is very low. On occasions gluten
J			may be not detectable.
$\sqrt{}$	Job's tears (millet)	$\sqrt{}$	Maltol
•		<b>7</b>	Maltitol (hydrogenated glucose syrup)
K		$\checkmark$	Maltose
	Karaya gum	$\checkmark$	Mandarin (juice)
√ <u>,</u>	Karaya gum	/	Manganese sulphate
√ <b>&lt;</b>	Kasha (buckwheat)	√ ./	Mango
ζ,	Kawmut (polish wheat)	<b>v</b> /	Manioc (cassava)
√.	Kippers	$\sqrt{}$	Mannitol
$\checkmark$	Kiwi fruit	V	
$\sqrt{}$	Kudzu		Manufactured ham/meat
X	Kumat <sup>®</sup>	$\sqrt{}$	Margarine
		$\sqrt{}$	Marjoram
I		<b>7</b>	Marsala wine*
	Lactase		* May contain caramel color.
√,	Lactic acid		Marzipan
√,			Mayonnaise
√ X	Lactose		Meat
X <sub>,</sub>	Lager		
√ <u>.</u>	Lamb		Meat (processed)
$\checkmark$	Lard	$\sqrt{}$	Melon
$\checkmark$	L-carnitine	$\sqrt{}$	Menthol
/	L-cystine	$\sqrt{}$	Methyl cellulose
)	L-cysteine	$\sqrt{}$	Milk
./	Lecithin		Milk (curd)
`/	Leeks	<b>1</b>	Milk (full cream)
V <sub>/</sub>	Legumes	•/	Milk (skim, skim concentrate)
ν,	Lemon essence/oil	./	Milk (skim dried)
ν,		<b>v</b> /	Milk fat
√,	Lemon juice concentrate	<b>v</b> /	Milk protein
√,	Lemons	$\sqrt{}$	
√.	Lentil flour	$\sqrt{}$	Milk solids
√.	Lentils	$\sqrt{}$	Millet
$\checkmark$	Lettuce	$\sqrt{}$	Millet meal
Ì	Licorice	$\sqrt{}$	Mineral oils
/	Licorice extract		Mineral salts
./	Lima beans		Mineral water
./	Lime	$\sqrt{}$	Mint
<b>v</b> /	Linoleic acid	1/	Mint essence
ν,	Linseed	./	Mint flakes
√,		<b>v</b> /	Mint leaves
√_	Linseed oil	<b>v</b> <sub>/</sub>	Modified starch (corn, maize, potato, tapioca)
1	Liqueur*	×	Modified starch (wheat)
	* May contain caramel color.		, ,
$\checkmark$	L-methionine		Molasses
./	Lobster	$\sqrt{}$	Monoglyceride
./	Locust bean gum (carob bean)	X	Monosodium glutamate (MSG) (if from wheat)
<b>v</b> /	Loganberry	$\checkmark$	Mushrooms
v <sub>/</sub>	Lupin	ý	Mussels
<b>v</b> ,		•/	Mustard (pure)
ν,	Lupin fiber	√ □	Mustard (prepared)
$\checkmark$	Lychees		Mustard flour
		<b>v</b> /	Mustard seed
М		$\mathbf{v}_{_{/}}$	Mutton
	Macadamia	$\checkmark$	WIGHT
$\sqrt{}$			
\ \	Macaroni (rice)		
√ √ <b>X</b>	` ,	N	
√ × ./	Macaroni (rice) Macaroni (wheat) Mackerel	N √	Niacin

The following ingredient labels are used on food products: gluten free, X contains gluten, can sometimes be manufactured from or contain ingredients derived from a gluten-containing grain, and **7** no detectable gluten even if derived from a gluten-containing grain. Noodles/egg noodles (rice) Peas (chick) Noodles/egg noodles (wheat) Peas (garbanzo) Nori Peas (gram) Nutmeg Peas (green) Nuts Peas (split)  $\sqrt{}$ Nuts (plain) Peas (yellow) Nuts (dry roasted) Pecan nuts Nuts (mixed) Pectin Peel (mixed)\* 0 \* May contain glucose syrup. Χ Oatmeal Pepper (black) Oats Pepper (white) Oats (rolled) Pepper extract Oat bran Peppercorns Oat flour Peppermint oil Oat gum Pepperoni Olives (black, green) **Peppers** Onion Peppers (chilli) Onion (green) Peppers (green) Onion (kibbled) Peppers (hot) Onion juice Peppers (jalapeno) Onion powder Peppers (red) Phylloquinone Orange √ □ X **Pickles** Orange juice concentrate Oregano Pilcorn (oats) Oregano flakes Pimento Oyster juice extract Pine nuts Oyster sauce Pineapple Pineapple juice concentrate Oysters (smoked) Plum pulp Plum puree Plums Palm oil Poi (fermented taro) Papain Polenta Papaya Polydextrose\* Papaya juice Paprika \* Manufactured in the USA from dextrose and sorbitol. Cereal origins almost certainly maize. Parsley Poppy seeds Parsley juice Pork Parsnip Pork livers Passionfruit  $\checkmark$ Port\* Passionfruit juice/concentrate ☑ Port wine\* Pasta (corn, rice) \* May contain caramel color. Pasta (unless labeled gluten free) Paw paw Χ Porter Paw paw puree Potassium iodate Pea juice Potato (dried instant mashed) Peaches Potato flour Peach juice/puree Potato granules Potato starch Peanuts Peanuts (dry roasted) Potatoes Peanut butter\* Praline **Prawns** \* May contain glucose syrup or maltodextrin. Preservatives Peanut oil **Propellants** Pear juice Protease enzyme Pears Prunes/juice Peas (besan) Psyllium Peas (channa)

/	Pulse fiber	/	Sesame meal
<b>v</b> /	Pyridoxine hydrochloride	<b>v</b> /	Sesame seeds
V	T yriddxille Trydrodilloridd	<b>v</b> /	Shallots
^		√ <b>✓</b>	Sherry*
Q	Outro	٠	* May contain caramel coloring.
$\sqrt{}$	Quince	,	-
$\sqrt{}$	Quinine	$\sqrt{}$	Shortening
$\sqrt{}$	Quinoa	$\sqrt{}$	Shortening (vegetable)
		$\sqrt{}$	Shrimp
R			Shrimp paste
$\sqrt{}$	Radish	$\Box$	Shrimp powder
$\sqrt{}$	Rapeseed		Snow peas
1/	Raspberry	$\Box$	Soba noodles
\ <u>\</u>	Red currant	$\sqrt{}$	Sodium acid pyrophosphate
./	Rennet		Sodium bicarbonate
./	Riboflavin	$\sqrt{}$	Sodium caseinate
./	Ribonucleotides	V	Sodium citrate
./	Rice	V	Sodium metabisulphite
<b>v</b> /	Rice beverages*	1/	Sodium molybdate
<b>v</b> /	Rice bran	<b>v</b> /	Sodium nitrate
<b>v</b> /	Rice cereal	·/	Sodium nitrite
<b>v</b> /	Rice extract*	×/	Sorbic acid
<b>v</b> /	Rice ground/flour/starch	$\checkmark$	Sorbitol
<b>v</b> /	<u> </u>	. /	Sorghum
$\sqrt{}$	Rice noodles	×	Sour dough (unless identified gluten free)
$\checkmark$	Rice syrup*	,	Soy bran
	* May be manufactured using amylase derived from malted cereals –	$\sqrt{}$	Soybeans
,	residual gluten traces seem unlikely.	√ <sub>/</sub>	
$\sqrt{}$	Rice vermicelli	√ <sub>/</sub>	Soy fiber
$\checkmark$	Rice (glutinous)*	<b>√</b> ,	Soy flour
	* Despite the similar sounding term, this does not contain gluten.	$\sqrt{}$	Soy grits
./	Rice (malted)*		Soy isolate
V	* May be manufactured using amylase derived from malted cereals –		Soy milk (soy drink, soy beverage)
	residual gluten traces seem unlikely.	$\bigvee_{\square}$	Soy protein
/	Rice (wild)		Soy sauce
<b>v</b> /	Rockmelon	X	Spaghetti (unless labeled gluten free)
<b>v</b> /		$\sqrt{}$	Spaghetti (corn, rice)
<b>v</b> ,	Rose hip juice		Spearmint oil
$\checkmark$	Rosemary	X	Spelt (dinkel or German wheat)
V	Rum*		Spice extract
	* May contain caramel color		Spice oils
X	Rye		Spices (pure)
Χ	Rye flour/meal	V	Spinach
<b>7</b>	Rye whisky*	V	Spinach powder
	* May contain caramel color.	1/	Spirit (fermented)
Χ	Rye (kibbled/sour)	\ <u>\</u>	Starch (corn, modified corn, pregel corn)
		V.	Starch (maize, modified maize, pregel maize)
S		/	Starch (potato, modified potato, pregel potato)
7	Saccharin	\ \ \	Starch (tapioca, modified tapioca, pregel tapioca)
<b>v</b> /		×	Starch (wheat, modified wheat, pregel wheat)
<b>v</b> /	Saffron		Stock
	Sago	. /	Strawberry
	Salami	<b>v</b> /	Sucralose
$\sqrt{}$	Salmon	$\sqrt{}$	Sucrose
$\sqrt{}$	Salt	√ <sub>/</sub>	
	Sardines	$\checkmark$	Sugar
$\sqcup$	Sauces	<b>√</b> ,	Sugar
$\checkmark$	Sauerkraut		Sugar (brown)
X	Sausages (unless identified as gluten free)	$\checkmark$	Sugar (caramelized)
$\sqrt{}$	Sausages (gluten free)	$\sqrt{}$	Sugar (caster)
	Scotch whisky*		Sugar (icing) (pure)
<b>7</b>		<b>\</b>	Sugar (icing/mixture - maize, corn, rice starch)
<b>7</b>	* May contain caramel color.	. v	
		X	Sugar (icing/mixture - wheat starch)
<ul><li>✓</li><li>✓</li></ul>	* May contain caramel color.  Seasoning Seaweed	X √	Sugar (inverted)
	Seasoning	X √	,

	The following ingredient labels are used on food products:		
	gluten free,		
	X contains gluten,		
	can sometimes be manufactured from or contain i	naredier	nts derived from a gluten-containing grain, and
	✓ no detectable gluten even if derived from a gluten	-	
$\sqrt{}$	Swede	X	Vegetable protein extract (wheat, barley, malt)
$\sqrt{}$	Sweet potato	$\checkmark$	Vegetable protein hydrolyzed (soy)
$\sqrt{}$	Sweetener	X	Vegetable protein hydrolyzed (wheat)
	Szechuen sauce	$\sqrt{}$	Vegetable (dehydrated)
		$\sqrt{}$	Verbena
Т		×	Vermicelli (rice)
$\sqrt{}$	Tallow	Χ	Vermicelli (wheat)
/	Tallow (beef refined)	$\sqrt{}$	Vinegar (balsamic)
$\bigvee$	Tamari	$\checkmark$	Vinegar (white distilled)
/	Tamarind	$\sqrt{}$	Vinegar (wine)
<b>\</b> /	Tapioca (flour)	<b>7</b>	Vinegar (cider)*
<b>V</b> /	Taro (dasheen, eddo)	<b>7</b>	Vinegar (distilled)*
<b>\</b> _/	Tartaric acid		* May contain caramel color.
<b>v</b> /	Taurine	Χ	Vinegar (grain)
<b>v</b> <sub>/</sub>	Teff	X	Vinegar (malt)
<b>V</b> ,	Textured vegetable protein (soy)		Vitamins
V	• • • • • • • • • • • • • • • • • • • •	$\sqrt{}$	Vodka*
X	Textured vegetable protein (wheat)	V	* Distilled from fermented grains.
<b>v</b> ,	Thiamin (hydochloride) This leave (including 1400, 1450)* (corp. maize	W	Biotiliod Horri formonica graino.
$\checkmark$	Thickener (including 1400–1450)* (corn, maize,	,	Wolnuto
V	potato, tapioca) Thisk pay (including 1400, 1450)* (wheet)	$\sqrt{}$	Water sheets to
Χ	Thickener (including 1400–1450)* (wheat)	$\checkmark$	Water chestnuts
	* The number on the thickener indicates how the starch is processed not the source.	X	Wheat
/		X	Wheat flakes (malked malled)
$\sqrt{}$	Thyme	X	Wheat flakes (malted, rolled)
<b>√</b> ,	Tofu	X	Wheat germ (concentrated)
$\sqrt{}$	Tomato juice/paste/powder	<b>✓</b>	Wheat at a target
<b>√</b> ,	Tomatoes	X	Wheat starch
<b>√</b> ,	Tragacanth gum	X	Wheat (cracked)
$\sqrt{}$	Treacle	X	Wheat (kibbled)
$\sqrt{}$	Triglycerides	X	Wheat (puffed)
X	Triticale	X	Wheatmeal
<b>√</b> ,	Tuna	$\sqrt{}$	Whey powder
$\sqrt{}$	Turkey	<b>7</b>	Whisky (rye)*
$\sqrt{}$	Turkey meat (unprocessed)	<b>7</b>	Whisky (scotch)*
$\sqrt{}$	Turmeric	$\sqrt{}$	Wine (red)
$\checkmark$	Turnip	$\checkmark$	Wine (white)
		<b>7</b>	Wine (fortified)*
U			* May contain caramel color.
$\sqrt{}$	Urd (urad)		Worcestershire sauce
		v	
V		X √	Xanthan gum
$\sqrt{}$	Vanilla	V	Adminan gum
$\sqrt{}$	Vanilla bean	v	
$\sqrt{}$	Vanilla bean extract	Y	V.
$\checkmark$	Vanilla essence	$\sqrt{}$	Yams
$\sqrt{}$	Vanilla essence (imitation)		Yeast
$\sqrt{}$	Veal	√ X	Yeast extract (from molasses)
X	Vegetable extract (wheat, barley, malt)		Yeast extract (malt, barley)
$\checkmark$	Vegetable fat		Yogurt
$\sqrt{}$	Vegetable fat (hydrogenated)	$\sqrt{}$	Yogurt culture
X	Vegetable fiber (wheat)	$\checkmark$	Youngberries
$\sqrt{}$	Vegetable fiber (lupin, soy)		
$\sqrt{}$	Vegetable gum	Z	
$\checkmark$	Vegetable oil	$\checkmark$	Zinc sulphate
$\sqrt{}$	Vegetable oil (brominated, soy)	$\sqrt{}$	Zucchini

general not labeled with an ingredient statement. Whisky, brandy, fortified wines (such as sherry and port), and some liqueurs may contain caramel color, which can be derived from starch and contain no detectable gluten.

See also: Celiac Disease. Cereals: Overview; Protein Chemistry. Fortification of Grain-Based Foods. Gluten and Modified Gluten. Labeling of Grain-Based Foods. Maize: Foods from Maize. Nutrition: Guidelines for Grain-Based Foods; Soy-Based Foods.

# **Further Reading**

Nilson B (ed.) (1970) The Coeliac Handbook. London: The Coeliac Society.

#### **Relevant Websites**

http://www.coeliac.org.au - Coeliac Society of Australia Inc.

http://www.celiac.com - Celiac Disease and Gluten-Free Diet Online Resource Center.

http://www.glutenfreemall.com - Gluten-Free Mall

http://www.niddk.nih.gov/health/digest/pubs/celiac/ index.

htm - National Digestive Diseases Information Clearinghouse, part of the US National Institutes of Health.

# **APPENDIX 3**

# **Commercial Websites**

http://just-food.com - EU: Food labels may require full ingredients listings (2002).

http://www.abb.com.au — Web page for an Australian marketing agent. This site also describes barley receival standards for Australia.

http://www.abf.co.uk - Associated British Foods, UK.

http://www.acti.de – A web page for an international grain merchant.

http://www.ambainc.org — The primary purpose of The American Malting Barley Association, Inc. (AMBA) is to ensure an adequate supply of high-quality malting barley for the malting and brewing industry, through development of malting barley varieties with improved agronomic and quality characteristics.

http://www.amylum.com

http://www.apvbaker.com

http://www.arvalisinstitutduvegetal.fr – ARVALIS Institut du végétal, France.

http://www.auto-bake.com

http://www.avebe.com — Website of Avebe Company, an international company specializing in potato starch products, has useful information on starch applications.

http://www.barilla.com - Barilla, Italy.

http://www.brewingresearch.co.uk - BRI (Brewing Research International), Nuffield, UK.

http://www.bri.com.au — BRI Australia Ltd., Australia (previously, the Bread Research Institute of Australia).

http://www.buhlergroup.com — Bühler, with head offices in Switzerland, is a leading manufacturer of food processing and chemical processing equipment. They are the largest manufacturer of milling equipment. The website provides information on their most recent equipment and milling innovations.

http://www.burcon.ca - Information on a new commercial canola protein isolate can be found at this website.

http://www.campden.co.uk — The Campden and Chorleywood Food Research Association, Chipping Campden, UK.

http://www.canola.com, http://www.canolainfo.org, and http://www.canola-council.org — A description of canola processing can be found at these sites.

http://www.cargill.com - Web page of an international grain handler and merchant.

http://www.carlsberg.com - Carlsberg Brewers.

http://www.cbot.com - Chicago Board of Trade.

http://www.centralsoya.com – Central Soya Company, Fort Wayne, IN, USA.

http://www.cerestarfoodandpharma.com – Cargill Cerestar BVA, Mechelen, Belgium.

http://www.cheerios.com - General Mills, Inc.

http://www.conagramilling.com - ConAgra Grain Processing Company in Omaha, NE, USA.

http://www.corn.org — This is the website for the Corn Refiners Association, Inc. (CRA), which is the national trade association based in Washington, DC representing the corn refining (wet milling) industry of the US. CRA conducts programs of research and technical service, public relations, and government relations for the association membership. It is the primary source of educational material on corn and products from corn for schools, government, journalists, agriculture, and agribusiness. CRA publishes "The Corn Annual," which documents yearly data on corn industry statistics.

http://www.cwb.ca — Web page for the Canadian wheat and barley marketing agent.

http://www.esake.com – A site based in the USA at which premium saké can be purchased.

http://www.foodstarch.com, http://www.carbohydratenutrition.com — National Starch and Chemical Company, Bridgewater, NJ, USA.

http://www.franceexportcereales.org

http://www.fritolay.com - Frito-Lay, Inc.

http://www.gafta.com - This site indicates the extent of contracts available for the delivery of grain.

http://www.gbsgroupspa.com — The GBS Group is headquartered in Italy. It markets milling, feed milling, and related equipment under the brand names Sangati-Berga and Golfetto. All of their milling equipments are described in detail in this website.

http://www.generalmills.com - General Mills Whole Grains 101.

http://www.genmills.com - General Mills.

http://www.gmabrands.com - Grocery Manufacturers of America.

http://www.goodmanfielder.com.au – Goodman Fielder, Australia.

http://www.grainscanada.gc.ca - A web page on sampling.

http://www.grdc.com.au - Australian Grains Research and Development Corporation, Australia.

http://www.gwmfg.com - Great Western Manufacturing, based in Kansas, USA is a leading manufacturer of flour mill sifters. The website describes the latest sifters in detail.

http://www.healthychoice.com - ConAgra, Inc.

http://www.heartlandfields.com - This is a vegetarian site that supplies soy-based foods to the wholesale market.

http://www.hgca.co.uk - Web page of a UK promoter of cereal grains.

http://www.ilovepasta.org - Some background information about pasta, history, recipes, nutritional information.

http://www.interbrew.com - Interbrew, European Union.

http://www.japan-guide.com — A site all about Japan including travel and living as well as food. Japanese alcoholic beverages can be purchased.

http://www.jibt.com - The Japan Institute of Baking Technology, Tokyo, Japan.

http://www.katzen.com - Katzen International, Inc. with headquarters in Cincinnati, OH, provides innovative and advanced design concepts to a wide variety of industries, including the ethanol industry. As a technology company, Katzen's goal is to provide value-added utilization of renewable resources through development and commercialization of new and innovative technologies.

http://www.kelloggs.com - Kellogg Company in Battle Creek, MI, USA.

http://www.kice.com - Kice Industries headquartered in Kansas, USA is an industry leader in mill automation and pneumatic conveying. They also market a modular short flow mill and grain handling and storage products. Kice also services and supplies GBS Group equipment. The website has detailed descriptions of their products.

http://www.kraftfoods.com – Kraft Foods/Post Cereals.

http://www.leatherheadfood.com - Leatherhead Food International, UK.

http://www.limagrain.com - Groupe Limagrain, France.

http://www.lmc.co.uk - This is the website for LMC International Ltd., an economic and business consulting company specializing in economic, marketing, and planning services in the field of agricultural products, their downstream markets, and their synthetic substitutes. Its headquarters are in Oxford, England, and an office in New

York, NY which serves USA, Canada, and Mexico. LMC has a specialist team of economists who provide essential analysis and advice for the global starch and fermentation products industry. It publishes a monthly bulletin on "Starch and Fermentation Analysis.".

http://www.manildra.com.au - A major manufacturer of wheat starch, vital dry gluten and modified gluten products.

http://www.marukome.co.jp - A Japanese site dealing with miso and its uses.

http://www.mazola.com - Mazola Oils.

http://www.midwestgrain.com - MGP Ingredients Inc., Atchison, KS, USA.

http://www.monsanto.com - Monsanto Company, St. Louis, MO, USA.

http://www.nationalstarch.com - Website of National Starch and Chemical Company, Bridgewater, NJ, USA, a division of ICI. It has some useful technical information on starch functionality.

http://www.newport.com.au - Newport Scientific Pty Ltd., Warriewood, NSW, Australia, manufacturer of the Rapid Visco Analyzer and Dough Lab equipment.

http://www.nippin.co.jp - Nippon Flour, Japan. http://www.nisshin.com - Nisshin Flour Milling,

http://www.ocrim.com - Ocrim, headquartered in Italy, markets milling, handling, and automation equipment. Ocrim also have a Milling Training Centre which offers vocational training. The webiste describes all of Ocrim's equipment.

http://www.ohiocorn.org - Ohio Corn Marketing. http://www.peanutsusa.com - The American Peanut Council.

http://www.pos.ca - A description of improved methods for canola processing to give higher-quality meal may be found at this website.

http://www.pulseaus.com.au - This site provides information on production, breeding, and marketing of pulse crops in Australia and has links to other Australian institutions involved in pulse research.

http://www.pulseaus.com.au - R&D undertaken on chickpea in Australia: management and marketing. http://www.quakeroats.com — Quaker Oats Co.

http://www.remy-industries.be - Based in Belgium, Remy Industries is the world's largest producer of rice starch. General information on rice derivatives is presented on the website.

http://www.ricebranoil.biz - Rice Bran Oil.

http://www.riceland.com - Riceland Foods in Stuttgart, AR, USA.

http://www.saitoku.com — A Japanese site all about okara and its use as a food.

http://www.sake.nu – A site all about saké and where you can buy to suite your taste.

http://www.saskpulse.com — This website is maintained by Saskatchewan Pulse Growers. It has information on production and marketing of pulse crops. This site is updated regularly.

http://www.satake.co.uk — Satake UK Division. Satake (http://www.satake.com) is headquartered in Japan. For information in English, the Satake UK Division site is recommended. Satake have long been established as a leader in rice milling equipment, and entered the wheat milling equipment market in the 1990s. They were the first company to aggressively market preprocessing equipment for wheat milling under the brand name "Peritec."

http://www.solae.com - DuPont Protein Technologies, St. Louis, MO, USA.

http://www.sosland.com — Sosland Publishing Co., publishers of several useful trade magazines and baking science & technology.

http://www.soylife.com – Schouten USA, Minneapolis, MN, USA.

http://www.starchaust.com.au - Penford starches, Australia.

http://www.statpub.com — Worldwide market and production information on chickpea.

http://www.sv-m.com.

http://www.tepral-fr.com - Tepral, Strasbourgh, France.

http://www.tortilla-info.com – Tortillas Industry Association (2003) website provides information on member companies, current activities, and some educational materials.

http://www.unipi-pasta.it — This website gives useful information on economic aspects of pasta (in Italian).

http://www.wensfood.com - The site of Wensfood offering soy products for sale in the United States of America.

http://www.westonmilling.com.au - Weston Foods, Australia.

http://www.world-grain.com — The Sosland Publishing Company website contains a "focus" series that gives detailed information on wheat production in many countries.

http://www.zeochem.com — Zeochem is a major producer of molecular sieve adsorbents. Its US head-quarter is located in Louisville, KY. This website describes the types of molecular sieves and molecular sieve adsorbents (crystalline alumino-silicates) with applications in ethanol dehydration.

# **APPENDIX 4**

# Test Methods for Grain and Grain-Based Products

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#### Introduction

The great breadth of topics in grain science is indicated within this encyclopedia. For virtually all of these topic areas, there is the need for laboratory test methods to evaluate the quality characteristics of the grains produced and to monitor the processing quality of the final products that result. This appendix lists the many methods that have been devised to provide these testing requirements. The methods listed are those developed and published by two major associations of grain scientists:

- The American Association of Cereal Chemists (AACC) is based in St. Paul, MN, USA, with a worldwide membership of ~4000 scientists who have interests in the grain-based food industry.
- The International Association for Cereal Science and Technology (ICC), based in Vienna, Austria, is an international association committed to international cooperation through disseminating information and developing standard methods for the well-being of all people.

In addition, standard methods of grain testing have been developed by several other organizations, including the following. The interested readers are referred to their publications or websites (listed at the end of this text) for further details (*see* Scientific Societies Associated With Grain Science).

- The American Oil Chemists' Society (AOCS) comprises nearly 5000 members worldwide, providing "a global forum for the science and technology of fats, oils, surfactants, and related materials."
- The International Seed Testing Association (ISTA) is a worldwide, nonprofit association whose main activity is to provide methods and services for the testing of seed moving in international trade.
- AOAC International is an "internationally recognized organization with ∼120 years of experience in validating and approving analytical methods for foods and agriculture."

- The Cereal Chemistry Division of the Royal Australian Chemical Institute, based in Melbourne, Australia.
- The American Society of Agronomy (ASA) is "dedicated to the development of agriculture enabled by science, in harmony with environmental and human values. The Society supports scientific, educational, and professional activities to enhance communication and technology transfer among agronomists and those in related disciplines on topics of local, regional, national, and international significance."

# **Method Development**

The development of these methods is a painstaking and laborious process. It may start with the realization that there is the need for a specific method of analysis; a group of researchers may study the best means of achieving the desired aim. It may take a year or two of research to achieve their initial goal. When they reckon that they have a viable method, they would probably describe it in a research article that is submitted to a suitable scientific journal.

This might be a journal that covers topics of grain science, such as Cereal Chemistry and Cereal Foods World (both published by the AACC), or the *Journal* of Cereal Science (published by Elsevier Science, UK). Alternatively, they may choose to submit their paper to a journal that deals more broadly with food science or agriculture, or more specifically with analytical methodologies. On receipt of their research article, the editorial staff of the journal would send out the manuscript for "peer review," i.e., the paper is sent for critical assessment separately by two or more independent scientists who are recognized experts in the specific field covered by the paper. Depending on the opinions of these referees, the editorial staff may accept the article for publication; alternatively, it may be rejected, or it may be returned to the authors for revision until it is acceptable for publication.

Following the publication of the new method, other research groups may read it and realize its value, evaluating it as a procedure that would suit their analytical needs. If they find it suitable, they might add some improvements of their own. These other research groups might communicate with the original group of scientists, suggesting the need for collaborative evaluation of the method, either in its original or

modified format. A common forum for such collaborative evaluation is provided within associations of scientists with common interests, such as the AACC or ICC. Regular scientific meetings of the associations provide additional opportunities for the exchange of ideas and for arranging collaborative development of methods.

A further step in the formal development of a method comes when there is agreement on the need for the method, on a draft procedure, and on a process for collaborative evaluation of the draft method. This process would probably involve the provision of a set of relevant samples for distribution to all the laboratories involved. Results obtained by applying the draft procedure to these samples would be provided to a central coordinator for statistical evaluation, using guidelines provided in ICC method 203 and in AACC method 78-60, for example. In this process, collaborators may find deficiencies in the method, reporting on these with recommendations for improvement. These iterative processes of collaboration and improvement may be repeated a few times until the various collaborators are satisfied that the method is optimized, and that it is thus ready for adoption as a "standard method" of the association involved. This final step of adoption may require the formal agreement of a meeting of the association.

Following adoption, the details of the method are added to the association's manual of standard methods. These manuals are updated from time to time as new methods are added, and as superseded methods are removed. The most up-to-date lists of standard methods are likely to be the respective websites, with the possibility that methods can be purchased individually from the website.

This general process of method development has been followed, in some form or other, for the methods listed in this appendix (see Tables 1 and 2). Inevitably, there is some duplication between associations in the methods developed. In some cases, the method developed by one organization is adopted (possibly after modification) by another organization. In addition, lists of approved methods are likely to be developed by individual research or analytical laboratories or companies.

## The Range of Methods

The diversity of methods listed below covers the analysis of grains and of grain-based products. Additional to these are the many more analytical procedures needed for the earlier stages of grain production, such as soil and plant testing. Further details of

these agronomic needs can be accessed on relevant websites and via "Further Reading" below.

#### **Grain Testing after Harvest**

The methods listed below cover all the requirements of quality control from harvest through to the final production of the food or feed product. An essential initial step at any of these stages is the act of taking a sample. If the samples taken for analysis are not representative of the consignments that have been sampled, then there is little point in performing the required analyses. At least, there must be an awareness of the relationship of the sample for testing to the whole consignment that it is intended to represent. For the recommended sampling procedures, see ICC standard methods 101, 120, 130, and 138, and AACC methods grouped under 64.

In the sequence of grain production and processing, the analysis of the harvested grain comes first. Initial tests involve the determination of bulk density (as "bushel weight" in AACC method 55-10) and of nongrain material, called "extraneous matter" in the AACC methods (group 28) and as "besatz" in ICC methods 102 and 103. Grain analysis also involves determination of possible defects, such as the production of mycotoxins (AACC method group 45).

Another potential defect is the extent to which rain at harvest has triggered the production of the starchdegrading enzyme, α-amylase (see Cereals: Grain Defects). This may be determined by the range of methods listed in the ICC suite as numbers 107, 108, 126, 161, and 162, or by AACC methods in the enzymes group (22). Why is there the need for several methods to apparently achieve the same result? In this case, the various methods are suited to different situations, namely, for use in the laboratory by determining the actual enzymic activity by colorimetric means (ICC method 126, AACC method 22-02), or methods that measure the effect of the  $\alpha$ -amylase on the endogenous starch of the grain sample, either in the laboratory (as "falling number" in ICC method 107 and AACC method 56-81B) or when grain is received at the elevator (as "stirring number" in AACC method 22-08 and ICC method 161).

# **Chemical Composition**

Next in importance is the chemical composition of the grain, involving moisture content for all grains, protein content for most grains, and oil content for the oilseeds. Routine analyses of these constituents generally involve near-infrared (NIR) spectroscopy (AACC method group 39 and ICC methods 159 and 202), but this procedure requires reference sets

#### Table 1 ICC standard methods

Acidity

No. 145: Determination of acidity (according to Schulerud) for cereals and cereal products

α-Amylase activity (enzymes)

No. 107/1: Determination of "falling number" according to Hagberg-Perten as a measure of the degree of  $\alpha$ -amylase

activity in grain and flour

No. 108: Colorimetric method for the determination of  $\alpha$ -amylase activity

No. 126/1: Method for using the Brabender amylograph

No. 161: Determination of the "stirring number" using the Newport Rapid Visco Analyzer, as a measure of the degree of

 $\alpha$ -amylase activity in grain and flour

No. 162: Rapid pasting method using the Newport Rapid Visco Analyzer

Ash content

No. 104/1: Determination of ash in cereals and cereal products

No. 157: Ash determination by conductivity

Baking test

No. 131: Baking test for wheat flours

Besatz

No. 102/1: Determination of besatz of wheat No. 103/1: Determination of besatz of rye

Carbohydrates

Mono- and disaccharides

No. 132: Determination of saccharose in cereals and cereal products

Starch

No. 122/1: Determination of starch content by calcium chloride dissolution
No. 123/1: Determination of starch content by hydrochloric acid dissolution
No. 128/1: Procedure for the determination of starch after enzymic decomposition
No. 164: Measurement of damaged starch by using MEGAZYME enzymatic kit

No. 169: Method for using the Brabender viscograph

Durum wheat, semolina, flour, and pasta

No. 129: Method for determination of the vitreousness of durum wheat
No. 151: Determination of the sedimentation value SDS test of durum wheat

No. 152: Determination of the yellow pigment content of durum wheat semolina and flour

No. 153: Determination of total organic matter (TOM) in pasta

No. 158: Gluten index method for assessing gluten strength in durum wheat (*Triticum durum*)

Fat content

No. 136: Cereals and cereal products – determination of total fat content

Fiber (crude fiber, bran, dietary fiber)

No. 113: Determination of crude fiber value

No. 140: Enzymic determination of the bran content of cereals

No. 156: Determination of total dietary fiber

Gluten

No. 106/2: Working method for the determination of wet gluten in wheat flour

No. 137/1: Mechanical determination of the wet gluten content of wheat flour (glutomatic)

No. 155: Determination of wet gluten quantity and quality (gluten index according to Perten) of whole wheat meal and

wheat flour (Triticum aestivum)

No. 158: Gluten index method for assessing gluten strength in durum wheat (Triticum durum)

Heavy metals

No. 141: Determination of mercury in cereals

No. 154: Determination of cadmium and lead in cereals and cereal products

Infrared analyses

No. 159: Determination of protein by near-infrared (NIR) reflectance spectroscopy

No. 202: Procedure for near-infrared (NIR) reflectance analysis of ground wheat and milled wheat products (recommendation)

Microbiological tests

No. 125: Method of determining the count of aerobic mesophilic bacteria (plate count method)

No. 133: Determination of the germ count of aerobic and facultatively anaerobic, mesophilic bacteria (plate count method) in

cereals, cereal products, and animal feed

No. 134: Determination of the fungus germ count (plate count method) in cereals, cereal products, and animal feed

No. 139: Determination of fungus germ count (plate count method)

No. 144: Enumeration of spores of mesophilic bacteria
No. 146: Enumeration of yeasts and mold (spatula method)

No. 147: Enumeration of bacteria (spatula method)

No. 206: Microbiology – general guidance for microbiological examination (recommendation)

#### Moisture content

No. 109/1: Determination of moisture content of cereals and cereal products (basic reference method)

No. 110/1: Determination of moisture content of cereals and cereal products (practical method)

No. 135: Determination of the water content of whole maize kernels

No. 201: Test procedure for rapid moisture determination apparatus (recommendation)

#### Particle size

No. 127: Determination of the particle size distribution in flour by the Andreasen pipette method

#### Physical dough testing

No. 114/1: Method for using the Brabender extensograph
No. 115/1: Method for using the Brabender farinograph
No. 121: Method for using the Chopin alveograph
No. 126/1: Method for using the Brabender amylograph

#### Protein content

No. 105/2: Determination of crude protein in cereals and cereal products for food and feed No. 159: Determination of protein by near-infrared (NIR) reflectance spectroscopy

No. 167: Determination of crude protein in grain and grain products for food and feed by the Dumas combustion principle

#### Sampling

No. 101/1: Sampling of grains

No. 120: Mechanical sampling of grain

No. 130: Sampling of milling products (semolinas, flours, agglomerated flours, and by-products)

No. 138: Mechanical sampling of milled cereal products

#### Sedimentation test

No. 116/1: Determination of sedimentation value (according to Zeleny) as an approximate measure of baking quality

No. 118: Preparation of test flour from wheat samples for sedimentation test No. 151: Determination of the sedimentation value SDS test of durum wheat

#### Statistical evaluation

No. 203: Statistical analysis of the results of collaborative studies (recommendation)

## Variety identification

No. 143: Wheat identification of varieties by electrophoresis

#### Vitamins

No. 111: Chemical assay of nicotinic acid in cereal products
 No. 112: Microbiological assay of nicotinic acid in cereal products
 No. 117: Chemical determination of thiamine in cereal products

No. 119: Rapid method for the determination of thiamine in enriched flours and enrichment mixtures

Methods of the ICC are continuously revised and enlarged in accordance with recent needs and development. For additional information, contact the ICC Secretariat by e-mail or via the website.

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Table 2 Tenth edition of AACC methods (including the 2002 supplement)

02 Acidity 02-01A 02-02A 02-03A 02-04A 02-31 02-32A 02-52	Fat acidity — general method Fat acidity — rapid method, for small grains Fat acidity — rapid method, for corn Fat acidity — colorimetric method Titratable acidity Neutralizing value of acid-reacting materials Hydrogen-ion activity (pH) — electrometric method
04 Acids 04-10 04-11 04-14 04-20 04-21 04-22 04-27 04-28	Phosphoric acid — qualitative method Phosphoric acid — quantitative method Sulfuric acid — quantitative method Acetic, butyric, and lactic acids in rye flour Benzoic acid Citric and isocitric acids Tartaric acid — quantitative method Free or combined tartaric acid — qualitative method
06 Admixture 06-10 06-11	e of flours Admixture of rye and wheat flours Soy flour

Table 2 Co	ontinued
07 Amino ac	ids
07-01	Measurement of acid-stable amino acids
07-11	Measurement of sulfur amino acids
07-20	Measurement of tryptophan — alkaline hydrolysis
08 Total ash	
08-01	Ash – basic method
08-02	Ash – rapid (magnesium acetate) method
08-03	Ash – rapid (2 h, 600°) method
08-10	Ash in cacao products
08-11	Ash in dry milk products
08-12	Ash in farina and semolina
08-14 08-16	Ash in molasses, sugars, and syrups Ash in soy flour
08-10	Ash in starch
08-18	Ash in yeast foods
08-21	Prediction of ash content in wheat flour – near-infrared method
10 Baking qu	uality
10-05	Guidelines for measurement of volume by rapeseed displacement
10-09	Basic straight-dough bread-baking method — long fermentation
10-10B	Optimized straight-dough bread-baking method
10-11	Baking quality of bread flour — sponge-dough, pound-loaf method
10-13	Guidelines for testing a variety of products
10-15	Baking quality of angel-cake flour
10-31B	Baking quality of biscuit flour
10-50D	Baking quality of cookie flour
10-52 10-53	Baking quality of cookie flour — micro-method  Baking quality of cookie flour — macro-wire-cut formulation
10-55	Baking quality of cookie flour — micro-wire-cut formulation
10-90	Baking quality of cake flour
10-91	Use of layer cake measuring template
11 Biotechno	oloav
11-10	Bt cry1Ab-Modified corn in corn flour — ELISA method
11-20	StarLink corn in corn flour and corn meal — ELISA method
11-21	ELISA method for StarLink corn in corn flour and corn meal
12 Carbon d	ioxide
12-10	Residual carbon dioxide in baking powder
12-20	Total (gasometric) carbon dioxide in baking powder
12-21	Total carbon dioxide in prepared mixes and self-rising flours
12-29	Table: correction factors for gasometric determination of carbon dioxide
14 Color and	· ·
14-10	Pekar color test (slick test)
14-22 14-30	Color of pasta — reflectance colorimeter method  Agtron color test for flour
14-50	Determination of pigments
	, ,
20 Ingredien 20-01	Egg solids – digitonin cholesterol method
20-01	Egg solids in pasta products
20-20	Determination of isoflavones in soy and selected foods containing soy by extraction, saponification, and liquid
	chromatography
22 Enzymes	
22-02	Measurement of $\alpha$ -amylase in plant and microbial materials using the ceralpha method
22-05	Measurement of $\alpha$ -amylase in cereal grains and flours — amylazyme method
22-08	Measurement of $\alpha$ -amylase activity with the Rapid Visco Analyzer
22-10	Measurement of $\alpha$ -amylase activity with the amylograph
22-11	Measurement of gassing power by the pressuremeter method
22-12	Measurement of $\alpha$ -amylase activity in flour supplemented with fungal $\alpha$ -amylase — modified amylograph method
22-14	Measurement of gassing power by volumetric method
22-15 22-40	Measurement of diastatic activity of flour or semolina  Measurement of trypsin inhibitor activity of soy products — spectrophotometric method
22-40	Measurement of proteolytic activity — spectrophotometric method
22-80	Qualitative test for peroxidase in oat products
22-90	Measurement of urease activity

26 Experime	ental milling
26-10A	Experimental milling: introduction, equipment, sample preparation, and tempering
26-21A	Experimental milling – Bühler method for hard wheat
26-22	Experimental milling – batch method for hard wheat
26-30A	Experimental milling – Bühler method for soft wheat short-extraction flour
26-31	Experimental milling – Bühler method for soft wheat straight-grade flour
26-32	Experimental milling – batch method for soft wheat
26-41	Experimental milling – Bühler method for durum wheat
26-42	Experimental milling – batch method for durum wheat
26-50	Brabender quadrumat Jr. (quadruplex) method
26-95	Experimental milling: temper table
28 Extraneo	
28-01	Apparatus or materials for extraneous matter methods
28-02	Reagents for extraneous matter methods
28-03	A special technique for extraneous matter methods
28-06	Cinder and sand particles in Farina — counting method
28-07	Cinder and sand particles in Farina – gravimetric method
28-10A	Macroscopic examination of external contamination in whole grains
28-19	External filth and internal insect infestation in whole corn
28-20A 28-21A	Microscopic examination of external contamination in whole grains  X-ray examination for internal insect infestation
28-21A 28-22A	Cracking-flotation test for internal insects in whole grains
28-30A	Macroscopic examination of materials hard to hydrate
28-31A	Pancreatin sieving method, for insect and rodent filth in materials hard to hydrate
28-32A	Sieving method, for materials hard to hydrate
28-33A	Pancreatin nonsieving method for insect and rodent filth in materials easy to hydrate
28-40	Acid hydrolysis method for insect fragments and rodent hairs — wheat—soy blend
28-41B	Acid hydrolysis method for extracting insect fragments and rodent hairs — light filth in white flour
28-43	Glass plate method, for insect excreta
28-44	lodine method, for insect eggs in flour
28-50	Decantation method, for rodent excreta
28-51A	Flotation method, for insect and rodent filth
28-60A	Tween-Versene method, for insect fragments and rodent hairs in rye flour
28-70	Defatting-Digestion method, for insect fragments and rodent hairs
28-75A	Sieving method, for light filth in starch
28-80	Flotation method, for insect and rodent filth in popped popcorn
28-85	Ultraviolet light examination, for rodent urine
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of samples whose composition has been determined by the basic methods of oven drying for moisture (AACC method group 44 and ICC method 109), elemental analysis of nitrogen content for protein (ICC method 167 and AACC method group 46), and solvent extraction for oil and fat (ICC method 136, AACC method group 30). These methods have also been adapted to analyses of milled and baked products. For flour and food products, a wider range of compositional analyses may be required, including the content of starch (AACC method group 76, ICC methods 122, 123, and 128), nonstarch polysaccharides and fiber of various types (ICC methods 113, 140, and 156; AACC

method group 32), specific types of lipids (fats and oils) (AACC method group 58), vitamins (ICC methods 111, 112, 117, and 119; AACC method group 86), and heavy metals (ICC methods 141 and 154).

#### **Simulation of Grain Processing**

Many of the methods of analysis are designed to predict how a consignment of grain or flour will perform in processing. Most obvious are methods that are small-scale versions of the real process. For example, equipment and procedures have been developed for laboratory-scale milling (AACC method group 26). Following milling (laboratory- or full-scale), a common method to determine the quality of the resulting flour is to assay the flour for its ash content (AACC method group 08, ICC methods 104 and 157), i.e., the mineral residue remaining after burning off the combustible materials, such as protein, fat, and starch.

Likewise, small-scale versions are available for the analysis of wheat to mimic the processes of making bread, pasta, noodles, and other food products (*see* Cereals: Overview). Predictive of these product-related methods are the significant aspects of flour performance that influence processing, especially dough properties (ICC methods 114, 115, 121, and 126; AACC methods group 10). Further groups of methods determine specific aspects of composition that are known to provide a correlation to performance in the real process. These include gluten strength (ICC methods 155 and 158; AACC method group 38) and sedimentation tests (AACC methods 56-60 to 56-63 and 56-70; ICC methods 116, 118, and 151).

#### **Methods for Food Processing**

Further methods describe the means of testing the quality of a range of ingredients that may be used in the preparation of grain-based foods (AACC method groups 20 and 58). Other methods relate to the evaluation of finished products according to their color (AACC method group 14) and their taste and mouth feel (sensory analysis, AACC method 33-50). Paramount for food production is the need to test for food safety, i.e., the need for assurance of the absence of pathogenic microorganisms (ICC methods 125, 133, 134, 139, 144, 147, and 206; AACC method group 42).

### **Methods for the Diversity of Grains**

The main accent in the AACC and ICC standard methods is on wheat and wheat-based foods, but there are also analyses of product and processing quality for rice; these mainly involve the quality of rice starch (AACC method group 61). Methods for

barley testing are provided by the European Brewing Commission (EBC).

#### **Oilseed Methods**

Greater accent on fats and oils is provided by standard methods of the American Oil Chemists' Society (AOCS). These are available as a loose-leaf manual of 1200 pages or online at the AOAC website. Their methods include sections on vegetable-oil source materials (section A), oilseed by-products (section B), commercial fats and oils (section C), and various other methods for the wider range of fats, oils, and surfactants. Specific methods of relevance to oilseed analysis include, e.g.,

- determination of oil content in oilseeds,
- oil content of oilseeds by nuclear magnetic resonance,
- oil in oilseeds: supercritical fluid extraction method,
- determination of oil, moisture and volatile matter, and protein by NIR reflectance, and
- total hexane content in extracted meals.

# **Plant Breeding**

Many of these testing methods are also essential to the needs of plant breeders, who must evaluate the suitability for specific end uses of the many cross-bred lines coming through their breeding programs, whatever the grain species (see Canola: Genetics and Breeding. Maize: Breeding. Rice: Breeding. Soybean: Germplasm, Breeding, and Genetics. Wheat: Breeding). In the late stages of breeding, there may be  $\sim 1$  kg of grain for advanced lines, sufficient for test milling and test baking if, for example, the aim were to produce a bread wheat. At earlier stages of breeding, the amount of grain is much less, and very small scale methods have been devised to suit these specific purposes, so that lines that perform poorly for end-use quality can be eliminated from the breeding program, thereby avoiding the unnecessary propagation of poor

#### Conclusion

Suitable methods of analysis are essential to the ongoing development of the grain-processing industries, both internationally and locally. These methods permit the comparison of test results between laboratories and between countries. Without this ability, it would be impossible to establish codes for the hygiene and composition of grains and of foods made from them. New methods are now taking advantage of the biotechnology era, as well as addressing the

challenges of biotechnology. For example, AACC methods in the biotechnology group (11) provide the means of determining the presence of genetically modified corn (*see* Genetically Modified Grains and the Consumer), using methods from the new biotechnology, namely, immunological assays. These parallel trends are likely to expand, with the increasing need to identify and segregate grains that have resulted from genetic modification. On the other hand, molecular biology is delivering a wide range of efficient testing methods, whereby analysis can be conducted by targeting the presence or absence of specific marker genes or marker proteins (*see* Variety Identification of Cereal Grains).

See also: Canola: Genetics and Breeding. Cereals: Overview; Grain Defects. Maize: Breeding. Rice: Breeding. Scientific Societies Associated With Grain Science. Soybean: Germplasm, Breeding, and Genetics. Wheat: Breeding.

# **Further Reading**

- AACC (2002) AACC Methods, 10th edn. St. Paul, MN: American Association of Cereal Chemists.
- AOAC (2002) AOAC Official Methods. Gaithersburg, MD: AOAC International.
- AOCS (2001) Official Methods and Recommended Practices of the AOCS. Champaign, IL: American Oil Chemists' Society.
- AOCS (2002) Standard Methods of the American Oil Chemists' Society. Champaign, IL: American Oil Chemists' Society.
- CCFRA (2002) Manual of Methods for Wheat and Flour Testing, Guideline No. 3, 3rd edn. Chipping Campden, Gloucester: CCFRA Technology Ltd.
- Dendy DAV (ed.) (1995) Sorghum and Millets: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.
- Fabriana G and Lintas C (eds.) (1988) *Durum Wheat: Chemistry and Technology*. St. Paul, MN: American Association of Cereal Chemists.
- Henry RJ and Kettlewell PS (eds.) (1996) Cereal Grain Quality. London: Chapman and Hall.

- International Association for Cereal Science and Technology (2002) *Standard Methods*. Vienna, Austria: International Association for Cereal Science and Technology (ICC).
- International Seed Testing Association (1999) *International Rules for Seed Testing*, 1999. Zurich, Switzerland: International Seed Testing Association.
- Juliano BO (ed.) (1985) Rice: Chemistry and Technology, 2nd edn. St. Paul, MN: American Association of Cereal Chemists.
- Kent NL (ed.) (1975) Technology of Cereals, with Special Reference to Wheat, 2nd edn. Oxford: Pergamon Press.
- McGregor AW and Bhatty RS (eds.) (1993) *Barley: Chemistry and Technology*. St. Paul, MN: American Association of Cereal Chemists.
- Pomeranz Y (ed.) (1988) Wheat: Chemistry and Technology, 3rd edn. St. Paul, MN: American Association of Cereal Chemists.
- Watson SA and Ramsted PE (eds.) (1987) Corn: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists.
- Webster FH (ed.) (1985) Oats: Chemistry and Technology.
  St. Paul, MN: American Association of Cereal Chemists.
- Westcott T and Ross D (eds.) (2002) Official Testing Methods of the Cereal Chemistry Division of the Royal Australian Chemical Institute. Melbourne, Australia: Royal Australian Chemical Institute.

#### Relevant Websites

http://www.aaccnet.org - American Association of Cereal Chemists.

http://www.icc.or.at — International Association for Cereal Science and Technology.

http://www.aoac.org - AOAC International.

http://www.aocs.org — Standard Methods of the American Oil Chemists' Society (AOCS).

http://www.seedtest.org — International Seed Testing Association (ISTA).

http://www.agronomy.org - American Society of Agronomy.

# **APPENDIX 5**

# **Units of Grain Science**

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## Introduction

Until the whole world shares one common "language" for units of measurement, there will be a need for conversion tables, such as are provided in this appendix. There is a bewildering array of units, many of them are no longer in common use; examples include tons and tonnes, fathoms and furlongs, pennyweights and hundredweights, stones and scruples, gills and grains, drams and pecks, poles and perches, rods and roods, and leagues and bags. An ounce is a different measure depending on whether it is a dry or liquid measure, and depending on whether it is an ounce Troy, an Apothecaries' ounce or an ounce Avoirdupois! Agreement on units of measurement is no less important today for grain science, in the many disciplines that make their contributions, than such agreement has been through the ages.

Throughout this encyclopedia, the standard is to quote units as metric, and indeed this is usual throughout the scientific community worldwide. However, it is appropriate that there should be the provision of conversion tables between metric and US units in this appendix, because the metric system of units is still not in common use in some English-speaking countries. This is especially so for the USA, although the USA is officially on the metric system. All US units are defined in terms of the SI system of metric units (Systeme Internationale d'Unites).

The use of customary US units continues to be permitted in trade, but they are not mandated. In many cases, including consumer foods, e.g., canned foods and packaged baking mixes, they are dual-labeled in US customary units and in their metric equivalent. In medicine, metric units are used almost exclusively. Metric units are also used for some alcoholic beverages. The provision of conversion tables is also needed for many of the current and older generations in countries that have adopted the metric system relatively recently, as they may still think in imperial terms for infrequently used units.

Added problems for the use of units of measurement relate to the lack of standardization of units in nonmetric systems. Many of the British imperial units are differently defined in the USA. Some of these problems are discussed and explained in the text below. However, the table of conversions below is confined to conversion between the US system and metric units.

In addition, explanation is needed for a range of nonstandard units used in some branches of grain science. Such instances are discussed in this appendix, thereby providing a focus for this information, which is also spread throughout the encyclopedia.

# Historical Perspective — The Metric System

Agreement on units of measurement has been necessary since the beginnings of civilization. For example, it was all very well for the cubit to be used to measure short lengths, based on the distance from the elbow to the fingertip, but when accuracy mattered, whose elbow and whose fingertip? According to the Oxford dictionary, this measurement ranged from 18 to 22 inches. So, presumably, the matter of standardization was resolved by decree: the standard elbow and fingertip would be the king's arm!

Anatomical origins obviously also apply to the foot, still in limited use officially, but discontinued in France following the French Revolution of 1789. Prior to that date, the familiar unit was the *piedde-roi* (king's foot), similar, but not identical, to the British foot. However, even within France, the *piedde-roi* varied from province to province. The foot of Paris was 11% longer than that in Strasbourg, but 10% shorter than the foot measure in Bordeaux. Such impediments to trade were addressed by the French scientific community following the French Revolution. The French *Academie des Sciences* was given the responsibility of producing a unified system of weights and measures by act of Parliament in 1790.

Although France and England were officially at war at the time, the decree of 1790 stated that the King of England should be approached for England to join in the process of standardizing the system of weights and measures. It is still unclear whether this invitation was ever relayed to the English or, if it was, what response was provided. If Anglo-French cooperation had proceeded at that time, we would probably be a more unified world a few centuries later, in view of the subsequent spread of the British imperial system.

Furthermore, the basis of the unit of length might also be much simpler, because a pendulum system was proposed for the joint action with the Royal Society of Britain. According to this system, the basic measure was the length of a pendulum with a period of one second (as familiar as the grandfather clock) giving a system of kilopendules, millipendules, and cubic pendules.

Instead, however, the *Academie des Sciences* settled for the meter as one ten-millionth part of one-quarter of the Earth's meridian. Initially, this was to be based on accurate measurement of the distance from Dunkirk to Barcelona. The interim project, begun in 1793, involved measurement only to Collioure in the south of France. This measure has stood the test of time much better than others in the original package, such as a 10 h clock, a circle divided into 400 grades (still finding some use), and a Republican calendar, starting on September 22nd (the autumnal equinox in the northern hemisphere) with 12 months, each divided into three 10 day periods, plus a 5 day holiday period (or 6 day holiday period in a leap year).

# Imperial Units – The British and US Versions

Following the transfer of the British imperial system of weights and measures to the USA a few centuries ago, separate evolution has occurred on either side of the Atlantic ocean, introducing further inconsistencies between the two versions of what is known as the imperial system of Units. The differences include the following examples:

- the US gallon (3.7851) is smaller than the British gallon (4.5461);
- the US "short" ton of 2000 pounds (907.2 kg) is shorter measure than the British "long" ton of 2400 pounds (1016 kg), made up of 20 hundredweight (cwt) of 112 pounds each;
- the US bushel (35.24 l) consists of 32 dry quarts, or 8 US gallons, or 4 pecks. It differs from the British "Winchester" bushel (8 British gallons), which is 36.37 l.
- the British "bag" of 3 bushels (0.109 m³) is not in general use in the USA, but it is a farmers' unit in former British colonies such as Australia.
- the US hundredweight (cwt) (45.36 kg) is 100 pounds (as the name suggests), significantly less than the British hundredweight of 112 pounds (50.80 kg). One story to explain this anomaly refers to the shipping of wool from Australia to Britain in the nineteenth century. Bales of wool started as 100 pounds on the dry Australian sheep station, but they gained moisture during the long ocean voyage

in the wool clippers to become 112 pounds on arrival in Britain.

To the extent that metric units have been used in USA, there have been differences in spelling to further confound the possibility of standardization. For example, the tonne, litre, metre and gramme of the SI metric system are the metric ton, the liter, the meter and the gram in US use, respectively. Potentially, international adoption of the SI metric system is a unifying principle to overcome these anomalies, but habitual use of traditional systems dies hard. On the assumption that the use of nonmetric units is more prevalent in the US than elsewhere, the table of conversions to metric units (Table 1) relates primarily to US units, using US spelling for the metric units.

# The International System (SI) of Units

The SI system of units is the modern version of the metric system. The General Conferences on Weights and Measures (1954–71) agreed on seven base units for seven basic quantities as the foundation of the SI system. These are:

- the meter (m) for length;
- the kilogram (kg) for mass;
- the second (s) for time;
- the ampere (A) for electric current intensity;
- the kelvin (K) for thermodynamic temperature;
- the mole (mol) for amount of substance; and
- the candela (cd) for luminous intensity.

A much longer set of derived SI units was based on these. The basic SI units for area and volume are thus obtained by raising the unit for length to the second and third powers, respectively. A more complex case is the set of derived units for energy, which may be expressed as the meter squared kilogram per second squared (m² kg s<sup>-2</sup>), also called the joule (J), the newton meter (N m), and the volt ampere second (V A s). These three terms for energy are equivalent; the user may choose which is appropriate to use in a specific context.

General Conferences on Weights and Measures (1960–75) adopted a set of decimal multiples as SI prefixes. The more common ones are:

- giga (G) for 10<sup>9</sup>,
- mega (M) for  $10^6$ ,
- kilo (k) for 10<sup>3</sup>,
- hecto (h) for  $10^2$ ,
- deci (d) for  $10^{-1}$ ,
- centi (c) for 10<sup>-2</sup>
- milli (m) for 10<sup>-3</sup>.
- micro ( $\mu$ ) for  $10^{-6}$ , and
- nano (n) for  $10^{-9}$ .

**Table 1** Conversion between metric units (mostly SI units) and US units

US units	
Metric units	US units
Length 1 centimeter (cm) 25.4 millimeter (mm) 1 meter (m) 0.3048 m 1 m 0.9144 m 1 kilometer (km) 1.6093 km	0.3937 inch (in) 1 in 3.2808 feet (ft) 1 ft 1.094 yard (yd) 1 yd 0.6213 mile 1 mile
Area 1 square centimeter (cm²) 645.2 square millimeters 1 square meter (m²) 1 m² 1 m² 1 hectare (ha) 0.4047 ha 1 ha 259.0 ha	0.1550 square inch (in²) 1 in² 10.76 square feet 1.196 square yard 0.0002471 acre (ac) 2.471 acres 1 acre 0.00386 square mile 1 square mile (1 "section")
Volume 1 cubic centimeter (cc) 16.38 cc 1 milliliter (ml) 29.57 ml 1 liter (l) 3.785 l 1 hectoliter (hl) 1 hl 35.24 l 1 l 0.9463 l	0.0610 cubic inch 1 cubic inch 0.3382 fluid ounce 1 fluid ounce 0.2642 US gallon 1 US gallon 26.42 US gallons 2.838 US bushels 1 US bushel 0.02838 US bushel 1 quart (liquid)
Mass 1 gram (g) 28.35 g 1 kilogram (kg) 0.4536 kg 1 quintal (q) (100 kg) 45.36 kg 1 tonne (t) (metric ton) 0.9072 t 1 t 1.016 t	0.0353 ounce 1 ounce 2.205 pounds (lb) 1 lb 220.5 lb 1 US hundredweight (cwt) (100 l) 1.102 US short ton (2000 lb) 1 US short ton 0.9843 US long ton (2205 lb) 1 US long ton
Grain yield 1 q ha <sup>-1</sup> 0.1235 tonne/ hectare (t ha <sup>-1</sup> ) 1 t ha <sup>-1</sup> 6.73 t ha <sup>-1</sup> 1 t ha <sup>-1</sup>	0.79 cwt/acre 1 bag/acre  14.86 bushels/acre for wheat 100 bushels/acre for wheat 13.87 bushels/acre for maize 11.89 bushels/acre for barley
Bulk density 1 kilogram/ hectoliter (kg hl $^{-1}$ ) 1.287 kg hl $^{-1}$	0.7770 pound/US bushel 1 pound/bushel
Power 1 kilowatt (kW) 0.7457 kW	1.341 horsepower (hp) 1 hp

Table 1 Continued

Metric units	US units
Energy	0.000 colorio (col)
1 joule (J) 4.187 J	0.239 calorie (cal) 1 cal
1 kilojoule (kJ) 1.055 kJ	0.945 British thermal unit (BTU) 1 BTU
Temperature 0°C (celsius) 20°C 100°C	32°F (fahrenheit) 68°F (about "room temperature") 212°F
Pressure 100 pascals (Pa)	1 millibar (mbar)
Dynamic viscosity 1 pascal second	1 poise

## **Standard Measures in Grain Science**

#### **Units of Grain Harvesting**

In science generally, and in grain science specifically, the SI system of metric units is happily accepted. Yet, at harvest, farmers might still measure their grain yields in "bushels per acre," or even in "bags to the acre," with grain volume given in US bushels, Winchester bushels, hectoliters, or in quintals.

In "dry measure" of volume, the US bushel is 2150 cubic inches or 8 gallons or 4 pecks or 32 dry quarts. One dry quart equals 67.20 cubic inches or 1.101 l. In the metric system, grain yield is measured in tonnes per hectare (t ha<sup>-1</sup>), i.e., units of mass per unit of area. However, the US tradition has been to measure yield in bushels per acre, i.e., units of volume (not mass) per unit of area. The use of volume measurement units for grain in the US makes it difficult to provide direct conversion between metric and US units for grain yield. To overcome this problem, the US government has defined the bushel for grains in commerce in terms of weight, irrespective of the true test weight. Standard volume—mass values for the various cereals are:

- 1 bushel of wheat = 60 pounds;
- 1 bushel of maize = 56 pounds;
- 1 bushel of oats = 32 pounds;
- 1 bushel of barley = 48 pounds; and
- 1 bushel of rye = 56 pounds.

Thus, it is possible to convert grain yields, depending on the grain being considered. When the grain in question is wheat, 100 bushels per acre is 6000 pounds per acre equal to 2723 kg for 0.4047 ha, or 6.73 t ha<sup>-1</sup>. A rough "rule-of-thumb" is to take the yield of wheat in pounds per acre and increase it by 10% to get kg ha<sup>-1</sup>. Correspondingly, 1 t ha<sup>-1</sup> equates to 14.86 bushels per acre for wheat, 13.87 bushels per acre for

maize, 7.92 bushels per acre for oats, 11.89 bushels per acre for barley, and 13.87 bushels per acre for rye.

With the British "bag" measuring 3 bushels,  $1 \, \text{tha}^{-1}$  ( $\sim 15$  bushels per acre) approximates to five bags to the acre – a farmer's on-the-spot measure of wheat yield.

# **Units for Temperature**

Temperature is another case of difficulties for conversion, because the fahrenheit and celsius (centigrade) scales do not correspond at any reasonable temperature (they equate at  $-40^{\circ}$ ). Conversion between the scales involves taking into account the differences between the scales in the slope (ratio of 9:5) and the freezing point of water (0°C and 32°F). To convert a temperature from celsius to fahrenheit, multiply °C by 9, divide the result by 5, and add 32. To convert a temperature from fahrenheit to celsius, deduct 32 from the temperature in °F, multiply by 5, and divide by 9.

#### **Cereal-Based Foods and Nutrition**

Several articles and a major appendix in this encyclopedia relate to the food end of the grain production—utilization chain. It is thus necessary to explain units in this area, which eventually involves the home kitchen. These articles use units such as cups (CU), tablespoons (TB), teaspoons (tsp), and fluid ounces (FO). This last unit is defined in Table 1. Household units are more difficult to define. Their use in this encyclopedia is provided below.

These US measurements may use the same word for two measurements that are different. An ounce can be one-sixteenth of a pound (weight measure) or one-sixteenth of a pint (volume measure). A fluid ounce and an ounce of weight may be two different amounts, depending on the density of the ingredients being measured. In countries outside of the USA, cooks usually measure solid ingredients by weight. US measurements are based on fluid measures used for water or milk, and for dry ingredients such as flour and sugar, and for solid ingredients such as shortening. Commonly used equivalents are:

- 1 tablespoon (TB) = 3 teaspoons = 15 ml;
- 1 teaspoon (tsp) =  $\frac{1}{3}$  tablespoon = 5 ml;
- 8 tablespoons =  $\frac{1}{2}$  cup or 4 ounces or 1 teacup;
- 16 tablespoons = 1 cup; and
- 1 cup (CP) =  $\frac{1}{2}$  pint or 8 fluid ounces = 237 ml.

The names of some British units have the same names as US measurements, but are not identical to US units. Overall, weights are equivalent but volumes differ. An English measuring cup (English breakfast cup) is  $\sim$ 32% larger than the US cup, measuring  $\sim$ 312 ml.

#### **Chemistry Units**

Standard chemical units are used throughout the encyclopedia. These include estimates of the amounts and sizes of molecules, especially macromolecules, such as proteins. The mole (abbreviated to mol) is described above as one of the seven basic units of the SI system, being (approximately) the amount of the substance expressed as its molecular mass in grams. The size of a molecule is given as its molecular mass in daltons (abbreviated to Da) or its relative molecular mass ( $M_r$  with no units). In both cases, the number indicates approximately the size of the molecule as a multiple of the size of the hydrogen atom, taken to be 1 Da. More correctly, however, the unit is taken to be one-twelfth of the mass of the carbon nuclide  $^{12}$ C.

The Svedberg unit (S) is a less common unit used in relation to estimating the size of macromolecules. In particular, it is an estimate of the size of a protein molecule, based on how fast it sediments under the very large centrifugal force of the ultracentrifuge. This rate of sedimentation is measured in sedimentation units. One Svedberg unit equals  $10^{-13}$  sedimentation units. The Svedberg unit is often used, for example, to characterize the proteins of the legume grains. Examples are vicilin and legumin, which are indicated to be 7S and 11S, respectively (see Protein Chemistry of Dicotyledonous Grains).

# **Units for Cereal Chemistry Testing**

It is still normal practice for cereal chemists to measure the resistance to extension of a dough in Brabender units, and to determine the activity of  $\alpha$ -amylase (the enzyme that hydrolyzes starch) in Farrand units or in stirring number units. Such nonstandard units may not be a problem to people working in the same topic area. They have worked during the development of these methods of analysis. In some cases, these units may be intentionally perpetuated, because a manufacturer or inventor sees the names for these units as a valuable means of advertising.

Although these units are meaningful and familiar for those who are using the test systems all the time, standard units would be more appropriate for the newcomer to the field. The adoption of standard units would also be conducive to international standardization of the method. This surely is the main reason for the use of SI units, namely, that they form a sound basis of universal understanding of what analytical results mean from one time to another and from one lab to another. In this encyclopedia,

there is therefore the need to explain the basis for the nonstandard units of grain science, as well as providing conversion tables for interchange between the metric and US Systems.

#### The Moisture Basis for Grain and Flour Data

Irrespective of what units are used, it is important to be aware of the moisture basis on which composition data are based for grain and flour samples. For example, protein content may be quoted in various forms, namely, on a dry-weight basis, on the basis of a specified moisture content (e.g., 14.5% moisture), or on an "as is" basis (the moisture content of the sample as it was at the time of testing). Figure 1 provides the means of converting values for protein content from a known moisture basis to either 11.0% or 13.5% moisture content.

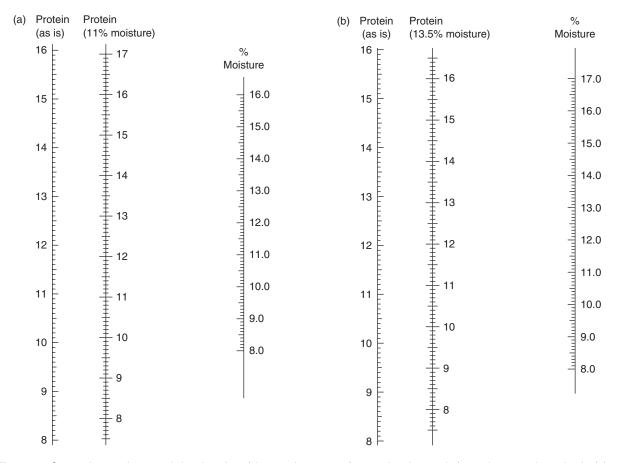
Furthermore, when flour milling is under consideration, "extraction" terms may need to be clarified. For example, some mills base 100% extraction and mill

capacity on the dry weight of wheat, whereas others base such estimates on the weight of wheat after the process of tempering, also known as conditioning, during which its moisture content is adjusted to a suitable level, often 14.5% moisture. Alternatively, mill capacity may be based on the production capacity for all flour products. A US flour mill making 78 000 pounds of flour from 100 000 pounds of wheat grain would be described as a 780 cwt mill in the US, but not in Europe.

# Measurement Units of Dough Testing

# **Resistance Units and the Mixograph**

When American scientists, Swanson and Working, developed the recording mixograph dough mixer in the early 1930s, the vertical axis of the mixograph trace was left unlabeled, providing no quantitative indication of the resistance of the dough to mixing. In their 29-page article, they described how an early



**Figure 1** Conversion graph to permit the alteration of the protein content of a cereal-grain sample from a known moisture basis: (a) to an 11% moisture basis or (b) to a 13.5% moisture basis. To do so, a ruler is used to join the observed protein content of the sample on the left-hand scale to the moisture content of the sample (right-hand scale) is used. The point where the ruler crosses the inner scale is the protein content of the sample, based on either (a) 11% or (b) 13.5% moisture. (Adapted from the Cereal Chemistry Division Official Methods, Royal Australian Chemical Institute, Melbourne, Australia.)

# **Traditional and Newer Dough-Testing Equipment**

The Brabender farinograph and extensigraph use a combination of SI units and "Brabender units" (BUs) in the testing of various aspects of dough testing. No standard units have become adopted for the BU, so it remains as a measure that is meaningful for cereal chemists, who regularly use the equipment, or for those who are involved in interpreting the results, e.g., wheat breeders.

Recently developed research instruments for emulating the dough-test processes of the Brabender farinograph and extensigraph with very small dough samples (a few grams) have been designed specifically for automatic data capture, to correlate with the results from more traditional instruments, and to provide results in SI units, namely, in grams (vertical axis of the mixing curve) and centimeters (horizontal axis).

Other recently developed dough-testing instruments are calibrated and read out in SI units. In the case of the doughLAB (Figure 2), an instrument similar to the Brabender farinograph, data are reported in either newton meters (N m), or farinograph units ("FU"). The relationship between these units is unfortunately not constant, but differs with the choice of bowl, namely, that for the 300 g bowl, 500 FU =  $4.90 \, \text{N}$  m and for the 50 g bowl, 500 FU =  $0.98 \, \text{N}$  m.

# Measurement Units of Enzymic Activity and Starch Viscosity

# The Falling Number Unit

One of the strange names for units in grain science is the "falling number unit," akin to the (also strange) stirring number unit. The falling number method was developed in Sweden in response to the need for a means of quantifying the degree of sprout damage to grain as a result of premature germination caused by rain at harvest. It provides a measure of the enzymic action of  $\alpha$ -amylase on the endogenous starch for the germinating grain. It is applicable to several cereal

species, especially wheat and rye. The results are provided as the number of seconds taken for a plunger to fall through a heated mixture of ground grain and water; a short falling time, e.g., 100 s, indicates extensive sprout damage. One falling number unit is thus simply equal to 1 s of falling time, but nevertheless the name falling number unit is still in common use in grain trading.

# **The Stirring Number Unit**

The "stirring number method" was developed in 1984 and 1985 following widespread rain damage to the Australian wheat crop. Research was initiated to develop an instrument that could detect this damage, like the established falling number method, but would be robust and rapid, thus better suited to the harsh conditions of the Australian silo (grain-receival site). The instrument produced was called the Rapid Visco Analyzer (RVA), and a 3 min test was devised to detect sprout damage. The test works by using stirring action to mix and measure the viscosity of a slurry of wheatmeal and water as it is heated. Apparent viscosity is measured continuously as the power requirement of the stirring motor. The viscosity of raindamaged samples is markedly lower than that of undamaged grain because of starch hydrolysis by the sprouting enzyme.

The stirring number unit (later renamed the "rapid visco unit") was chosen for routine output from the method to provide results in a range that would make the measurement easily accessible to farmers and grain traders. The range chosen was from near zero (poor-quality damaged grain) to  $\sim 150$  (good-quality sound grain).

#### The Rapid Visco Unit and Centipoise

Nevertheless, at the outset of developing the RVA, the use of standard units of viscosity was seen as essential. Initially, the RVU was devised as a unit for the more general use of the RVA in characterizing starch properties. This unit of apparent viscosity can be related to international units through the relationship 1 RVU = 12 centipoise (cP). Traceable calibrations were then developed for the RVA, which now provides data in centipoise (1 centipoise = 1 millipascal second (an SI unit) abbreviated to mPa s).

#### Conclusion

While there may be general agreement that it is desirable to standardize units of measurement, the world is still far from uniform in this respect. There may be less general agreement that the SI/metric system is the only one by which standardization can be



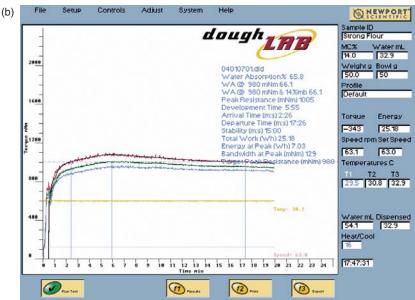


Figure 2 (a) The doughLAB equipment, designed to provide dough-test results in SI units (newton meters). Dough is mixed in the tiny chamber in front. (b) Mixing curve for a strong flour from the doughLAB, showing the vertical axis in millinewton meters. The mixing action produces a broad band of torque, whose width is indicated by the two outer traces. The inner trace shows the mean of the mixing curve. Resistance to mixing starts from zero and rises as the dough forms, reaching a maximum (for this sample) after mixing for  $\sim$ 6 min. Thereafter, the resistance to mixing decreases only slightly, showing that this dough would have a good tolerance to over-mixing in the bakery. A much greater decrease in "breakdown" after the peak would be indicative of a weak flour, which would also have a shorter time to the peak of mixing torque. (Illustrations provided by Newport Scientific, Sydney, Australia.)

achieved; there is no other real contender. Until international standardization is adopted in practice, it will be necessary to provide conversion tables, such as are given in Table 1. Nevertheless, the general agreement in the scientific community on SI units sets an example for the rest of the world, demonstrating the added efficiency of uniformity in units of measurement.

See also: Cereals: Grain – Quality Attributes. Labeling of Grain-Based Foods. Nutrition: Mineral Composition;

Vitamin Composition. **Starch**: Analysis of Quality. **Wheat**: Dough Rheology. **Appendix**: Grain Composition Table; Test Methods for Grain and Grain-Based Products.

## **Further Reading**

International Standards Organization (1993) ISO Standards Handbook: Quantities and Units, 3rd edn. Geneva, Switzerland: International Standards Organization.

Lentner C (1981) Geigy Scientific Tables. Volume 1. Units of Measurement, Body Fluids, Composition of the Body, Nutrition. Basle, Switzerland: Ciba-Geigy Ltd. Standards Australia (1998) The International System of Units (SI) and Its Application. AS ISO 1000-1998. Sydney, Australia: Standards Australia (See also equivalent publications in other countries.).

# **Relevant Websites**

http://online.standards.com - National standards organizations provide information on units. An example is the Australian Standards website. http://www.iso.ch International Standards Organization.