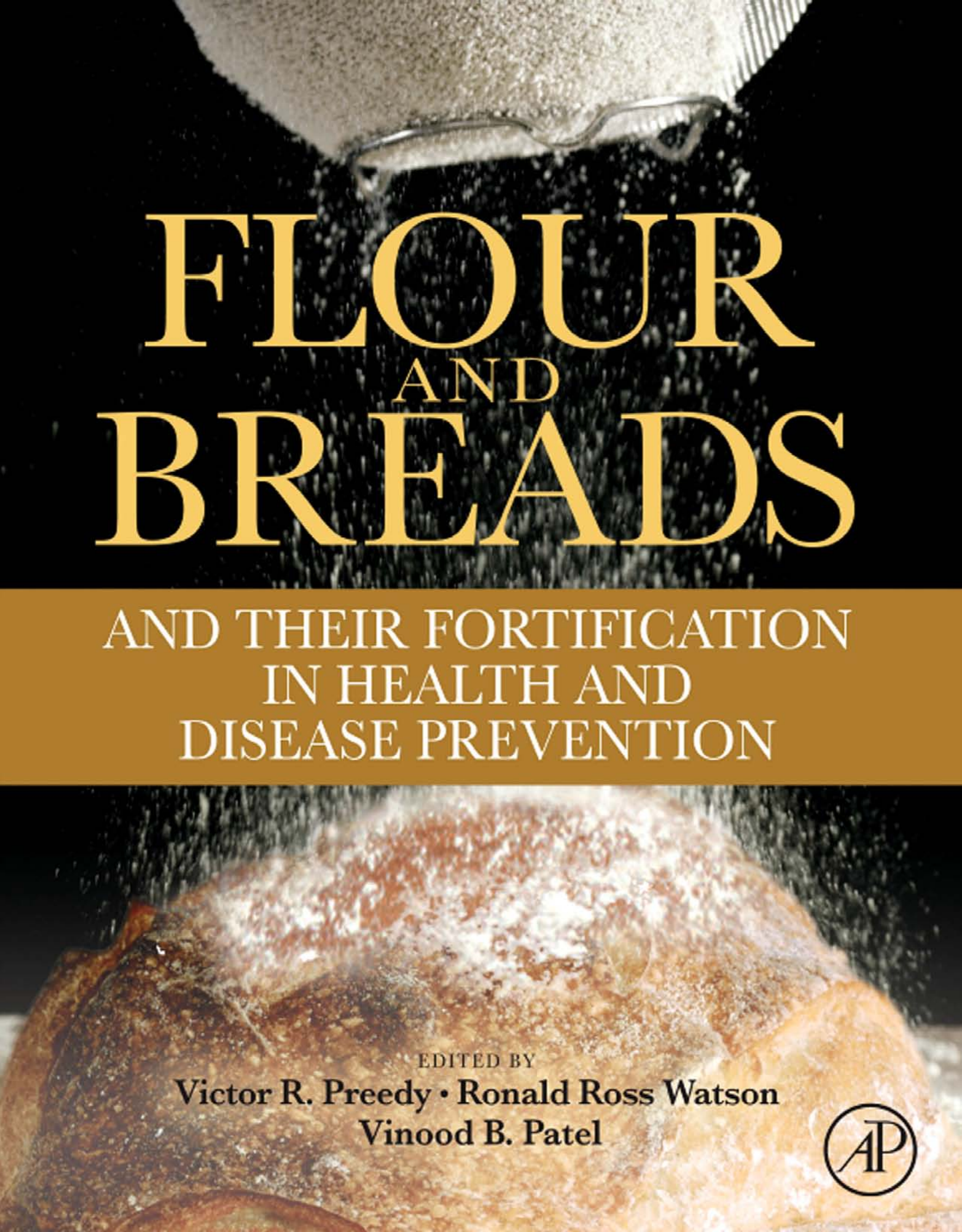

FLOUR AND BREADS

AND THEIR FORTIFICATION IN HEALTH AND DISEASE PREVENTION

EDITED BY

Victor R. Preedy • Ronald Ross Watson
Vinood B. Patel

Flour and Breads and their Fortification in Health and Disease Prevention

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PREFACE

The historical pictorial evidence for bread making dates back 8000 years, but it is probable that bread was consumed in the unleavened form (without yeast) earlier than this, going hand-in-hand with the cultivation of crops. In some cultures, bread is an integral part of sacred and religious ceremonies.

Currently, bread is an important part of the diet for millions of people worldwide. Its complex nature provides energy, protein, minerals, and many other macro- and micronutrients. However, consideration must be taken of four major aspects related to flour and bread. The first is that not all cultures consume bread made from wheat flour. There are literally dozens of flour types, each with its distinctive heritage, cultural roles, and nutritive contents. Second, not all flours are used to make leavened bread in the traditional (i.e., Western) loaf form. There are many different ways that flours are used in the production of staple foods. Third, flour and breads can be fortified either to add components that are removed in the milling process or to add components that will increase palatability or promote health and reduce disease per se. (In this book, the term "fortification" is used holistically to include statutory and nonstatutory additions.) Finally, there are significant groups of individuals who have intolerance to flours such as wheat, barley, or rye flours.

Finding all this knowledge in a single coherent volume is currently problematical, and *Flour and Breads and their Fortification in Health and Disease Prevention* addresses this.

This book is divided into two main sections:

1. Flour and Breads
2. Fortification of Flour and Breads and their Metabolic Effects

The editors are aware of the difficulties imposed by assigning chapters to different sections and their order, but the navigation of the book is enhanced by an excellent index. The book is also extremely well illustrated, with tables and figures in every chapter.

Where applicable, information on adverse effects or responses is provided. Emerging fields of science and important discoveries relating to flour and bread products are also incorporated in the book. Contributors are authors of international and national standing and leaders in the field.

This book represents a comprehensive coverage of material relating to flour and bread and their constituents. It is essential reading for policymakers, food technologists, marketing strategists, nutritionists, food chemists, health care professionals, research scientists, as well as those interested in flour and breads in general or working in the food industry.

Victor R. Preedy, Ronald Ross Watson,
and Vinood B. Patel

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SECTION 1

Flour and Breads

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CHAPTER 1

The Science of Doughs and Bread Quality

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CHAPTER OUTLINE

INTRODUCTION

Cereals and cereal-based products have constituted the major component of the human diet throughout the world since the earliest times. Cereal crops are energy dense, providing approximately 10–20 times more energy than most juicy fruits and vegetables. Major cereal crops include wheat, rice, corn, and barley. The cereal crop most produced is corn (or maize) (31%), but it has relatively less importance than wheat and rice because it is not directly used for human consumption. Wheat and rice are the most important cereals with regard to human nutrition, and they account for 55% of the total cereal production. Nutritionally, they are important sources of dietary protein, carbohydrates, the B group vitamins, vitamin E, iron, trace minerals, and fibers. It has been estimated that global cereal consumption directly provides approximately 45% of protein and energy necessary for the human diet and only approximately 7% of the total fat (Table 1.1). The specific contribution of wheat to daily food intake corresponds to approximately 20% of the required energy and protein for the human diet (see Table 1.1).

Cereals have a variety of uses as food, although only two cereals, wheat and rye, are suited for the preparation of leavened bread. Nevertheless, wheat is a unique cereal that is suitable for the preparation of a wide diversity of leavened breads that meet consumer demands and requirements worldwide (Figure 1.1) (Rosell, 2007a). Among baked goods, bread has been a staple food for many civilizations. Even today, bread and cereal-based products constitute the base of the food pyramid, and its consumption is recommended in all dietary guidelines. Bread has a fundamental role in nutrition due to the adequate balance of

Flour and Breads and their Fortification in Health and Disease Prevention. DOI: 10.1016/B978-0-12-380886-8.10001-7

TABLE 1.1 Contribution of Cereals to the Daily Food Intake

	Food Consumption (kg/Capita/Year)	Food Consumption (kcal/Capita/Day)	Protein Consumption (g/Capita/Day)	Fat Consumption (g/Capita/Day)
Total		2808.87	75.72	79.63
Cereals	151.07	1302.75	31.62	5.49
Wheat	67.00	518.00	15.34	2.18
Milled rice	54.21	541.92	10.07	1.28
Barley	1.13	8.04	0.23	0.03
Maize	18.54	152.72	3.66	1.22
Rye	0.98	7.42	0.20	0.03
Oats	0.52	2.94	0.12	0.05
Millet	4.05	33.26	0.89	0.35
Sorghum	3.90	32.72	0.97	0.33
Other cereals	0.74	5.73	0.16	0.02

Source: Food and Agriculture Organization (2007).

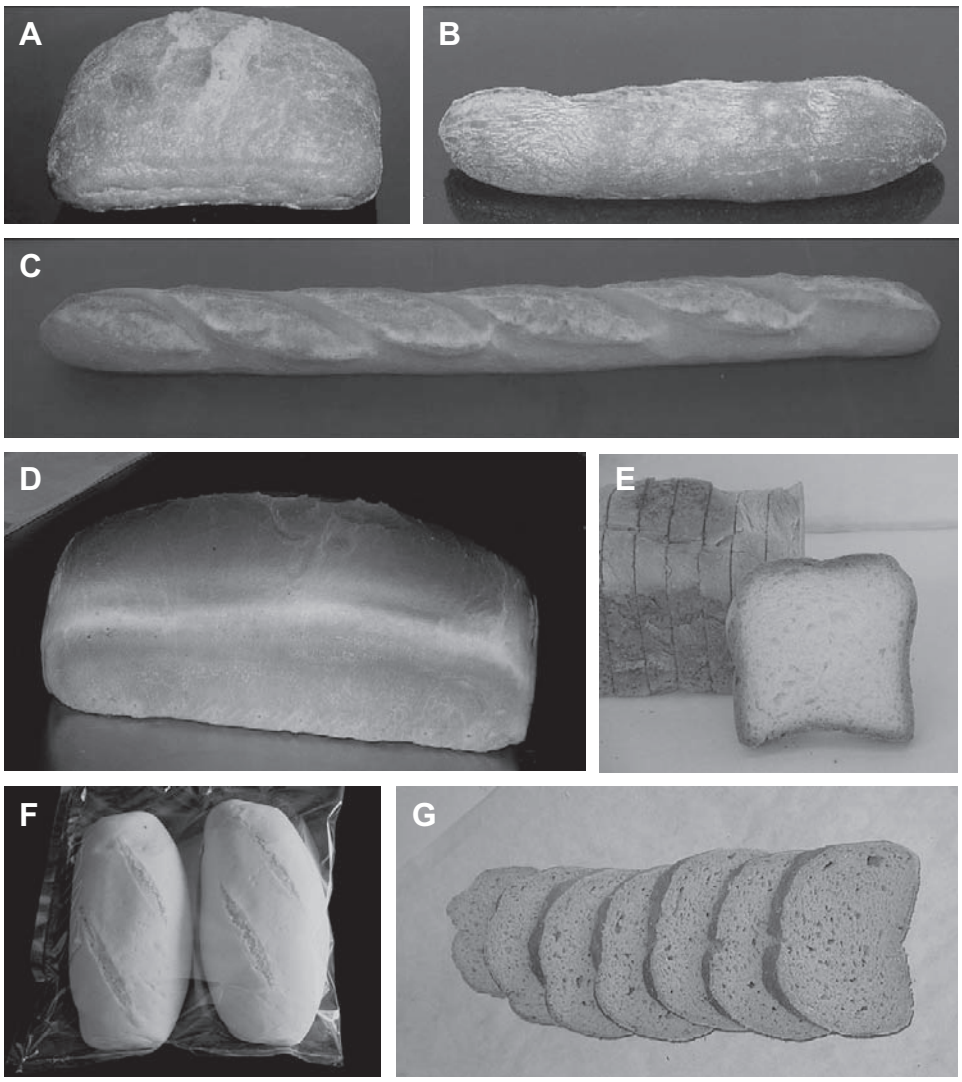

FIGURE 1.1
Different types of breads. There is a wide diversity of leavened breads that meet consumer demands and requirements worldwide. (A and B) Crusty bread named ciabatta, (C) baguette, (D and E) pan bread, (F) partially baked bread, and (G) fiber-enriched bread.

macronutrients in its composition; in addition, it provides some micronutrients and minerals.

NUTRITIONAL VALUE OF CEREALS AND THE IMPACT OF MILLING

All cereal grains have a fairly similar structure and nutritive value, although the shape and size of the seed may be different. In this chapter, wheat is used as a reference because it is the base of more foods than any other grain and the basis for the preparation of leavened bread; hereafter, the discussion refers to wheat grain.

The chemical components of cereals are not evenly distributed in the grain. Table 1.2 provides the nutritive value of the three main different parts in wheat. Bran, which represents 7% of the grain, contains the majority of the grain fiber, essentially cellulose and pentosans. It is a source of B vitamins and phytochemicals, and 40–70% of the minerals are concentrated in this outer layer. The endosperm, the main part of the grain (80–85%), contains mostly starch. It has lower protein and lipid content than the germ and the bran, and it is poor in vitamins and minerals. The germ, the small inner core that represents approximately 21% of the grain, is rich in B group vitamins, proteins, minerals such as potassium and phosphorous, healthful unsaturated fats, antioxidants, and phytochemicals. Cereals are rich in glutamic acid, proline, leucine, and aspartic acid, and they are deficient in lysine. The amino acid content is mainly concentrated in the germ.

Generally, cereal grains are subjected to different processes to prepare them for human consumption. These processes significantly affect their chemical composition and consequently their nutritional value.

The majority of wheat is milled into flour, which can be used to make many types of breads that differ in shape, structure, and sensory characteristics. Milling removes the fibrous layers of the grain; therefore, refined cereals do not have the same nutritional and health benefits as the grain or wholemeal (see Table 1.2). Without the bran and germ, approximately 45% of the grain proteins are lost, along with 80% of fiber, 50–85% of vitamins, 20–80% of minerals, and up to 99.8% of phytochemicals. In addition, important losses of amino acids (35–55%) occur during refining. Some fiber, vitamins, and minerals may be added back into refined cereal products through fortification or enrichment programs, which compensates for losses due to refining, but it is impossible to restore the phytochemicals lost during processing (Rosell, 2007b).

BREAD DOUGH MODIFICATIONS DURING THE BREAD MAKING PROCESS

A brief description of the bread making process is included so that the reader will understand the physical and chemical constraints to which the cereal main biopolymers, constituents of the dough, are exposed during the process (for more detailed information, see Cauvain, 2003). Different alternatives have been developed for adapting bread making to consumer demands and for facilitating the baker's work (Figure 1.2). Bread making stages include mixing the ingredients, dough resting, dividing and shaping, proofing, and baking, with great variation in the intermediate stage depending on the type of product. During mixing, fermenting, and baking, dough is subjected to different shear and large extensional deformations (including fracture), which are largely affected by temperature and water hydration (Rosell and Collar, 2009). Several physical changes occur during the bread making process, in which gluten proteins are mainly responsible for bread dough structure formation, whereas starch is mainly implicated in final textural properties and stability.

In bread making, mixing is one of the key steps that determine the mechanical properties of the dough, which have a direct consequence on the quality of the end product. Mixing evenly

TABLE 1.2 Proximate Composition (%) of Wheat and the Effect of the Milling Process on Nutrient Composition

	Wheat Grain	Bran	Flour	Germ
Energy (kcal)	329.0	216.0	364	360
Total carbohydrate (g)	68.0	64.5	76.3	51.8
Dietary fiber (g)	12.0	42.8	2.7	13.2
Total fat (g)	1.9	4.3	1	9.7
Saturated fat (g)	0.3	0.6	0.2	1.7
Monounsaturated fat (g)	0.3	0.6	0.1	1.4
Polyunsaturated fat (g)	0.8	2.2	0.4	6
Protein (g)	15.4	15.5	10.3	23.1
Amino acids				
Tryptophan (mg)	195	282	127	317
Threonine (mg)	433	500	281	968
Isoleucine (mg)	541	486	357	847
Leucine (mg)	1038	928	710	1571
Lysine (mg)	404	600	228	1468
Methionine (mg)	230	234	183	456
Cystine (mg)	404	371	219	458
Phenylalanine (mg)	724	595	520	928
Tyrosine (mg)	441	436	312	704
Valine (mg)	679	726	415	1198
Arginine (mg)	702	1087	417	1867
Histidine (mg)	330	430	230	643
Alanine (mg)	555	765	332	1477
Aspartic acid (mg)	808	1130	435	2070
Glutamic acid (mg)	4946	2874	3479	3995
Glycine (mg)	621	898	371	1424
Proline (mg)	1680	882	1198	1231
Serine (mg)	663	684	516	1102
Vitamins				
Vitamin A (IU)	9	9	—	—
Vitamin E (mg)	1.0	1.5	0.1	—
Vitamin K (μg)	1.9	1.9	0.3	—
Thiamin (mg)	0.5	0.5	0.1	1.9
Riboflavin (mg)	0.1	0.6	—	0.5
Niacin (mg)	5.7	13.6	1.3	6.8
Vitamin B_6 (mg)	0.3	1.3	—	1.3
Folate (μg)	43	79	26	281
Pantothenic acid (mg)	0.9	2.2	0.4	2.3
Choline (mg)	31.2	74.4	10.4	—
Minerals				
Calcium (mg)	25	73	15	39
Iron (mg)	3.6	10.6	1.2	6.3
Magnesium (mg)	124	611	22	239
Phosphorus (mg)	332	1013	108	842
Potassium (mg)	340	1182	107	892
Sodium (mg)	2	2	2	12
Zinc (mg)	2.8	7.3	0.7	12.3
Copper (mg)	0.4	1.0	0.1	0.8
Manganese (mg)	4.1	11.5	0.7	13.3
Selenium (μg)	70.7	77.6	33.9	79.2

Source: Gramene (2009).

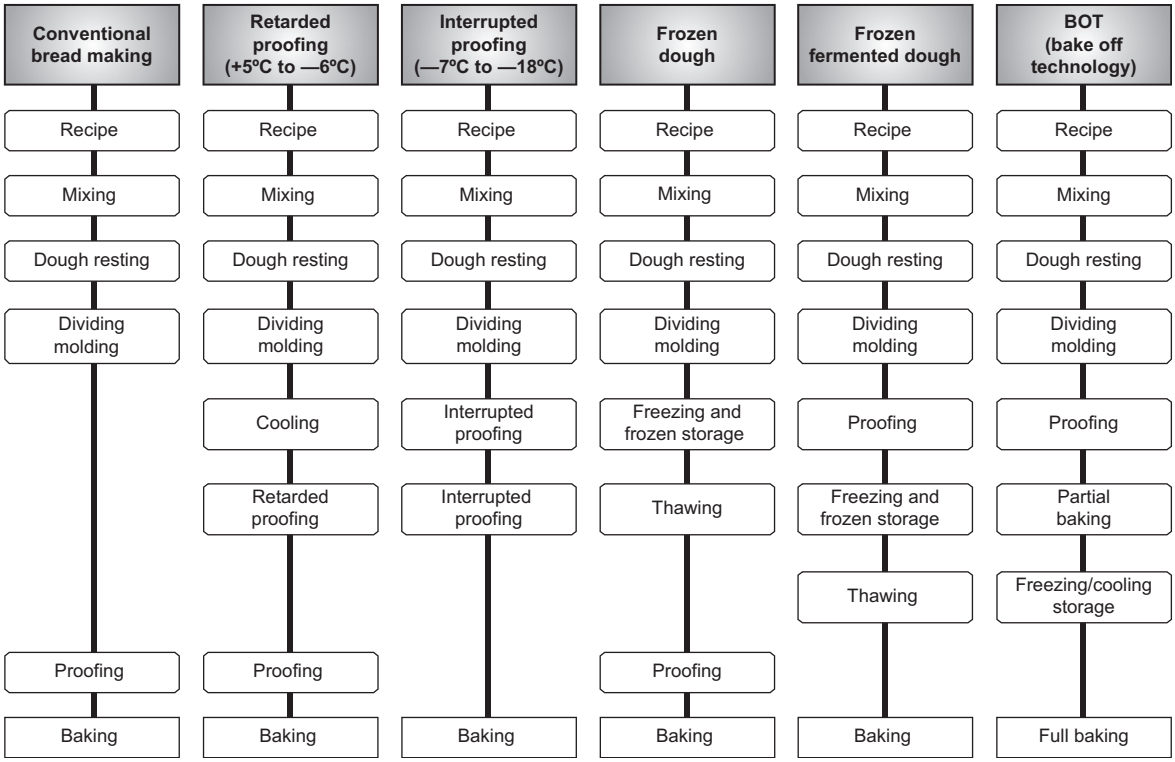

FIGURE 1.2
Current methods of bread making. Different alternatives have been developed for adapting bread making to consumer demands and for facilitating the baker's work. Bread making stages include mixing the ingredients, dough resting, dividing and shaping, proofing, and baking, with great variation in the intermediate stage depending on the type of product.

distributes the various ingredients, hydrates the component of the wheat flour, supplies the necessary mechanical energy for developing the protein network, and incorporates air bubbles into the dough. Each dough has to be mixed for an optimum time to fully develop, and at this stage it offers maximum resistance to extension. The period of barely constant torque determines the dough stability, which is dependent on the flour and mixing method used. Undermixing may cause small unmixed patches that interfere in the proofing stage. Conversely, if the mixing is excessive, dough properties change from good (smooth and elastic) to poor (slack and sticky) (Sliwinski *et al.*, 2004), and a decrease in the consistency is observed, which is attributed to the weakening of the protein network. Bread dough is a viscoelastic material that exhibits an intermediate rheological behavior between a viscous liquid and an elastic solid. Bread dough must be extensible and elastic enough for expanding and holding the released gases, respectively.

During initial mixing, wheat dough is exposed to large uni- and biaxial deformations. Moreover, the material distribution, the disruption of the initially spherical protein particles, and the flour component hydration occur simultaneously, and together with the stretching and alignment of the proteins, this leads to the formation of a three-dimensional viscoelastic structure with gas-retaining properties. The rheological properties of wheat flour doughs are largely governed by the contribution of starch, proteins, and water. The protein phase of flour has the ability to form gluten, a continuous macromolecular viscoelastic network, but only if enough water is provided for hydration and sufficient mechanical energy input is supplied during mixing. The viscoelastic network plays a predominant role in dough machinability and affects the textural characteristics of the finished bread (Collar and Armero, 1996). The viscoelastic properties of the dough depend on both quality and quantity of the proteins, and the size distribution of the proteins is also an important factor. Two proteins present in flour

(gliadin and glutenin) form gluten when mixed with water and give dough these special features. Gluten is essential for bread making and influences the mixing, kneading, and baking properties of dough. According to MacRitchie (1992), two factors contribute to dough strength: the proportion of proteins above a critical size and the size distribution of the proteins. The properties of this network are governed by the quaternary structures resulting from disulfide-linked polymer proteins and hydrogen bonding aggregates (Aussenac *et al.*, 2001). Dough mixing involves large deformations that are beyond the linearity limit, which correlates with nonlinear rheological properties. The characterization of the viscoelastic behavior exceeding the linear viscoelasticity requires specialized devices that record dough consistency when subjected to mechanical stress and/or dual mechanical and temperature constraints (Rosell and Collar, 2009). The stability of failure in single dough bubble walls is directly related to the extensional strain hardening properties of the dough, which plays an important role in the stabilization of bubble walls during baking.

During proofing or fermentation, yeast metabolism results in carbon dioxide release and growth of air bubbles previously incorporated during mixing, leading to expansion of the dough, which inflates to larger volumes and thinner cell walls before collapsing. The growth of gas bubbles during proof and baking determines the characteristics of the bread structure and thus the ultimate volume and texture of the baked product. The yeast breaks carbohydrates (starch and sugars) down into carbon dioxide and alcohol during alcoholic fermentation. Enzymes present in yeast and flour also help to speed up this reaction. The carbon dioxide produced in these reactions causes the dough to rise (ferment or proof), and the alcohol produced mostly evaporates from the dough during the baking process. During fermentation, each yeast cell forms a center around which carbon dioxide bubbles are released. Thousands of tiny bubbles, each surrounded by a thin film of gluten, grow as fermentation proceeds. Kneading or remixing of the dough favors the release of large gas bubbles, resulting in a more even distribution of the bubbles within the dough.

The size, distribution, growth, and failure of the gas bubbles released during proofing and baking have a major impact on the final quality of the bread in terms of both appearance (texture) and final volume (Cauvain, 2003). As the intense oven heat penetrates the dough, the gases inside the dough expand, with a concomitant increase in the size of the dough. As the temperature rises, the rate of fermentation and production of gas cells increases, and this process continues until the temperature of yeast inactivation is reached (approximately 45°C). When proteins are denatured, the gluten strands surrounding the individual gas cells are transformed into the semi-rigid structure that will yield the bread crumb. Endogenous enzymes present in the dough are inactivated at different temperatures during baking. The sugars and breakdown products of proteins released from the enzyme activity are then available to sweeten the bread crumb and participate in Maillard or nonenzymatic browning reactions, which are responsible for the brown color of the crust.

In the past several decades, bread making processes have been adapted to consumer demands, and subzero and low temperatures have been included in flow diagrams for interrupting the processes before or after fermentation, or when partial baking is completed, for obtaining partially baked breads (see Figure 1.1) (Rosell, 2009). These technologies have facilitated the launching of a great number of fresh-baked goods available at any time of the day, and overall they help bakeries bring new products to the market quickly and successfully.

BIOCHEMICAL CHANGES DURING BREAD MAKING

Bread making is a dynamic process with continuous physicochemical, microbiological, and biochemical changes caused by mechanical–thermal action and the activity of the yeast and lactic acid bacteria together with the activity of the endogenous enzymes. The changes in the flour biopolymeric compounds take place during mixing, proofing, and baking. During mixing, dough is exposed to large uni- and biaxial deformations and a continuous protein

network is formed, which is stabilized by disulfide bonds and modified thiol/disulfide interchange reactions. The input of mechanical energy that takes places during kneading confers the necessary energy for distributing flour components, favoring the protein interaction and the formation of covalent bonds between them, which finally leads to the formation of a continuous macromolecular viscoelastic structure. Depolymerization and repolymerization of the sodium dodecyl sulfate-unextractable polymers occurs by the repeated breaking and reforming of disulfide bonds within and between gluten proteins, where glutenin subunits are released in a nonrandom order, indicating a hierarchical structure (Aussenac *et al.*, 2001). Also in this structure, tyrosine cross-links contribute to dough elasticity, suggesting that a radical mechanism involving endogenous peroxidases might be responsible for dityrosine formation during bread making (Tilley *et al.*, 2001).

There is general agreement that gluten is the main contributor to the unique properties of wheat dough properties, affecting dough characteristics and, consequently, the quality of the fresh bread. Gluten is a non-pure protein system, and although the nonprotein components have significant effects, the rheological properties of gluten derive from the properties and interactions among proteins. Gluten proteins comprise two main subfractions: glutenins, which confer strength and elasticity, and gliadins, which impart viscosity to dough. Proteins mainly involved in the viscoelastic properties of the dough are the high-molecular-weight glutenin subunits, which affect dough viscoelasticity in a similar and remarkable way as the water content (Cauvain, 2003). Namely, the mixing process induces an increase in the amount of total unextractable polymeric protein and large unextractable monomeric proteins (Kuktaite *et al.*, 2004). Specifically, the amount of high-molecular-weight glutenins increases with a parallel decrease in the amount of low-molecular-weight glutenins, gliadins, and albumins/globulins (Lee *et al.*, 2002). Mixing also promotes the solubilization of arabinoxylans due to mechanical forces, and this solubilization proceeds further during resting due to endoxylanase activity, in addition to xylosidase and arabinofuranosidase activities (Dornez *et al.*, 2007).

The other large biopolymer that plays an important role in the bread making process is starch. Amylose and amylopectin are the constituents of the starch granule. This biopolymer provides fermentable sugars to yeast and has a significant contribution to dough rheology, especially during the baking process (Cauvain, 2003). Pasting performance of wheat flours during cooking and cooling involves many processes, such as swelling, deformation, fragmentation, disintegration, solubilization, and reaggregation, that take place in a very complex media primarily governed by starch granule behavior. During heating, the native protein structure is destabilized, and unfolding may facilitate sulfhydryl—disulfide interchange reactions and oxidation together with hydrophobic interactions, leading to the association of proteins and, consequently, to the formation of large protein aggregates. Nevertheless, as the temperature increases, the role of the proteins becomes secondary, and changes involving the starch granules become predominant. During this stage, starch granules absorb the water available in the medium and they swell. Amylose chains leach out into the aqueous intergranular phase, promoting the increase in viscosity that continues until the temperature constraint leads to the physical breakdown of the granules, which is associated with a reduction in viscosity. During cooling of the loaf, the gelation process of the starch takes place, in which the amylose chains leached outside the starch granules during heating are prompted to recrystallize. The reassociation between the starch molecules, especially amylose, results in the formation of a gel structure. This stage is related to the retrogradation and reordering of the starch molecules.

In addition to these changes, it must be considered that bread making is a dynamic process with continuous microbiological and chemical changes, motivated by the action of the yeast and lactic acid bacteria, which occur during proofing and the initial stage of baking. Yeasts and lactic acid bacteria contain different enzymes responsible for the metabolism of microorganisms that modify dough characteristics and fresh bread quality. Therefore, wheat flour,

yeasts, and bacterial population of sour doughs are sources of different endogenous enzymes in bread making processes and exert an important effect on dough rheology and on the technological quality of bread (Rosell and Benedito, 2003). Different processing aids, namely enzymes, are also used in bread making to improve the quality of the baked products by reinforcing the role of gluten, providing fermentable sugars, and/or contributing to stabilize the hydrophobic–hydrophilic interactions (Rosell and Collar, 2008).

Numerous biochemical changes occur during bread making that have direct effects on the sensory attributes and nutritional quality of the finished product. The contribution of low-molecular-weight proteins to the taste and flavor of bread depends on the content of peptides rich in basic and hydrophobic amino acids released during fermentation and baking, the proportion of hydrophilic peptides in unfermented bread, and the balance of endo- and exoprotease activities during those stages. Changes in the total or individual content of amino acids and peptides during the different steps of bread making modify the organoleptic characteristics of the bread (Martinez-Anaya, 1996). Amino acids are absorbed by yeast and lactic acid bacteria and metabolized as a nitrogen source for growth, resulting in an increase in the amount of gas produced, raising the alcohol tolerance of yeast and improving the organoleptic and nutritional quality of bread. They can also be hydrolyzed by the action of proteolytic enzymes from both flour and microorganisms on proteins as well as by yeast autolysis. The amino acid profile during bread making reveals that the total amino acid content (particularly for ornithine and threonine) increases by 64% during mixing and then decreases 55% during baking, with the most reactive amino acids being glutamine, leucine, ornithine, arginine, lysine, and histidine (Prieto *et al.*, 1990). Free amino acids in wheat flour and dough play an important role in the generation of bread flavor precursors through the formation of Maillard compounds during baking. In fact, leucine, proline, isoleucine, and serine reacting with sugars form typical flavors and aromas described as toasty and breadlike, whereas excessive amounts of leucine in fermenting doughs lead to bread with unappetizing flavor (Martinez-Anaya, 1996). The specific metabolic activities of fermentation microorganisms are responsible for the dynamics in nitrogen compounds, showing different metabolic rates for acidic, basic, aliphatic, and aromatic amino acids. Lactic acid bacteria contain proteases and peptidases, which release into the media amino acids and peptides that are easily metabolized by yeast and lactic acid bacteria, showing different nutritional requirements and exoproteolytic and endoproteolytic activities depending on the strain of lactic acid bacteria (Collar and Martinez-Anaya, 1994). In general, wheat doughs started with lactic acid bacteria show a gradual increase in valine, leucine, and lysine during fermentation, and there is also an increase in proline but only during the initial hours of proofing. In addition, the action of proteinases and peptidases from lactic acid bacteria on soluble polypeptides and proteins results in an increase in short-chain peptides that contribute to plasticize the dough and give elasticity to gluten. Jiang *et al.* (2008) observed a decrease in 17 amino acids in steamed bread; alanine underwent the highest loss (17.1%), followed by tyrosine (12.5%), and leucine was the least affected amino acid.

Protein–lipid interactions in wheat flour dough also play an important role because both lipids and proteins govern the bread making quality of flour. Lipids have a positive effect on dough formation and bread volume, namely polar lipids or the free fatty acid component of the nonstarch lipids, whereas nonpolar lipids have been found to have a detrimental effect on bread volume (MacRitchie, 1983). During mixing, more than half of the free lipids in flour are associated with gluten proteins, although there is no consensus about the type of interactions between lipids and proteins. However, evidence has been presented that nonpolar lipids are retained within the gluten network through hydrophobic forces, involving the physical entrapment of lipids within the proteins (McCann *et al.*, 2009). The same study suggests that glycolipids are associated with glutenins through hydrophobic interactions and hydrogen bonds, whereas the phospholipids presumably interact with either the gliadins or the lipid-binding proteins.

Vitamin content is also affected during the bread making process. The yeasted bread making process leads to a 48% loss of thiamine and 47% loss of pyridoxine in white bread, although higher levels of these vitamins can be obtained with longer fermentations (Batifoulier *et al.*, 2005). Native or endogenous folates show good stability in the baking process, and even an increase in endogenous folate content in dough and bread compared with the bread flour was observed by Osseyi *et al.* (2001). Nevertheless, the bread making process with yeast fermentation is beneficial for reducing the levels of phytate content with the subsequent increase in magnesium and phosphorus bioavailability (Haros *et al.*, 2001).

BREAD QUALITY: INSTRUMENTAL, SENSORY, AND NUTRITIONAL QUALITY

Bread quality is a very subjective term that greatly depends on individual consumer perception, which in turn is affected by social, demographic, and environmental factors. The perception of bread quality varies widely with individuals and from one bread to another. Scientific reports focused on the bread making process or recipes usually refer to instrumental methods for assessing quality, whereas studies focused on consumer preferences highlight the significant relationship between sensory quality and consumer perception. Alternatively, healthy concepts related to nutritional value are emerging as fundamental quality attributes of bread products (Table 1.3). Therefore, the global concept of bread quality could be integrated by instrumental attributes, those that can be objectively measured; sensory sensations including descriptive attributes related to consumer quality perceptions; and nutritional aspects related to healthiness and functionality of the bread products.

Regarding instrumental quality (see Table 1.3), due to the existence of a great variety of breads derived from different wheat grains, bread making processes, and recipes, it is almost impossible to identify specific features for assessing bread quality. Consequently, different features have been defined and quantified to evaluate breads, including volume (rapeseed displacement), weight, specific volume, moisture content, water activity, color of crust and crumb, crust crispiness, crumb hardness, image analysis of the cell distribution within the loaf slice, and volatile composition. All these instrumental measurements have been extensively used for investigating the impact of different flours, ingredients, processing aids, and bread making processes on baked products (Cauvain, 2003; Rosell and Collar, 2008). These measurements provide objective values that, although they do not reflect consumer preferences or freshness perception, are very useful for comparison purposes when the aim is the improvement of intrinsic bread features perceived as bread quality attributes.

The perceived quality of bread is a complex process associated with sensory sensations derived from product visual appearance, taste, odor, and tactile and oral texture. Generally, perceived quality of bread is intimately linked to freshness perception. Consumer test provides an important tool for understanding the consumer expectations of different bread varieties. A number of surveys have been conducted to determine consumer perceptions of and preferences for bread products (Dewettinck *et al.*, 2008; Heenan *et al.*, 2008; Lambert *et al.*, 2009). A descriptive sensory analysis carried out on 20 commercial bread types allowed consumer segmentation into three clusters: (1) preference for porous appearance and floury odor; (2) preference for malty odor and sweet, buttery, and oily flavor; and (3) preference for porous appearance, floury and toasted odor, and sweet aftertaste (Heenan *et al.*, 2008). In a European survey on consumer attitudes toward breads, two main groups were defined: frequent (daily) buyers with a focus on quality and pleasure and less frequent buyers (once a week) with a more pronounced interest in nutrition, shelf life, and energy (process) (Lambert *et al.*, 2009). The first group was called the "crust group" and the second one the "crumb group."

Consumers are becoming more conscious about the relationship between nutrition and health. Currently, innovations in bread are mainly focused on nutritionally improving bread

TABLE 1.3 Overview of the Parameters That Can Be Used for the Quality Assessment of Breads

Instrumental quality	Sensory analysis
Specific volume	Visual appearance
Crust color	Odor
Crumb texture fresh	Tactile and oral texture
Hardness	Taste
Springiness	Overall acceptance
Cohesiveness	Nutritional quality
Chewiness	Proximate composition
Resilience	Carbohydrates
Crust indentation	Proteins
Hardness	Fat
Area	Dietary fiber
Crust thickness	Glycemic index
Water activity	Load index
Moisture content	
Width:height ratio	
Crumb cell analysis	
Number of alveoli	
Average area	
Average diameter	
Circularity	
Volatile compounds	

through enrichment or the use of different flours (Collar, 2007; Rosell, 2007b). Particularly, older consumers and those who are attentive to their health are the most concerned about nutritional aspects of bread (Lambert *et al.*, 2009). Although labels related to the composition of bread are mandatory only for packed breads (regulatory constrain), the majority of consumers would prefer to have that information for all bread varieties. Despite the fact that the nutritional composition of bread varies with the type of bread, bread is an energy-dense product due to the carbohydrate content in the form of starch. It also provides important amounts of protein and dietary fiber and does not contain cholesterol (Table 1.4). Bread is the

TABLE 1.4 Nutritional Information of Different Commercial Bread Varieties

	Nutritional Composition					
Bread Variety	**Energy Value (kcal/100 g)**	**Carbohydrates/ Sugars (g/100 g)**	**Fats/Saturated (g/100 g)**	**Proteins (g/100 g)**	**Dietary Fiber (g/100 g)**	**Sodium (g/100 g)**
White loaf	268	53/2.5	1.8/1.0	9.8	1.8	0.5
Baguette	279	53/1.9	1.8/0.7	9.9	6.6	0.7
White wheat pan bread	232	43/4.3	3.2/0.4	7.9	2.5	0.5
Whole wheat pan bread	247	41/6.0	3.0/1.0	13.0	7.0	0.5
Fiber-enriched pan bread	221	43/4.3	1.0/0.2	9.6	4.2	0.7
Protein-enriched wheat bread	245	44/1.0	2.0/0.0	12.0	3.0	0.5
Reduced-calorie wheat bread	198	44/3.0	2.0/0.0	9.0	12.0	0.5
Mean	229	43/3.7	2.2/0.3	10.3	5.7	0.5
SD	20.1	1/1.9	0.9/0.4	2.1	3.9	0.1

most important source of dietary fiber, although the content of this macronutrient decreases significantly during the refining process; as such, wholemeal breads are the recommended bread type for healthy diets.

CONCLUSION

Bread dough is a versatile matrix that, after proofing and baking, yields a variety of bread products. Traditionally, bread has been seen as a staple food, with nearly ubiquitous consumption worldwide, because it constitutes an important source of energy and provides most of the nutrients and important micronutrients. However, changes in consumer eating patterns have resulted in the modification of the perception of bread from a basic food to a nutritious and healthy product, a vehicle of functional ingredients, or the target product when nutrition deficiencies are detected in the population. Namely, bread not only contains traditional nutrients but also provides other compounds that are beneficial to health and well-being. The nutritive and sensory values of cereal grains and their products are, for the most part, inferior to those of animal food products. Nevertheless, genetic engineering, amino acid and other nutrient fortification, complementation with other proteins (notably legumes), milling, heating, germination, and fermentation are methods employed for improving the nutritive value of breads. Research has also introduced novel flour and traditional grains, such as amaranth, quinua, sorghum, or spelt, to improve the nutritional value of baked products and also to meet the demands and requirements of targeted groups with special food needs.

SUMMARY POINTS

- Worldwide, bread is one of the most consumed foodstuffs.
- Bread making stages include mixing the ingredients, dough resting, dividing and shaping, proofing, and baking, with great variation in the intermediate stage depending on the type of product.
- Bread making is a dynamic process with continuous physicochemical, microbiological, and biochemical changes.
- A global concept of bread quality could be integrated by instrumental attributes objectively measured, sensory sensations, and nutritional aspects.
- Bread has a fundamental role in nutrition derived from the adequate balance of macronutrients in its composition; moreover, it provides some micronutrients and minerals.
- Some fiber, vitamins, and minerals may be added back into refined cereal products through fortification or enrichment programs.

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CHAPTER 2

Monitoring Flour Performance in Bread Making

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

MCC Multivariate control chart
NIR Near-infrared
PCA Principal component analysis
PLS Partial least squares
RMSECV Root mean square error in cross-validation

INTRODUCTION

The food industry needs to keep the product quality perceived by the consumer as constant as possible. This is not easy to achieve given the inner unevenness of raw materials, which can depend on several sources of variability. The baking industry is influenced by the irregularity of wheat flour properties: During the year, flour batches present high variability in terms of rheological parameters, which depends on wheat varieties, employed as pure or in mixtures of different proportions, and on the harvesting time, weather conditions, and agronomic techniques—all of which play a role, sometimes not completely understood, in

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determining wheat performance (Carcea *et al.*, 2006). Thus, flour batch variability influences dough and bread properties to a great extent. Moreover, the possibility to know in advance which flour batches could lead to a defective final product and for which peculiar flour characteristics could allow adapting bread recipes to recover final product acceptability.

Commonly, a restricted pool of flour rheological properties are considered as "performance indicators" to act on the bread recipe and process conditions of mixing, leavening, and baking phases and correct them on an "experience basis" to maintain acceptable final product quality. One of the most limiting aspects of this approach is that the technological parameters are considered in a univariate way, thus losing the effect of the correlation of these properties on flour performance. Also, their effect on bread is usually evaluated in a "trial-by-error" approach by adjusting process parameters and recipe, verifying the outcome on the subsequent production, and repeating the modifications until bread properties become optimal.

Li Vigni *et al.* (2009) proposed an approach, based on multivariate control chart (MCC) methodology (Kourti, 2006), that allows monitoring of flour quality and early identification of flour batches potentially leading to poor performance in production. Using this approach, all rheological properties of incoming flour batches are evaluated multivariately, and these values are projected on a model based on historical data, thus highlighting potential deviances from optimal flour batches employed in the past.

In this chapter, we extend this strategy to a more general framework that considers routine flour quality control at the miller and routine control of incoming flour batches at the bakery:

1. The determinations routinely performed at millers' laboratories are used to elaborate an MCC based on flour variability in terms of rheological properties (rheoMCC) to evaluate if a new delivered sample presents technological characteristics that are either comparable to or significantly different from previous flour deliveries. This chart has to be modeled on a sufficiently wide period of data collection to be robust both to harvesting year and to flour mixture composition variations. Moreover, contribution plots allow the identification of the rheological properties responsible for these deviances. This information will help millers to control the quality of the flour they produce.
2. The rheoMCC can be used by bakeries to orient bread recipe modification at a very early stage of production. However, taking into account the steps involved in flour storage and delivery, a greater benefit may come from the use of an MCC elaborated on the basis of near-infrared (NIR) spectra acquired *in situ* for each flour delivery. This fast, noninvasive technique allows for monitoring of every incoming flour batch directly at delivery.
3. Both kinds of information can be matched with the quality parameters monitored for bread products.

This approach offers an interesting tool to detect anomalous flour batches; however, the relationship between technological parameters and bread properties is often poorly known. Several studies have dealt with the influence of flour composition on bread quality (Goesaert *et al.*, 2005), focusing on the role of the protein fraction because it is well-established that the gluten network determines dough extensibility and tenacity. Different studies have noted that it is not the global content of proteins that influences flour performance but, rather, the amount of certain protein subfractions (Peña *et al.*, 2005), such as glutenins [high molecular weight (HWM) and low molecular weight (LMW)] and gliadins (α, β, γ, and ω components), and their ratio (Uthayakumaran *et al.*, 1999). Thus, Li Vigni *et al.* (2010) addressed the study of the influence of flour batch properties on bread quality by monitoring the protein content of flour batches employed in real industrial production during a period of 2 years. Here, the main results are matched to flour quality from a technological point of view.

TECHNOLOGICAL ISSUES

A principal issue regarding wheat flour and bread quality is the rapidity with which one can gather this information, process it, and determine how to intervene in the process—for example, how to optimize process steps considering flour natural variability so as to maintain bread properties as constant as possible. Bread quality can be measured quickly by imaging techniques, which convert bread pictures in parameters such as dimensions, texture, and color. In this application, an on-line image acquisition system was used (Q-Bake, EyePro System S.R.L.) to measure diameter, height, and upper and lower color of bread and to purge defective product automatically.

At millers' laboratories, flour chemical and rheological properties are routinely analyzed both as a traditional way to evaluate flour performance and to comply national regulations on bread wheat commercial classification. Therefore, these determinations can be used as an early index of wheat flour potential performance. The rheological properties considered here were determined using a Brabender Farinograph and Extensigraph, a Chopin Alveograph, and a Newport Rapid Visco Analyser. Chemical properties (protein content, ashes, and humidity) were measured using a Foss NIRsystems 5500. Rheological properties are laborious and time-consuming to obtain, and a faster method to assess incoming deliveries, such as NIR spectroscopy, should be considered. Measurements were performed with a Bruker Vector-22N FT-NIR spectrophotometer equipped with an optical fiber (spectral working region, 9000 cm^{-1} to 3940 cm^{-1}; resolution, 2 cm^{-1}; 32 scans).

Although rheological properties are routinely measured to characterize flour deliveries, the microscopic correspondence to these macroscopic measurements in terms of flour chemical composition, and gluten components ratios in particular, has not been ascertained. Thus, a detailed investigation (Li Vigni *et al.*, 2010) of gluten composition, in terms of gliadin and glutenin subfractions, was conducted on flour batches according to the procedure proposed by Wieser *et al.* (1998) for protein subfraction separation and characterization by means of reverse-phase high-performance liquid chromatography. Because this procedure is laborious and time-consuming, it cannot be used routinely to obtain information on flour; however, it helps in the evaluation of gluten quality and its role in flour performance.

To elaborate MCC, several approaches can be chosen according to multivariate statistical process control methods. Rheological properties and NIR spectra are punctual measurements on flour batches and deliveries; thus, they can be processed with principal component analysis (PCA) for model creation. PLS Toolbox 5.2 (Eigenvector Research) has been used for PCA. Bread quality data, instead, are recorded on-line and are subject to the phase variability (alternation of raw materials loading, processing, and product exit) of a batch process, thus requiring a batch statistical process control approach (Camacho *et al.*, 2008; Wold *et al.*, 2009). Partial least squares (PLS) batch modeling was conducted by SIMCA-P+ 11 (Umetrics AB).

MULTIVARIATE CONTROL CHART METHODOLOGY

A more effective way to control an industrial process is to develop MCCs instead of classical univariate control charts. MCCs are built by means of a multivariate projection method (i.e., PCA or PLS) applied to a set of reference data in such a way as to consider different variables and the possible interactions among them at the same time. A key step in the definition of an MCC is the choice of the target process conditions, corresponding to a constant or optimal performance—that is, when the system is under control and almost stable through time. The evaluation of the distance of new data—projected on the model—from the model allows one to follow the process evolution. If the statistics for new samples fall within the T^2 and/or Q confidence limit, the new batch is considered in control; otherwise, the process has changed from its usual behavior.

In particular, two cases are possible:

- T^2 out of control: The model is still able to describe the process, but the new batch presents unusual values for some of the variables. In this situation, care should be used because, for example, the new sample may be a prediction outlier or the model may need to be updated.
- Q out of control: The new data present a particularity, which was not considered and described by the model.

Contribution plots (Westerhius *et al.*, 2000) report the contribution of each of the original variables to the calculated statistic, thus giving information on the causes of process deviation. To capture the variables responsible for deviation, a confidence interval has to be associated with the contribution values—that is, problematic variables will have a value of contribution outside the confidence interval. In the current work, the 95th and 99th percentile values of the contributions to Q and T^2 distances were considered as limits to have a distribution-free estimate. Moreover, they have been calculated on test set samples falling below the critical Q and T^2 values at the 95% confidence limit to consider the natural variability of the rheological properties for model-independent, not extreme, samples.

Here, we propose MCCs for flour quality control and to evaluate whether the performance in production of incoming wheat flour batches is comparable to (or significantly different from) that of previous deliveries. To this aim, we considered different sets of data, namely "bread quality data," "flour properties data," and "NIR data." Flour properties data are collected by the miller and used for the elaboration of rheoMCC. NIR data can be collected at the bakery for every incoming flour batch at the delivery stage and used to elaborate NIR-based MCCs (nirMCC) for postdelivery quality control of the main raw material. Moreover, flour batches showing a baking behavior considered "in control" can be indicated *a posteriori* by inspecting MCCs based on bread quality data to highlight flour-peculiar features influencing baking. This scheme is very general and may be applied in different milling and bread production contexts.

As an example, we illustrate the application in the production of a particular kind of bun, whose quality is assessed in terms of height, diameter, and color of the upper and lower part of the bun.

Bun quality data are batch data, monitored on-line during baking for each bun produced while different batches of flour are loaded. In this case, a total of 79 844 observations (every 2 min) of the four quality properties were recorder for the 58 batches reported in Table 2.1. A batch PLS model was developed for the centered and scaled data matrix using the time period in which one flour batch is continuously used in bun production as y variable. Such a model allows information to be obtained on how each batch has progressed in time, and Q and T^2 distances can be considered to detect which flour batches of the bun had the most production time above the considered confidence limits.

The goal of rheoMCC and nirMCC is to rapidly evaluate how and why a given flour delivery is distant from the model built on the historical data set; thus, a wide data set was collected to comprise different harvesting years and flour compositions (see Table 2.1). Moreover, in the training set for MCCs, batches and deliveries with extreme properties should be kept out so that the model is able to learn from past flour batches with a common profile.

The training set was chosen—with both flour properties data and NIR data—using the following probabilistic approach:

- The total number of collected samples, for each data set, was randomly divided into training and test sets according to a 2:1 partition, and the corresponding PCA model was computed. This procedure was repeated 500 times: As a result, all the samples were considered in the training set in approximately 70% of the total runs and in the test set for the remaining 30%. Model dimensionality was chosen as the root mean square error in cross-validation (RMSECV; leave-one-batch-out) minimum.

TABLE 2.1 Prospects of the Wheat Flour Batches Sampled[a]

Harvest	Dates	Symbol	Batches (Deliveries)	Batch ID	NIR (Samples)	Protein Analysis (Batches)	Mixture Composition			
							W1	W2	W3	Others
2007	3/20/07 to 5/19/07	Circle	4 (16)	—	—	—		80%	20%	
	5/12/07 to 9/11/07	Square	13 (53)	1–4, 6	34	—		74%	26%	
	7/28/07 to 4/9/08	Diamond	28 (91)	5, 7–10	29	—	75%	25%		
				11–24	—	—				
				25–31	42	25–31				
	6/6/08 to 7/30/08	Downward triangle	7 (16)	32–38	—	—	30%	30%	40%	
2008	7/22/08 to 8/14/08	Upward triangle	2 (6)	39	—	—	80%	20%		
	8/8/08 to 9/2/08	Cross	3 (9)	40	—	—	100%			
	9/4/08 to 9/17/08	Leftward triangle	2 (7)	41–42	—	—	98%			2% gluten
	9/18/08 to 12/30/08	Rightward triangle	10 (41)	43–52	—	—	69%	10%	20%	1% gluten
2009	1/3/09 to 3/7/09	Six-pointed star	6 (30)	53–54	—	—	70%			30% (W2 + others)
				55–58	35	55–58				
Total			75 (269)	58	140	11				

[a]*The table reports the number of flour batches and deliveries (column 4) divided by harvest year (column 1) and period of employment in production (column 2). ID numbers (column 5) are used in figures to identify batches. The last four columns indicate the flour formulation in terms of wheat varieties.*

- From the 500 PCA models, the list of samples outside the critical Q and T^2 values at 95% confidence limits was collected. For each sample, the frequencies of each sample above the critical Q and the critical T^2 were computed both when a given sample was in the training and in the test set. These frequencies were employed to identify samples that were particularly extreme in comparison to the mean of the model.
- The training set for the PCA model on which the MCC is based was chosen by excluding those samples that were extreme in more than 50% of the runs, according to Q or T^2 values, and also randomly excluding a number of samples in order to respect the 1:2 proportions with the training set. Random choice was done so that each batch was represented with a maximum of 20% of its samples in order to evaluate model robustness in correctly accepting samples with properties generally similar to those of the mean of the model.

Thus, of the total 269 samples of the rheological data, the test set included the 30 samples presenting Q values and the 8 samples presenting T^2 values above the confidence limit for more than 50% of the total occurrences and 52 additional samples randomly chosen among the remaining ones. The rheoMCC training and test sets were composed of 179 and 90 samples, respectively. Regarding the 140 samples of the NIR data, the test set included the 13 samples presenting Q values and the 6 samples presenting T^2 values above the confidence limit for more than 50% of the total occurrences and 27 additional samples randomly chosen among the remaining ones. The nirMCC training and test sets were composed of 94 and 46 samples, respectively.

MONITORING FLOUR PERFORMANCE ON THE BASIS OF CHEMICAL AND RHEOLOGICAL PROPERTIES

Figure 2.1 shows the rheoMCC Q chart with the test set samples projected on the considered model; less than 2% of all the samples fall above the 99% confidence limit, and less than 7% fall above the 95% confidence limit, for the T^2 chart (not shown). The model is quite robust in

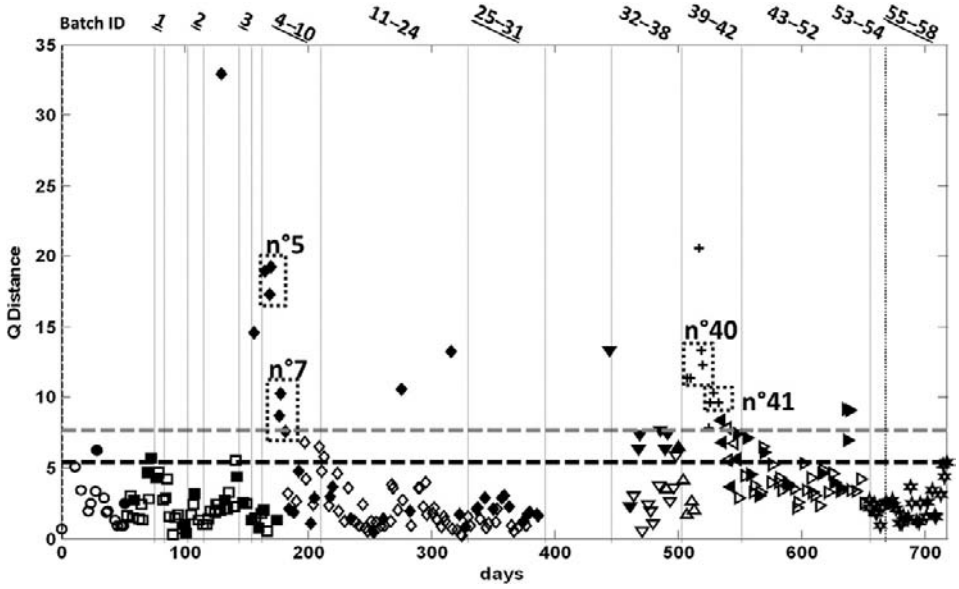

FIGURE 2.1
MCC based on wheat flour properties. *Q* distance to PCA model (five PCs as a minimum RMSECV—CV method: leave-one-batch-out). Samples ordered for delivering days; symbols correspond to wheat mixture (see Table 2.1). Black symbols, test set samples; horizontal gray and black lines, Q_{crit} at 99% and 95% confidence limits, respectively; vertical dotted lines, flour batch IDs introduced in Table 2.1.

terms of false positives (samples that present a significant *Q* distance from the model while not in the test set or among the randomly chosen, "normal" deliveries): All of the samples of the training set fall below the critical values of *Q* when the 99% confidence limit is chosen.

The samples highlighted in Figure 2.1 correspond to flour batches that present a *Q* distance from the model significantly higher than the critical values at the selected confidence limits for all their deliveries. This means that all the deliveries for batches 5, 7, 40, and 41 present rheological properties that are particularly different from those of the flour batches previously employed in production at the bakery. The operator can thus have immediate information on this, which would not have been possible if only a few properties were evaluated one at time, and raise a warning about the potential behavior in production of this batch from its first delivery.

These batches had already been employed in production at the time when the model was elaborated, which means that an indication of their performance in production can be assessed by considering the multivariate bread quality chart shown in Figure 2.2. It is possible to notice that most of the flour batches have led to a portion of bread production that scores above the *Q* confidence limits (a similar behavior is observed for the T^2 statistic; not shown). This can be explained by considering that the recipe is modified empirically during use of a flour batch on the basis of the outcome of the first deliveries whenever the product does not meet the required specifics: These modifications can strongly influence, both positively and negatively, the quality of bread. However, it is clear that batches whose deliveries fall above the confidence limit in rheoMCC usually lead to bread whose properties are mostly above the critical *Q* values. In particular, batches 5, 7, and 39—43 show both extreme values for the technological properties and a non-optimal performance in bread production.

Accessing the contribution plots for the two sets of data allows the interpretation of which kind of defectiveness in bread causes the production to fall above the *Q* critical value and which flour properties are peculiar to these batches. As an example, Figure 2.3 shows the contribution plot corresponding to batch 7.

Considering the rheological properties (see Figure 2.3a), there is a significant positive contribution of ashes, whose high values in flour are reported to contribute to a darker color in baking products, and of farinographic properties of the dough, such as low water absorption (negative value contribution), which indicates that the flour can take up less

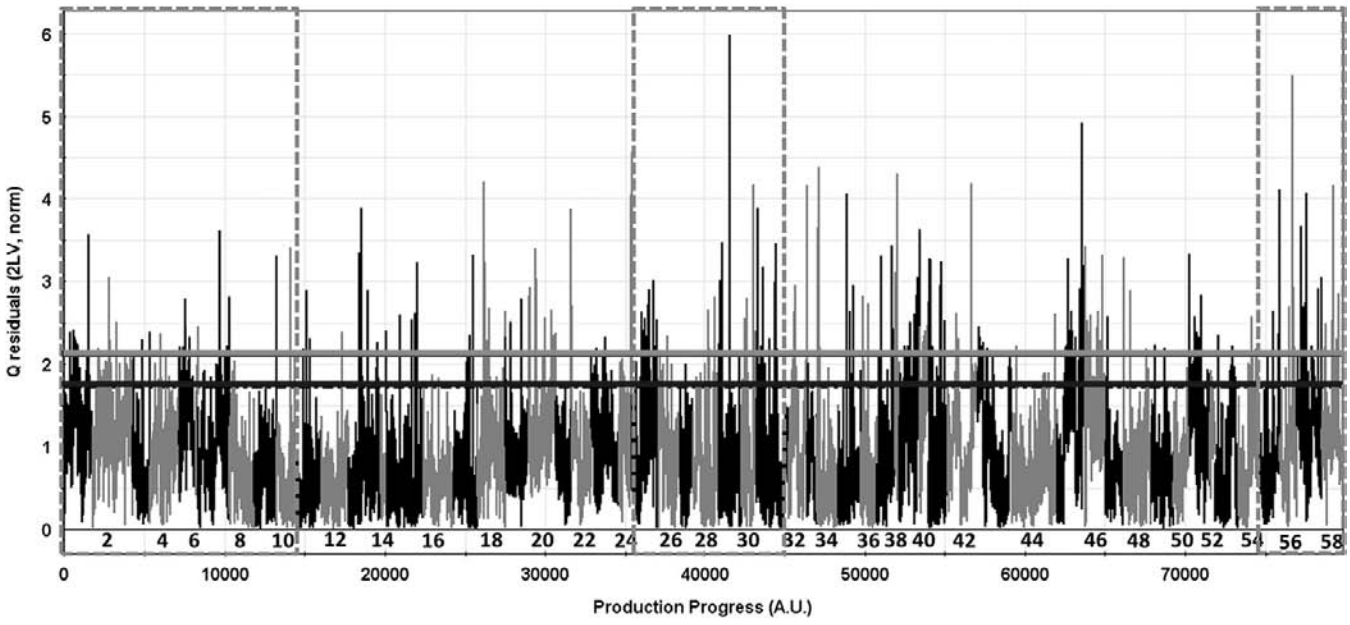

FIGURE 2.2
MCC based on bread quality data. *Q* distance from batch-PLS model (two PCs, explaining 56% of X-block variance). Dashed boxes, flour batches with corresponding NIR samples; horizontal gray and black lines, Q_{crit} at 99% and 95% confidence limits, respectively. Flour batches are numbered progressively in order of employment; alternated black and gray colors, and even batches numbering only, are used for clarity.

water. This, together with the significant contributions of the developing time and alveographic parameter *W*, which are associated with dough strength, can lead to non-optimal dough behavior in the leavening step so that the final product presents dimensions beyond specifications. The previously mentioned defectiveness can indeed be found in the bread produced from flour batch 7, as it is shown by *Q* contributions for the Q-Bake properties (see Figure 2.3b). Here, only the measurements falling above the *Q* critical value at the 95% confidence limit are represented as black dots: These points correspond to Q-Bake readings of several bread production batches obtained from that particular flour batch. Bread obtained from flour batch 7 shows a significant *Q* contribution of the upper color and bun diameter, both with a positive sign, which means a darker upper part and a larger diameter. It also shows a contribution of lower color with a negative sign, meaning that bread has a lighter color on its bottom part, and some bun batches with a smaller diameter (negative sign of the correspondent contribution).

FAST MONITORING OF FLOUR BATCHES BY NIR SPECTROSCOPY

The NIR spectra acquired on flour deliveries at the bakery have been considered to create the nirMCC, of which the *Q* chart is shown in Figure 2.4 (the T^2 distance, not shown, was below the critical value at the 99% confidence limit for all the deliveries). The flour deliveries that have been experimentally monitored belong to the flour batches indicated in Table 2.1 and highlighted in Figures 2.1 and 2.2. Some deliveries fall above the 95% critical limit, such as the first deliveries of batch 1, and in particular the deliveries with a *Q* value higher than the critical 99% confidence limit belong to batch 7 and batch 31. It is interesting that although the considered deliveries of batch 1 and 7 have a corresponding higher *Q* distance in the rheoMCC, batch 31 results are similar to the model based on historical rheological data. Multivariate bread quality evaluation (see Figure 2.2) and the comments of the personnel at the bakery indicated the batch was problematic, which suggests that either process and recipe modifications were not suitable for that batch or some modifications of the flour occurred during transportation or storage. The latter consideration suggests that the NIR spectrum is

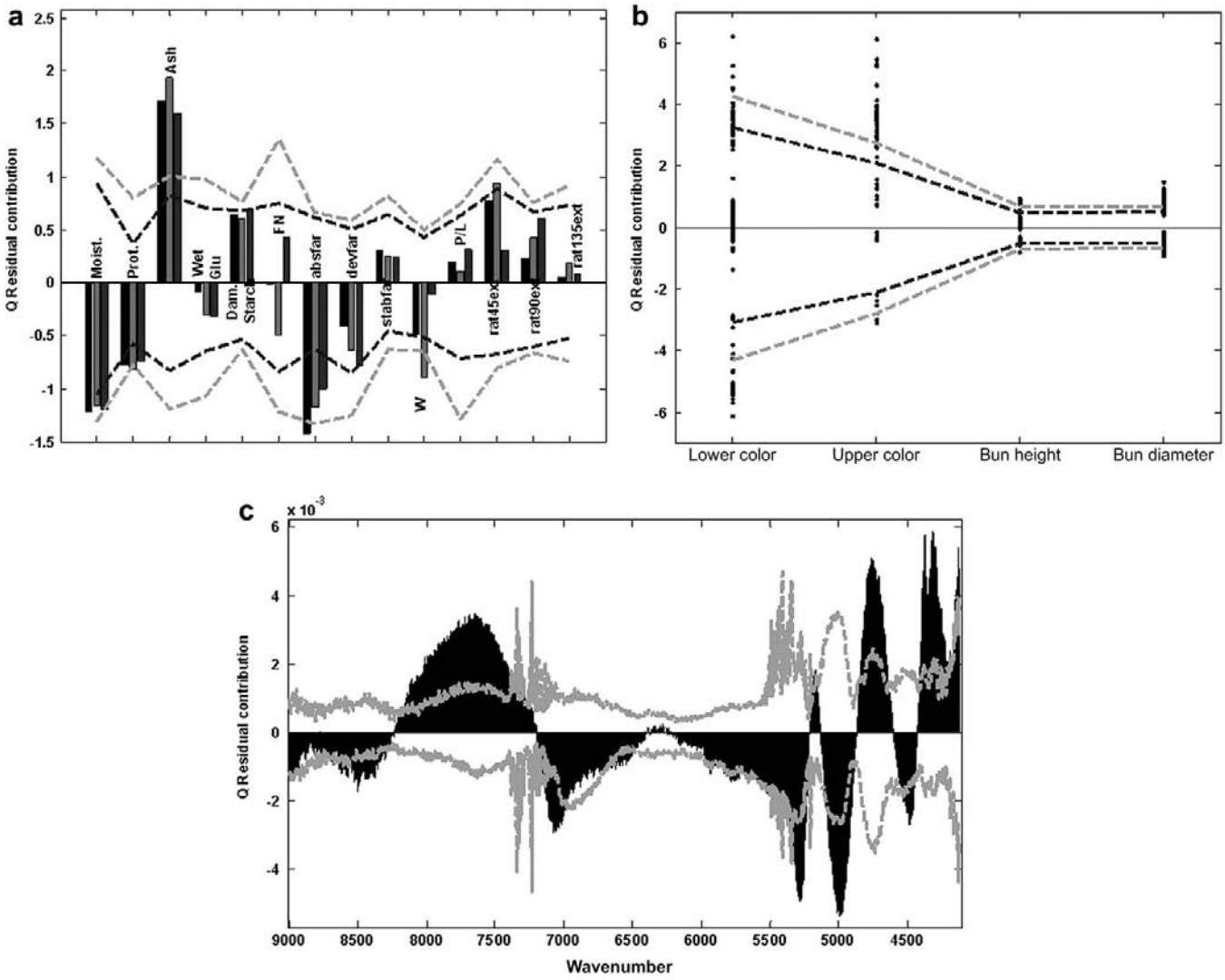

FIGURE 2.3
Contribution plots for selected flour batch samples. Contribution plots for batch no. 7, according to (a) rheoMCC (see Figure 2.1), (b) bread quality chart (see Figure 2.2), and (c) nirMCC (see Figure 2.4). Gray and black lines, 1st–99th and 5th–95th percentile confidence intervals, respectively.

able to record this modification and label as suspicious this batch because its acquisition has been done at a step that follows the arrival of flour at the bakery.

Also for the nirMCC chart, contribution plots can be used to individuate the spectral regions that mainly contribute to the high residuals shown by these samples. To complete the example, Figure 2.3c shows the contributions of the third delivery of batch 7, which result in higher than 95% percentile (I, III, and IV) and lower than 5% percentile (II) in the following regions:

I: 8200–7400 cm^{-1} (C–H second overtone and combination modes)
II: 5285 and 4990 cm^{-1} (C=O stretching second overtone)
III: 4760 cm^{-1} (O–H bending /C–O stretching combination)
IV: 4400–4250 cm^{-1} (starch and protein vibrational modes)

These contributions are commonly attributed to the starch and protein fractions (Shenk *et al.*, 2001). Although within the confidence limits for the contribution to *Q* residual in rheoMCC (see Figure 2.3a), damaged starch (index of starch quality, which increases as flour performance is reduced) had a high positive contribution for the samples of this batch. Regarding

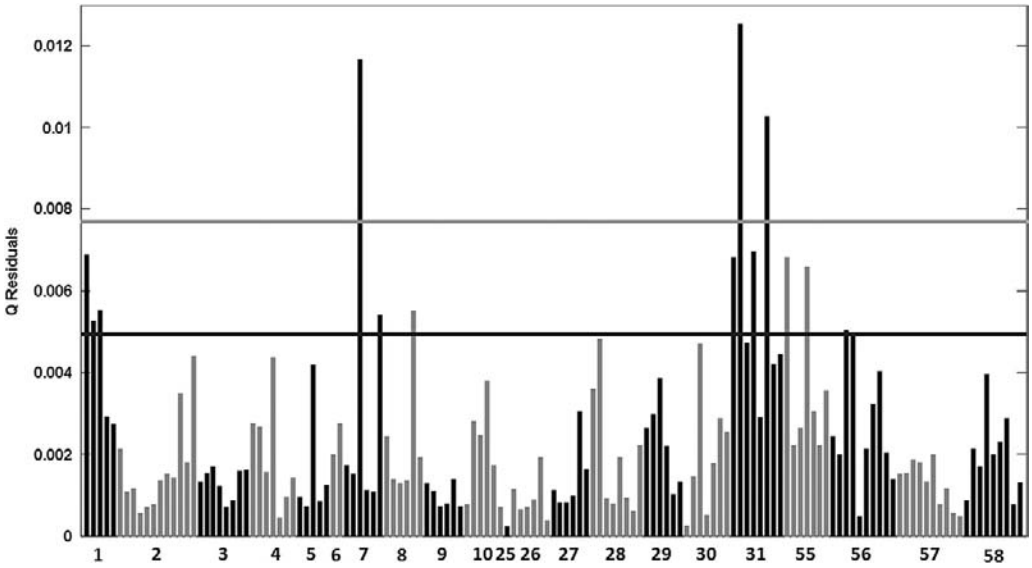

FIGURE 2.4
MCC based on NIR spectra of flour deliveries. PCA model for NIR spectra (two PCs, minimum RMSECV—CV method: leave-one-batch-out). *Q* distance is reported. Horizontal gray and black lines, Q_{crit} at 99% and 95% confidence limits, respectively. Flour batches are numbered progressively in order of employment; alternated black and gray colors are used for clarity.

proteins, the rheological contribution is coherent, referring to farinographic and alveographic properties that are related to protein quality.

EVALUATION OF PROTEIN PROFILE OF FLOUR BATCHES

The evaluation of the protein profile for wheat flour batches employed in production has been limited to the 11 batches identified in Table 2.1. The results of the analysis, reported in Li Vigni *et al.* (2010), show that the highest variability in terms of the protein subfractions content can be detected when considering flour batches from different years, which is in line with the well-known influence of the harvesting time and the crop history (e.g., weather conditions and agronomic treatments during its growth) on wheat protein content. However, the variability between subsequent deliveries of the same flour batch appears to be higher than expected, thus implying that the effect of the milling process is somehow relevant to the protein content differentiation of flour. In particular, some of the batches indicated as most problematic by the bakery, and presenting a production performance that falls above the confidence limits in the Q-Bake-based chart (see Figure 2.3), are characterized by a differentiation that generally corresponds to a higher content in gliadins, and lower content in glutenins, than the other, less problematic, batches. The balance of the two fractions, whose ratio for bread wheat should be close to 1 for genetic reasons, is important in determining gluten structure and, hence, its physical properties. A predominance of gliadin on the glutenin fraction generates a dough that has poor workability and non-optimal leavening properties: This situation is indicated by the gliadin-to-glutenin ratio, whose distribution for these batches is represented in Figure 2.5a, together with the HMW:LMW glutenins ratio (see Figure 2.5b). This ratio is generally reported as positively influencing dough strength, which increases when more of the HMW glutenins are present. The box and whisker representation offers an intuitive visualization of the distribution of the values for the considered flour batches; the gli:glu ratio intrabatch variability is manifest and similar for the 2 years of sampling, whereas the flour batches from 2009 show a ratio that is generally closer to 1 than do those from 2008. Batches with problems in production have either the highest gli:glu ratio in their year, such as batch 25, or several deliveries for which the ratio is significantly higher than 1 (batches 30, 31, and 57) and/or

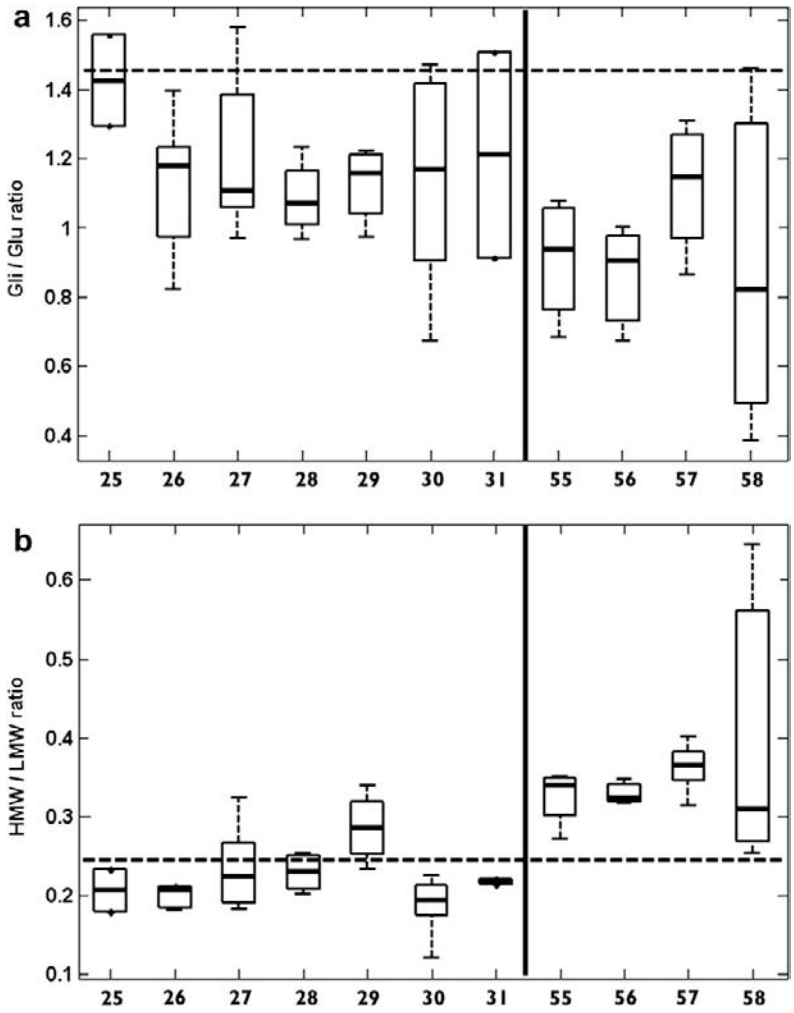

FIGURE 2.5
Box and whisker plot of protein ratios. The rectangle limits correspond to the 25th (lower) and 75th (upper) percentiles, and the internal line corresponds to the median. The dashed "whiskers" represent the total range of the values, if the extremes are not within the 50% variation. (a) Gliadin-to-glutenin ratio and (b) HMW-to-LMW glutenin subunits ratio for the 11 considered batches. Horizontal dotted lines represent the maximum (a) and the minimum (b) values indicatively reported in the literature for strong wheat flour. The vertical solid lines separate 2008 batches (left) from 2009 batches (right).

a great inner variability among different deliveries (batches 30, 31, and 58). Regarding the HMW:LMW ratio, strong bread wheat is reported to have values higher than 0.26, which indicates that almost all the samples from 2008 present a glutenin composition that indicates poor strength and performance of the flour, at least compared to samples from 2009, which have higher values for this ratio. A substantial similarity in median values can be found among the samples of the same year, although some batches have a higher variability range, such as batch 58 in 2009.

SUMMARY POINTS

- Multivariate evaluation of bread quality allows one to obtain a more compact and complete representation of production performance than considering univariate control charts for each property separately. Moreover, it allows a more realistic evaluation of product and departure from standards taking into account all different properties simultaneously.
- Evaluation of the rheological properties of incoming flour batches with an MCC approach helps in assessing the similarities and differences among new deliveries and historical data at a very preliminary step of the production chain so that rational planning of the best recipe to apply to exploit flour properties can be done at the beginning of production, instead of modifying it on the basis of the previous production outcome.

- NIR spectra can be easily and rapidly recorded on each delivery, and the information that can be obtained from nirMCC is generally similar to the rheoMCC findings, and often may also identify other sources of variability (e.g., storage and transfer).
- Characterization of protein composition allows the identification of the quantity and proportions of gluten components so that its quality can be assessed. Wheat flour batches that perform negatively in production mostly have a worse gluten quality, or a higher variability in terms of subfractions, which partially reflects on less stable rheological properties and causes more frequent changes in process conditions. Protein subfraction determination, however, is not suitable for routine analysis of flour batches; thus, the development of faster techniques, such as calibration models from NIR spectra, is desirable.

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CHAPTER 3

South Indian Parotta: An Unleavened Flat Bread

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

ABEV Apparent biaxial extensional viscosity
AR Arabic
BK Break stream
C Reduction stream
CG Carageenan
DATEM Diacetyl tartaric acid esters of monoglycerides
DBR Bran duster
G Index of swelling
GMS Glycerol monostearate
GR Guar gum
HPMC Hydroxypropyl methylcellulose

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LEC Lecithin
Ns Newton-second
Pa s Pascal-second
PS-60 Polyoxyethylene sorbitan monostearate
RSM Response surface methodology
SDS-SDV Sodium dodecyl sulfate-sedimentation value
SEM Scanning electron microscopy
SSL Sodium stearoyl-2-lactylate
STR Straight-run flour
W Deformation energy of dough
WPC Whey protein concentrate
XN Xanthan

INTRODUCTION

In India, the wheat flour from roller flour mills is used for the manufacture of bakery products and traditional foods such as South Indian parotta, nan, and batura. Either whole wheat flour or resultant atta is used for the preparation of traditional foods such as chapati, puri, phulka, tandoori roti, North Indian parotta, and other similar products.

South Indian parotta is wheat flour-based circular, unleavened, multilayered flat bread. It is one of the staple food items in the southern states of India (Tamil Nadu, Kerala, Andhra Pradesh, and Karnataka).

INGREDIENTS AND PROCESSING CONDITIONS

Basically, parotta is made from wheat flour, salt, water, and oil for spreading of the dough; however, optional ingredients such as sugar and egg are also used in the preparation of parotta (Indrani, 1998). Response surface methodology (RSM) is a popular method for product optimization within the sensory evaluation field. Using RSM, the levels of ingredients such as salt, sugar, egg, water, and oil are optimized for the preparation of parotta. Accordingly, the actual levels of ingredients required for the maximum estimated overall sensory quality score of 95.9 are as follows: wheat flour, 100 g; water, 56.3 ml; salt, 1.0 g; sugar, 0.8 g; egg, 9.6 g; and oil, 16.6 g (Indrani and Venkateswara Rao, 2001).

Parotta dough is prepared by mixing the ingredients, resting for 30 min, and then spreading 75 g of dough into a thin film while applying the refined oil. The film is folded into multiple layers and then coiled (Figure 3.1). After resting for 10 min, the coiled dough is sheeted again into a 15-cm-wide and 0.5-cm-thick circular disk. It is then baked on a hot plate for 2 min at 180°C, turning every 30 s.

QUALITY CHARACTERISTICS OF SOUTH INDIAN PAROTTA

A parotta with creamish white color, having light brown spots on the surface, a circular shape, a soft and pliable handfeel, a soft and slightly chewy texture, with distinct layers, optimum oiliness, easy breakdown in the mouth, and typical pleasant taste and aroma is considered to be highly desirable (Indrani and Venkateswara Rao, 2004).

PAROTTA INGREDIENTS AND RHEOLOGICAL CHARACTERISTICS

The rheological characteristics of dough are important because they affect both the machinability of the dough and the quality of the end product. The rheological characteristics of dough are influenced by the added ingredients (Table 3.1). Studies on the effects of ingredients of parotta—namely salt, sugar, egg, and oil—on the rheological characteristics of dough measured using a farinograph, extensograph, and Instron texturometer have been reported (Indrani and Venkateswara Rao, 2007). It has been observed that the use of an increasing

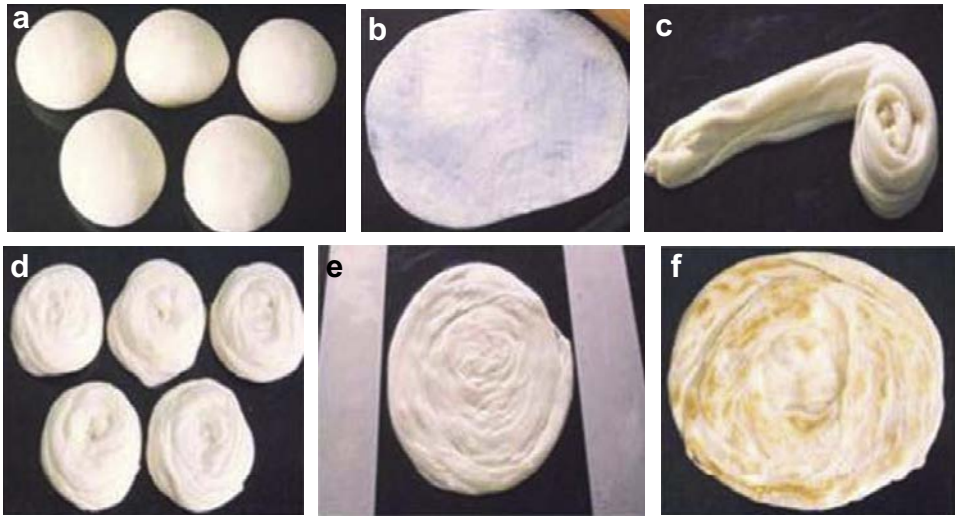

FIGURE 3.1
Method of preparation of parotta. (A) Rounded dough of 75 g each, (B) dough sheeted with oil into a very thin film, (C) coiling of sheeted dough, (D) resting of coiled dough, (E) final sheeting of coiled dough, and (F) baked parotta. *Source: Reprinted with permission from Indrani, D., and Venkateswara Rao, G. (2004). Effect of processing conditions on the quality of south Indian parotta—An Indian traditional food. Journal of Food Quality, 27, 55–72.*

amount of salt from 0 to 1.5% decreases the water binding capacity of wheat flour and increases dough stability. Galal *et al.* (1978) reported that salt decreases water absorption and increases the time until optimum development and stability of the dough as measured by farinograph. Use of sugar up to 1.5% does not alter the dough properties measured using farinograph and extensograph. The addition of increasing amounts of egg from 0 to 15% decreases the water absorption capacity, increases the strength, and modifies the elastic and extensible properties of the dough. When oil is added to the dough, it decreases the water binding capacity of flour, extends the time required for gluten hydration, increases dough development, resists the mixing action, and shows an increase in farinograph stability. The dough with oil lacks elasticity and strength; however, it exhibits higher extensibility. It is reported that the apparent biaxial extensional viscosity (ABEV) value of wheat flour dough measured using an Instron Universal Testing Machine (see Table 3.1) increases with the addition of 1.5% salt and 7.5% egg and decreases with the addition of 1.5% sugar and 20% oil, respectively. These results indicate that, in general, salt and egg increase the viscosity of the dough, whereas sugar and oil decrease the viscosity of the dough.

The dough hardness as measured by two-bite compression using Instron increases with the addition of salt and egg and decreases with sugar and oil. Thus, addition of salt or egg appears to increase the strength of the dough, and incorporation of sugar and oil to the dough

TABLE 3.1 Effect of Ingredients on the Rheological Characteristics of Wheat Flour Dough for Parotta[a]

Dough Characteristics	Ingredients[b]					SEM (±)[c]
	Control	Salt (1.5%)	Egg (7.5%)	Sugar (1.5%)	Oil (20%)	
Farinograph stability (min)	3.5[a]	9.5[d]	4.5[b]	3.5[a]	6.5[c]	0.25
Instron						
ABEV $\times 10^{-4}$ (Pa s)	5.041[b]	5.725[c]	6.069[d]	5.036[b]	3.779[a]	0.005
Hardness (N)	36.45[b]	39.52[c]	38.95[c]	36.05[b]	26.38[a]	0.300
Cohesiveness	0.782[b]	0.812[c]	0.811[c]	0.777[b]	0.604[a]	0.005
Adhesiveness (Ns)	26.54[c]	25.33[b]	25.02[b]	26.63[c]	18.80[a]	0.05

Source: Reprinted with permission from Indrani, D., and Venkateswara Rao, G. (2007). Rheological characteristics of wheat flour dough as influenced by ingredients of parotta. *Journal of Food Engineering*, 79, 100–105.
[a]*Values in the same row followed by a different letter differ significantly (*$P \leq 0.05$*).*
[b]*Addition of salt or egg increases strength, viscosity, and cohesiveness of dough, whereas the addition of sugar and oil decrease them.*
[c]*Standard error of mean at 15 degrees of freedom.*

separately results in soft dough. The decrease in hardness values is greater for the dough containing oil than that for the dough containing sugar. Compared to the cohesiveness of control dough (0.782), doughs containing varying amounts of salt and egg (0.812 and 0.811, respectively) are more cohesive than the doughs made with sugar and oil (0.777 and 0.604, respectively). This indicates that the dough with salt and egg offers more resistance to compression force, whereas doughs with sugar and oil are less cohesive. Dough adhesiveness decreases with incorporation of salt or egg or oil and increases with sugar, indicating the sticky nature of the dough with sugar (Indrani and Venkateswara Rao, 2007).

Chemical and rheological characteristics

There is consistent information on the role of different quality factors affecting the rheological characteristics of dough as well as on the quality of end products. Indrani and Venkateswara Rao (2000) identified the chemical and rheological parameters influencing the parotta making characteristics. The analysis of six different wheat flours (A–F) for chemical, rheological, and parotta making characteristics (Table 3.2) shows that among different wheat flours, sample C has the highest protein content, sodium dodecyl sulfate sedimentation value (SDS-SDV), farinograph dough stability, and extensograph area, followed in decreasing order by B, D, A, F, and E. The rheological properties of different flour samples measured using Instron show that dough from sample C is less extensible (ABEV value, 4.84×10^{-4} Pa s) and possesses higher consistency, elasticity, and cohesiveness as shown by the values of hardness (35.28 N), cohesiveness (0.812), and adhesiveness (26.85 Ns). On the other hand, dough from flour E is more viscous (2.86×10^{-4} Pa s), exhibiting low consistency and elasticity as evident by the hardness, cohesiveness, and adhesiveness values of 17.30 N, 0.68, and 27.72 Ns, respectively. The overall quality scores of 81 for sample C and 51 for sample E, with the maximum score of 100, show that sample C is suitable for preparation of parotta, whereas sample E produces inferior quality parotta. The correlation coefficient data between chemical, rheological, and overall quality scores of parotta show that dry gluten and protein and also SDS-SDV, indicating quantity and quality of protein, respectively, are the best indices for predicting the quality of parotta. The flour protein content shows the highest correlation coefficient with overall quality score ($r = 0.98$, $P \leq 0.001$), indicating that the protein content is the major factor influencing the quality of parotta. Among various rheological characteristics, farinograph dough stability, extensograph area, and Instron ABEV, hardness and cohesiveness are found to be correlated ($r = 0.98$, $P \leq 0.001$) to a greater extent to overall quality score. The previous results show that the parameters reflecting the strength of the dough are important in determining the quality of parotta.

ADDITIVES

Additives are substances that are added in small quantities to improve the rheological characteristics of dough as well as quality characteristics of the finished product.

Oxidizing agents

According to Bloksma (1968), oxidizing agents affect the rheological properties of dough by interchange reactions between sulfhydryl and disulfide groups present in the network. Use of oxidizing agents (potassium bromate, ascorbic acid, or potassium iodate) increases the strength of the dough, as shown by the increase in the values of farinograph stability of dough with 200 ppm of ascorbic acid from 3.5 to 4.5 min and Instron ABEV values from 5.041×10^{-4} to 5.954×10^{-4} Pa s (Table 3.3). Use of oxidizing agents also increases hardness and cohesiveness, and it decreases the adhesiveness of dough. The previously mentioned change in the dough characteristics due to oxidizing agents brings about an adverse effect on the quality of parotta, as shown by the decrease in the spread ratio from 29.4 to 26.8 and the increase in the force required to shear the parotta, a measure of the texture of parotta. Sensory evaluation shows that the parottas are hard to the feel (by hand) and possess thick layers, the coils appear

TABLE 3.2 Chemical, Rheological, and Parotta Making Characteristics of Wheat Flours[a]

Quality Characteristics	Wheat Flours[b]						SEM (±)[c]
	A	B	C	D	E	F	
Total ash (%)	0.50[b]	0.46[a]	0.51[b,c]	0.54[d]	0.52[c]	0.54[d]	0.04
Dry gluten (%)	9.46[c]	10.18[e]	10.30[e]	9.94[d]	7.48[a]	8.96[b]	0.75
Protein N × 5.7 (%)	9.60[c]	10.37[e]	10.86[f]	10.04[d]	8.04[a]	9.06[b]	0.80
SDS-SDV (ml)	55.0[c]	60.0[d]	63.0[e]	59.0[d]	31.0[a]	50.0[b]	0.95
Farinograph stability (min)	6[c]	7[e]	8[f]	6.5[d]	4[a]	5[b]	0.10
Extensograph area (cm^2)	165.8[d]	171.5[e]	173.8[f]	165.1[c]	145.2[a]	156.2[b]	0.35
Instron							
ABEV × 10^{-4} (Pa s)	4.58[c]	4.78[e]	4.84[f]	4.70[d]	2.86[a]	3.78[b]	0.10
Hardness (N)	33.68[c]	34.49[e]	35.28[f]	34.0[d]	17.30[a]	22.73[b]	0.44
Cohesiveness	0.786[c]	0.799[e]	0.812[f]	0.794[d]	0.681[a]	0.722[b]	0.01
Adhesiveness (Ns)	26.85[d]	25.29[b]	25.12[a]	26.09[c]	27.72[f]	27.10[e]	0.20
Overall quality score (100)	74[c]	79[e]	82[f]	76[d]	51[a]	61[b]	0.41

Source: Reprinted with permission from Indrani, D., and Venkateswara Rao, G. (2000). Effect of chemical composition of wheat flour and functional properties of dough on the quality of south Indian parotta. *Food Research International*, 33, 875–881.
[a]*Means in the same row followed by a different letter differ significantly (P ≤ 0.05).*
[b]*A–F, six different wheat flours. Among the different flours, sample C has the highest quantity and quality of protein, and dough strength is suitable for preparation of parotta.*
[c]*Standard error of mean at 18 degrees of freedom.*

thicker and separate after baking, and the parotta offers more resistance to bite and lacks easy breakdown. As a result, the overall quality score decreases from 75 to 69. The previous adverse effect is attributed to an excessive increase in the strength and elasticity of the dough (Indrani and Venkateswara Rao, 2006).

Reducing agents

The addition of reducing agents weakens the dough; it normally reduces extensograph resistance to extension and area and increases extensibility (Mita and Bohlin, 1983). Use of 100 ppm of L-cysteine hydrochloride or 50 ppm of potassium metabisulfite modifies the rheological characteristics of dough, reduces the strength and elasticity, and increases the dough extensibility, resulting in the dough becoming less cohesive and stickier in the presence of reducing agents (Indrani and Venkateswara Rao, 2006). These changes are seen by decreases, due to the addition of 50 ppm of L-cysteine hydrochloride, in the values of farinograph dough stability and Instron ABEV, hardness, and cohesiveness and by an increase in Instron adhesiveness values (see Table 3.3). The modification in the dough characteristics with the use of reducing agents results in an increase in the spread ratio, a decrease in the shear force, and an increase in the overall quality score of parotta from 75 to 83. However, the improvement in the quality of parotta is observed only with the use of low levels of 100 ppm of potassium metabisulfite or 50 ppm of L-cysteine hydrochloride; an increase in their concentration above these levels (200 and 100 ppm, respectively) adversely affects the overall quality of parotta.

Dry gluten

The addition of increasing levels of gluten from 0 to 3% significantly increases dough strength, elasticity, hardness, and cohesiveness and decreases adhesiveness (see Table 3.3). As a result of these changes, the diameter of the parotta decreases, thickness increases, shear force value increases, and the overall quality decreases. The adverse effect of gluten on the quality of parotta is attributed to the increase in elasticity and stiffness of dough (Indrani and Venkateswara Rao, 2006).

TABLE 3.3 Effect of Additives on Rheological Characteristics of Wheat Flour and Quality of Parotta[a]

Rheological and Parotta Quality Characteristics	Control	Oxidizing Agent: Ascorbic Acid (200 ppm)	Reducing Agent: Cysteine Hydrochloride (50 ppm)[b]	Dry Gluten (3%)	Enzymes: α-Amylase (10 ppm)	Enzymes: Protease (10 ppm)[b]	SEM (±)[c]
Farinograph stability (min)	3.5[c]	4.5[d]	2.5[a]	6.0[e]	3.0[b]	3.0[b]	0.10
Instron							
ABEV × 10^{-4} (Pa s)	5.041[c]	5.954[d]	4.351[a]	6.069[d]	4.580[b]	4.421[b]	0.10
Hardness (N)	36.45[c]	44.32[d]	29.55[a]	42.85[d]	33.68[b]	32.5[b]	0.35
Cohesiveness	0.782[c]	0.842[d]	0.651[a]	0.848[d]	0.721[b]	0.665[a]	0.01
Adhesiveness (Ns)	26.54[b]	24.12[a]	28.05[d]	23.41[a]	27.18[c]	27.55[a]	0.20
Spread ratio	29.4[b]	26.8[a]	30.4[c]	27.0[a]	29.8[b]	30.8[c]	0.25
Shear force (kg)	1.28[a]	1.51[b]	1.8[c]	1.4[b]	1.22[a]	1.15[a]	0.10
Overall quality score (100)	75[d]	69[b]	83[e]	65[a]	72[c]	81[e]	1.5

Source: Reprinted with permission from Indrani, D., and Venkateswara Rao, G. (2006). Effect of additives on rheological characteristics and quality of wheat flour parotta. *Journal Of Texture Studies*, 37, 315–338.

[a]*Values in the same row followed by a different letter differ significantly (*$P \leq 0.05$*).*

[b]*Addition of cysteine hydrochloride and protease enzyme is beneficial in modifying the dough properties and improving the quality of parotta.*

[c]*Standard error of mean at 18 degrees of freedom.*

Enzymes

α-AMYLASE

Use of α-amylase up to 20 ppm increases the spread ratio of parotta and decreases the shear force. Sensory scores for shape, handfeel, texture, layers, mouthfeel, and overall quality gradually decrease as the level of α-amylase increases from 0 to 20 ppm. Indrani and Venkateswara Rao (2006) indicated that addition of α-amylase has an adverse effect on handfeel, texture, layers, and mouthfeel. The parottas become fragile and manifest fused layers, their coils are not visible, and they lack typical chewiness; furthermore, they become sticky and doughy.

PROTEASE ENZYME

Proteolytic enzymes are added to reduce mixing time, increase extensibility, and improve the dough flow. The mechanism of action of proteolytic enzymes involves cleavage of peptide bonds, resulting in the formation of smaller fragments such as peptides and amino acids (Kulp, 1993). Addition of protease enzyme (up to 10 ppm) improves the sensory scores: Scores for shape, handfeel, texture, layers, and mouthfeel increase, resulting in a significant increase in overall quality score. However, at a protease concentration of 20 ppm, there is a significant decrease in the overall quality (Indrani and Venkateswara Rao, 2006).

XYLANASE

Addition of 10 ppm of xylanase improved both elasticity and extensibility of the wheat flour dough, as shown by the increase in the extensograph resistance to extension from 410 to 425 BU and extensibility from 148 to 155 mm. Parottas prepared with xylanase enzyme showed a significant increase in overall quality score from 74 for the control to 92 for the parottas with xylanase (Prabasankar *et al.*, 2004).

Surfactants

Surfactants are increasingly used to improve both the quality and the shelf life of bakery products. The surfactants aid in the development of less tacky, more extensible doughs that

process through machinery without tearing or sticking (Kamel and Ponte, 1993). Studies carried out by Indrani and Venkateswara Rao (2003) on the effect of the surfactants glycerol monostearate (GMS), lecithin (LEC), diacetyl tartaric acid esters of monoglycerides (DATEM), polyoxyethylene sorbitan monostearate (PS-60), and sodium stearoyl-2-lactylate (SSL) on the rheological characteristics of dough and quality of parotta show that use of the surfactants increases the farinograph stability value from 3.5 to 4.5 min (Table 3.4), indicating an increase in the strength of the dough. The largest increase in the stability value was observed with SSL and DATEM. Addition of surfactants reduces ABEV, hardness, and adhesiveness values and increases cohesiveness values. The largest effect on the dough occurs with SSL. These data indicate that use of surfactants decreases the hardness of the dough. However, it increases cohesiveness.

In general, parottas have a soft, pliable handfeel with the addition of surfactants. The texture is soft and slightly chewy. The layers are non-oily and distinct. The parottas easily disintegrate. The surfactants significantly improve the overall quality score. The highest improvement in the overall quality score from 75 to 90 is shown by SSL and PS-60, followed by DATEM (85), LEC (80), and GMS (78).

Hydrocolloids

The use of hydrocolloids in bakery and traditional products is increasing throughout the world because of the advantages they offer, such as increased water absorption, improved texture, and longer shelf life. Smitha *et al.* (2008) studied the effect of addition of the hydrocolloids arabic (AR), guar (GR), xanthan (XN), carageenan (CG), and hydroxypropyl methylcellulose (HPMC) on the rheological characteristics and quality of parotta. Addition of hydrocolloids increases the farinograph water absorption from 57 to 58−61%. The extensograph resistance to extension at 135 min increases with the addition of hydrocolloids. Addition of XN, GR, and CG decreases extensibility, whereas addition of AR and HPMC increases extensibility.

Addition of AR, GR, XN, CG, and HPMC significantly increases the spread ratio from 29.8 to 31.6−34.4; the greatest increase is observed in parottas containing HPMC and GR, followed by those containing CG, AR, and XN. Hydrocolloids reduce the shear force, indicating their softening effect on the parottas. In general, parottas with hydrocolloids have a soft, pliable handfeel. Parottas with GR have a soft and slightly chewy texture; the layers are thin and distinctly separate; and the parottas easily disintegrate in the mouth, giving a clean mouthfeel

TABLE 3.4 Effect of Surfactants on the Rheological Characteristics of Wheat Flour Dough and Quality of Parotta[a]

Rheological and Parotta Quality Characteristics	Surfactants (0.5%)[b]						SEM (±)[c]
	Control	GMS	LEC	DATEM	PS-60	SSL	
Farinograph stability (min)	3.5[a]	4.0[b]	4.0[b]	4.5[c]	4.5[c]	4.5[c]	0.1
Instron							
ABEV $\times 10^{-4}$ (Pa s)	5.04[f]	4.12[b]	3.89[a]	4.58[c]	4.69[d]	4.81[e]	0.04
Hardness (N)	36.5[f]	30.1[b]	29.6[a]	30.8[c]	31.5[d]	32.8[e]	0.50
Cohesiveness	0.782[a]	0.790[b]	0.796[c]	0.801[d]	0.828[e]	0.832[f]	0.002
Adhesiveness (Ns)	26.55[f]	26.10[e]	25.31[d]	24.21[c]	23.65[b]	23.12[a]	0.25
Spread ratio	29.4[a]	30.0[b]	30.2[b]	31.0[c]	31.4[c]	31.2[c]	0.26
Shear force (N)	12.5[d]	7.8[a]	8.3[a]	9.8[b]	11.9[c]	11.8[c]	0.03
Overall quality score (100)	75[a]	78[b]	80[c]	85[d]	90[e]	90[e]	0.45

Source: Reprinted with permission from Indrani, D., and Venkateswara Rao, G. (2003). Influence of surfactants on rheological characteristics of dough and quality of parotta. *International Journal of Food Science and Technology*, 37, 1−8.

[a]*Values in the same row followed by a different letter differ significantly* ($P \leq 0.05$).

[b]*Among the different surfactants, PS-60 and SSL significantly improve the quality of parotta.*

[c]*Standard error of mean at 18 degrees of freedom.*

without any lumps. Parottas with XN possess slightly thick layers and offer more resistance to bite. Sensory evaluation shows that the texture of parottas with HPMC is soft and chewy. The layers of parotta with CG are less distinct. Addition of AR brings about a marginal increase in the sensory scores for texture, layers, and mouthfeel. These data show that all the hydrocolloids studied improve the overall quality of parotta. However, the highest improvement in the overall quality score is brought about by GR, followed by HPMC, XN, CG, and AR.

MICROSTRUCTURE OF PAROTTA

Scanning electron microscopy (SEM) permits observation of three-dimensional structures. SEM evaluation of parotta dough (Smitha *et al.*, 2008) shows small and large starch granules enmeshed in protein matrix. In the microstructure of baked parotta, few partial outlines of large and small starch granules embedded in the protein matrix are seen. The outlines represent deformed starch granules due to gelatinization during baking. The outer layer of parottas shows more distorted starch granules than the middle layer. In the micrographs of dough treated with different hydrocolloids, the starch granules appear coated with gum, and the coating appears prominent in the case of doughs treated with GR and HPMC. Chaisawang and Suphantharika (2006) evaluated the SEM characteristics of native and anionic tapioca starches as modified by guar gum and xanthan gum, and they reported that xanthan gum completely wrapped the native starch granules.

EFFECT OF FLOUR MILL STREAMS

A variety of bakery and traditional products are manufactured using only one type of wheat flour. Because there is no specific flour for the production of good quality parotta, selection of streams and blending appears to be a practical approach. The results of an analysis of flours from different flour streams (Indrani *et al.*, 2003)—namely five break streams (1 BK−5 BK), one grader, seven reduction streams (C1−C7), bran duster (DBR), and straight-run flour (STR)—show that the ash increases from 0.562 to 1.12%, dry gluten content from 8.38 to 10.89%, and SDS-SDV from 53 to 64 ml with increasing numbers of breaks in the flour streams. The alveograph characteristics indicate that the average abscissa at rupture length of the curve increases with increasing reduction streams from C1 to C5, and also that the curves are better balanced when compared to 1 BK−5 BK. The parottas made from the first five break passages have a decreased spread ratio, dull brown color, fused layers, and lower overall quality score (43−79). The initial reduction streams (C1−C5) produce good quality parottas in terms of appearance, spread, layers, and texture. The specialty flour produced by combining C1−C5 streams produces parottas with the highest overall quality score of 93.5, with the maximum score of 100, compared to break streams (43−77), reduction streams (56−92), STR (81), grader (79), and DBR (56).

ALVEOGRAPH PARAMETERS

Indrani, Sai Manohar, *et al.* (2007) analyzed 25 commercial wheat flour samples for their chemical, alveograph, and parotta making characteristics. Correlation coefficients between chemical, alveograph, and parotta making characteristics show that among the chemical characteristics of wheat flours, ash content highly correlates to alveograph index of swelling (G), a measure of the square root of volume of air necessary to inflate the dough bubble until it ruptures ($r = 0.838$, $P \leq 0.01$). Gluten content and SDS-SDV correlate to maximum overpressure, a measure of dough elasticity ($r = 0.951$ and $r = 0.875$, $P \leq 0.01$), and to shear force ($r = 0.954$ and $r = 0.840$, $P \leq 0.01$). Among alveograph characteristics, maximum overpressure highly correlates to shear force of parotta ($r = 0.938$, $P \leq 0.01$). Average abscissa at rupture (L) correlates to spread ratio ($r = 0.754$, $P \leq 0.01$). The curve configuration ratio correlates to all physical and sensory characteristics of parotta. G correlates to spread ratio ($r = 0.914$, $P \leq 0.01$) and overall quality score ($r = 0.931$, $P \leq 0.01$). Deformation energy of dough (W), representing the energy necessary to inflate the dough bubble to the point of rupture, correlates to

spread ratio ($r = 0.825$, $P \leq 0.01$) and overall quality score ($r = 0.872$, $P \leq 0.01$), indicating that alveograph G and W can be considered as the indicators of the overall quality of parotta.

STORAGE

Changes in quality of bakery products during storage are attributed to the staling process. Parotta is generally prepared and consumed fresh in households and restaurants, as well as in roadside shops. Indrani *et al.* (2000) reported a decrease in alkaline water retention capacity from 257.4 to 127.7%; a decrease in total water solubles from 8.25 to 6.24%; a decrease in soluble starch from 2.11 to 1.19%; and decreases in amylose and amylopectin contents in soluble starch from 0.39 to 0.13% and 1.72 to 1.06%, respectively. This suggests that changes in both soluble amylose and amylopectin are involved in the staling of parotta. Organoleptic evaluation during storage indicates that the pliability of parottas decreases with time. The parotta exhibits typical chewiness up to 4 h of storage, after which there is a progressive loss of chewiness. The overall quality score gradually decreases from 75 to 43. The differential scanning calorimeter characteristics of stored parotta indicate an increase in the enthalpy of the endotherm with storage time, implying different degrees of starch retrogradation. Avrami methodology applied to determine Avrami exponents (index of crystal type) and time constants (reciprocal of rate constant) shows an Avrami exponent value of 1.18 and a time constant ($k \times 10^3$) of 24.56 $h^{-1.18}$ for parotta.

NUTRITIOUS PAROTTA

Use of whey protein concentrate

The protein content in whey protein concentrate varies from 65 to 75%. Whey proteins are the best quality proteins available and have a high protein efficiency ratio (3.6), and they possess almost all the essential amino acids. A study on the effect of replacement of wheat flour with 5, 10, and 15% whey protein concentrate (WPC) on quality of parottas has shown that use of WPC decreases farinograph water absorption (Indrani, Prabhasankar, *et al.*, 2007). An increase in the extensograph resistance to extension values up to 10% WPC and a decrease in the extensibility values are observed with the increase in the level of WPC from 0 to 15%. A marked decrease in the overall quality score of parottas containing more than 5% WPC is observed. Hence, replacement of wheat flour with 5% WPC is recommended for the preparation of nutritious parotta having increased protein content of 3 or 4%.

TECHNOLOGICAL ISSUES

The optimum ingredients, processing conditions, rheological characteristics of dough, and desirable quality characteristics of parotta highlighted in this chapter are useful in the mechanization of the process for large-scale production of parotta.

SUMMARY POINTS

- South Indian parotta is wheat flour-based unleavened, flat bread.
- The baked parotta is circular in shape and creamish white in color. It possesses a number of distinct layers, soft and pliable handfeel, and soft and slightly chewy texture, with typical pleasant taste and aroma.
- Protein quantity and quality are the best indices for predicting the quality of parotta.
- Alveograph index of swelling and deformation energy of dough are considered to be indicators of the overall quality of parotta.
- Use of additives such as L-cysteine hydrochloride, proteinase, xylanase, sodium stearoyl-2-lactylate, and guar gum separately modifies the rheological properties of the dough and improves the overall quality of parotta.
- Nutritional quality of parotta is improved with the use of whey protein concentrate.

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CHAPTER 4

Sourdough Breads

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

ACE Angiotensin I-converting enzyme
LAB Lactic acid bacteria
PDO Protected designation of origin
PGI Protected geographical indication

INTRODUCTION

The term "sourdough bread" refers to a bread leavened with a sourdough starter. Bread can be made with either baker's yeast or sourdough for dough leavening. Spontaneous sourdough consists of flour and water blended to make a "sponge," which is left at room temperature for several hours (Figure 4.1); endogenous fermenting microorganisms produce metabolites that affect the characteristics of the dough. The addition of new flour and water to the dough, defined as backslopping, allows a composite ecosystem of yeast and lactic acid bacteria (LAB) to take place inside the dough, giving it its typical sour taste. The yeast is mainly responsible for the production of CO_2, and the LAB are mainly responsible for the production of lactic and/or acetic acid; both microorganisms are responsible for the production of aromatic precursors of bread. Furthermore, the technological performance of the dough and the nutritional properties, aroma profile, shelf life, and overall quality of the bread are greatly affected by the metabolic activity of the sourdough microorganisms. Today, sourdough bread is produced mostly in retail or artisan bakeries for a wide variety of specialty products, whereas it is not used in the mechanized baking industry, in which baker's yeast is the main leavening agent.

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FIGURE 4.1
Sourdough starter. Spontaneous sourdough starter prepared with durum wheat flour and approximately 70% water. The production of CO_2 is shown by small points on the surface.

A BRIEF HISTORY OF SOURDOUGH BREADS AND THEIR CURRENT DIFFUSION

Because sourdough consists of a spontaneous fermentation process, it can undoubtedly be considered the primordial form of bread leavening. It is believed that the use of sourdough in bread leavening developed in ancient Egypt in approximately 3000 BC and from there spread gradually to Europe, throughout ancient Greece and the Roman Empire until the present.

Wheat and other grains cultivated in the Nile River Valley were used in ancient Egypt to manufacture leavened breads on a large scale—enough to feed thousands of people per day; this is supported by the discovery of desiccated bread and numerous wall pictures of the bread making process in ancient tombs. The ancient Greeks imported wheat grains from Sicily and Egypt, and continuous trading with Egyptians allowed them to become familiar with leavened breads. The Greeks began to bake bread during the night and made remarkable improvements to the technology and baking equipment. The Romans were avid bread eaters; bakers were initially freed slaves who turned into professional bakers, forming corporations that became increasingly important and indispensable to society until the bakers became public officials and, hence, employees of the state.

During the Barbarian migration period in Europe, bread was not the primary food of the Barbarians and industrial bread manufacturing disappeared. The technology of sourdough bread survived in the monasteries until the twelfth century when the profession of baker reappeared in France. After the Middle Ages, the technology of bread made new progress and, especially in northern Europe where breweries were widespread, the barm obtained from beer brewing was identified as a substitute for sourdough in the leavening process.

Since the nineteenth century, baker's yeast (also called compressed yeast) has almost completely replaced sourdough in the leavening of bread. The increased use of baker's yeast was due to its greater suitability for the requirements of modern baking processes, as a rapid and simple leavening process, and the adaptation to mechanized bread production. In fact, the sourdough baking process is time-consuming and requires a long fermentation time.

In recent years, sourdough bread has grown in popularity due to increased consumer demand for bread with pronounced flavor, high nutritional value, healthy properties, prolonged shelf life, and less additives; at last, the traditional aspects of sourdough breads have attracted consumers. Many sourdough breads are produced worldwide, most of them in Europe and

FIGURE 4.2
Loaves of sourdough bread produced in southern Italy. Pane di Altamura is a traditional PDO bread obtained from remilled durum wheat semolina and sourdough starter. It is produced in a limited area of southern Italy according to the bread making procedure defined by EU regulations (European Commission, 2003).

Mediterranean countries, others in North America. Sourdough was introduced from Europe to the San Francisco area during the California gold rush and to Alaska and western Canada during the Klondike gold rush. San Francisco sourdough bread is the most famous type currently produced in the United States. In the Mediterranean area, the sourdough baking process generally concerns artisanal bakeries; indeed, the "culture" and skill of sourdough baking have been lost by many bakers, and its use is perpetuated by specialists and enthusiasts, particularly bakeries producing traditional breads. PDO breads (protected designation of origin), such as Pane di Altamura (Figure 4.2) and Pagnotta del Dittaino (both produced in Italy), and PGI breads (protected geographical indication), such as Pan de Cruz de Ciudad Real and Pan de Cea (produced in Spain) and Pane di Matera, Pane di Genzano, and Coppia Ferrarese (produced in Italy), are protected by European regulations and must be leavened using sourdough. In northern Europe, sourdough is employed especially in the baking process of rye flour, for example, in Germany, the Baltic states, and Russia; this is also done in the United States. Because rye flour does not contain gluten proteins, which are responsible for the formation of a net structure in wheat dough, rye dough is not able to retain the CO_2 produced during fermentation. Rye flour mainly contains starch, which can absorb a high amount of water. Bread made with 100% rye flour is usually obtained by sourdough fermentation; the acidity of sourdough inactivates α-amylases, commonly present in rye flour, thus preventing excessive starch degradation, and it promotes solubilization of rye pentosans that enhance the water binding capacity of the dough, allowing the starch to gel and form a matrix during cooking. Therefore, dough acidification is essential to obtain a proper crumb structure and an increase of bread volume. In mixed wheat–rye breads, baker's yeast can be used for leavening, and if strong wheat is used, an increase in loaf volume can be obtained. Despite its distinctive flavor, taste, and eating quality, sourdough rye bread production is currently diminishing due to the large amount of labor and high cost of production, and most rye breads, especially in the United States, are manufactured using baker's yeast (Lorenz, 2003).

Semolina from durum wheat (*Triticum turgidum* subsp. *durum* L.), commonly used to make pasta and cous cous, is also used to produce leavened bread in Mediterranean countries. Two-layered flat breads are popular in the Middle East and Arab countries. In southern Italy, semolina is commonly used to produce traditional loaf breads, such as the previously mentioned PDO and PGI breads. Different types of leavened flat breads are produced in Sardinia (Figure 4.3), an island in the Mediterranean Sea, with Carasau bread—a crispy, flat, thin bread—being the most representative.

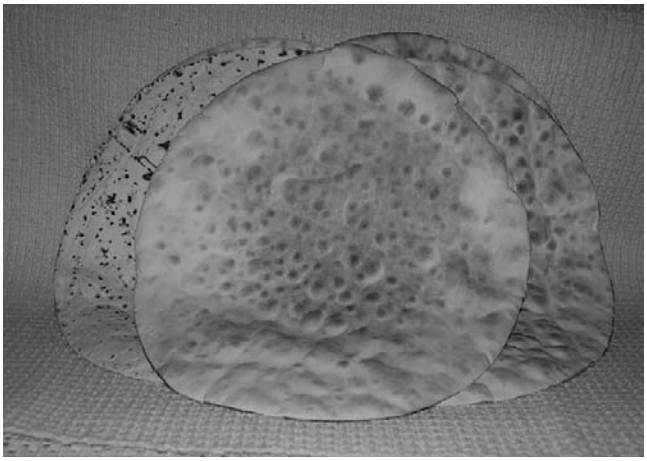

FIGURE 4.3
Sourdough flat bread. Two-layered leavened flat breads are produced in the Middle East, Arab countries, and Sardinia (the island in the Mediterranean Sea) using sourdough starter and durum wheat flour.

SOURDOUGH MICROORGANISMS

The presence of microorganisms in sourdough was discovered in approximately 1900. Spontaneous sourdough is a rich source of lactic acid bacteria and yeast (Table 4.1) belonging to different genera and species deriving from flour, the environment, or something being used as inoculum (e.g., fruits and yogurt). Thus far, hundreds of scientific papers have expanded the knowledge on sourdough microflora. Most of the isolated species of LAB belong to the genus *Lactobacillus*, but species of the genera *Pediococcus*, *Leuconostoc*, and *Weissella* are also commonly found. A few species of *Lactobacillus* were initially isolated from sourdough and are considered strictly related to the sourdough habitat—for example, *Lactobacillus sanfranciscensis*, *Lactobacillus pontis*, *Lactobacillus panis*, and *Lactobacillus rossii*—and some of them have never been isolated from other substrates. Other species isolated from sourdough (e.g., *Lactobacillus acidophilus*) may have an intestinal origin, probably due to cross-contamination, whereas others, such as *Lactobacillus plantarum* and *Lactobacillus brevis*, are quite common in other habitats and foods (Corsetti and Settanni, 2007).

TABLE 4.1 Species of Lactic Acid Bacteria (LAB) and Yeasts Isolated from Sourdough[a]

Obligate Heterofermentative LAB	Facultative Heterofermentative LAB	Homofermentative LAB	Yeasts
Lactobacillus sanfranciscensis	*Lactobacillus plantarum*	*Lactobacillus amylovorus*	*Saccharomyces exiguus*
Lactobacillus brevis	*Lactobacillus casei*	*Lactobacillus acidophilus*	*Saccharomyces cerevisiae*
Lactobacillus fermentum	*Lactobacillus rhamnosus*	*Lactobacillus farciminis*	*Candida holmii*
Lactobacillus reuteri	*Lactobacillus alimentarius*	*Lactobacillus delbrueckii*	*Candida krusei*
Lactobacillus panis			*Candida humilis*
Lactobacillus pontis			*Candida milleri*
Lactobacillus fructivorans			
Weissella confusa			
Weissella cibaria			
Leuconostoc citreum			
Leuconostoc mesenteroides			

[a]*The microorganisms most frequently found in sourdough. Most of the lactic acid bacteria belong to the genus* Lactobacillus*, the majority being heterofermentative species.* Saccharomyces cerevisiae *is rarely isolated in sourdough and is assumed to be a contamination from baker's yeast.*

LAB ferment maltose, the most abundant sugar in flour, and produce lactic acid when expressing homofermentative metabolism; in the case of heterofermentative metabolism, they produce CO_2, acetic acid, and/or ethanol in addition to lactic acid. The prevalence of species having one or the other metabolism influences the properties of the dough. Acetic acid is responsible for a hardening of gluten, whereas lactic acid can gradually account for a more elastic gluten. As a consequence, the texture and aromatic profile of the bread are also affected (Corsetti and Settanni, 2007).

Several species of yeast have been isolated from sourdough, but only a few of them are considered fundamental in the fermentation process. The most representative species belong to the genera *Saccharomyces* and *Candida*, with the species being *Saccharomyces exiguus*, *Candida humilis*, and *Candida krusei* (Corsetti and Settanni, 2007). The presence of *Saccharomyces cerevisiae* is not common in spontaneous sourdough, and its origin is often attributed to contamination with baker's yeast. Because the yeast is the major producer of CO_2 in sourdough, it is considered responsible for dough leavening (Corsetti and Settanni, 2007).

Lactobacillus sanfrancisco was the first species identified (by Kline and Sugihara in 1971) as responsible for the souring activity in San Francisco bread (it is now denominated *L. sanfranciscensis*). This species is strictly associated in sourdough with the yeast *S. exiguus*, also denominated *Candida holmii* in its anamorphic form. The trophic relationships between these two species are well documented (Gobbetti *et al.*, 1996); the microorganisms are not competitors for sugar uptake because *L. sanfranciscensis* utilizes maltose, the most important sugar in sourdough. During sourdough fermentation, maltose is hydrolyzed into two molecules of glucose, but only one is metabolized, whereas the other is excreted outside the cell, where it can be fermented by *S. exiguus*, a maltose-negative species. Furthermore, during sourdough fermentation the yeast's excretion of specific amino acids and small peptides provides an advantage to the lactobacillus that, in turn, produces lactic or acetic acid in the dough and decreases the pH, creating a favorable substrate for the growth of yeast.

The number and quality of sourdough microorganisms, both yeast and LAB, depend on several factors, such as the type of raw materials, the amount of water (dough yield), the fermentation temperatures, the environment, and the refreshment practices. The quality of sourdough bread is obviously affected by the presence of one species or another throughout the fermentation process (Figure 4.4). In spontaneous sourdough, the LAB:yeast ratio is generally 100:1 (Gobbetti *et al.*, 1994; Ottogalli *et al.*, 1996).

In approximately the past decade, numerous papers have described the microbial communities responsible for sourdough fermentation throughout the world, reporting new species and describing metabolic interactions between yeast and LAB. *Lactobacillus sanfranciscensis* is considered the predominant LAB, especially in type I sourdough, obtained using traditional techniques and daily refreshments at ambient temperature, but not in type II sourdough, a semi-fluid sourdough adapted for an industrial process at higher temperature (De Vuyst *et al.*, 2002). In Italy, *L. sanfranciscensis* dominates the microflora of sweet leavened baked products (Ottogalli *et al.*, 1996) and wheat sourdough (Gobbetti *et al.*, 1994), and the same species has been found in traditional Greek wheat sourdough (De Vuyst *et al.*, 2002). *Lactobacillus pentosus* and *L. plantarum* (Catzeddu *et al.*, 2006; Ricciardi *et al.*, 2005) dominate sourdough bread produced in southern Italy using durum wheat flour. *Lactobacillus brevis* is predominant in Turkish (Gül *et al.*, 2005) and Portuguese sourdough (Rocha and Malcata, 1999).

At the beginning of the twentieth century, commercial starters were developed for sourdough baking processes. Currently, several suppliers in North America and Europe offer different types of starters. Two main formulates can be distinguished: (1) starter cultures

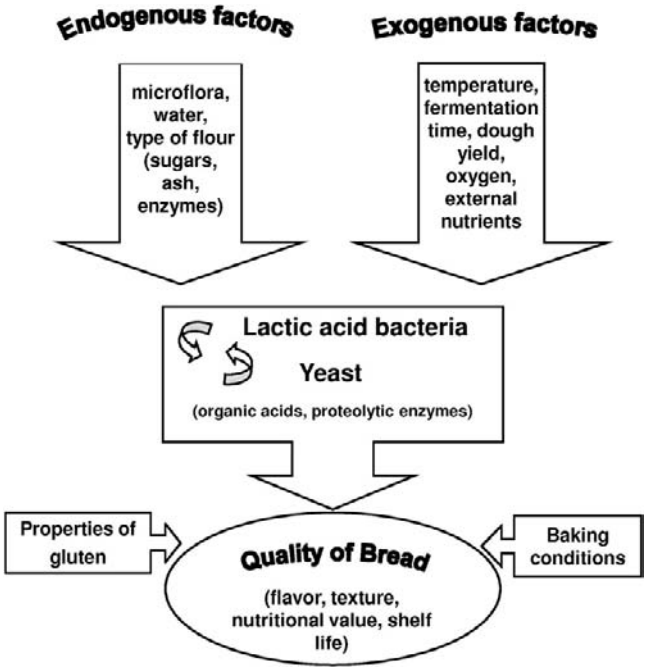

FIGURE 4.4
Factors affecting sourdough fermentation and bread quality. The fermentation process in sourdough is affected by numerous endogenous and exogenous factors that regulate the microflora composition (e.g., microbial species and LAB:yeast ratio) and hence the types of metabolites produced in the dough. The metabolites in sourdough, together with the baking conditions and the quality of flour proteins, affect the bread quality.

consisting of pure strains of LAB and/or yeast isolated from sourdough and (2) sourdough starters obtained by fermenting flour with yeast and/or LAB strains and supplied in semi-liquid or dry-powder form. Starter cultures are in dried, paste, or liquid form and contain a very high concentration of living cells. Sourdough starters are often microbiologically inactive, and they are not used as leavening agents but, rather, as dough acidifiers and flavor enhancers. Inactivation of microorganisms in liquid sourdough is performed by adding salt or by pasteurization, whereas in freeze-dried products some microorganisms do not survive the drying process; for example, *S. exiguus* does not tolerate freeze drying and fresh cells must be used to inoculate a dough. The aim of these products is to obtain a constant bread quality, overcoming the problems of artisanal sourdough, especially those regarding processing difficulties, the required skill and operational time, and the uncertainties of the preparation.

TECHNOLOGICAL ISSUES: THE QUALITY OF SOURDOUGH BREAD

Differences in sourdough bread and baker's yeast-leavened bread are well recognized and documented by many authors. Sourdough fermentation undoubtedly affects the dough and bread properties, extending the shelf life through inhibition of spoilage fungi and bacteria (Katina *et al.*, 2002; Ryan *et al.*, 2008), improving the bread flavor (Katina, Heiniö, *et al.*, 2006), enhancing the nutritional properties (Björck and Liljeberg Elmståhl, 2003), increasing loaf volume, and delaying staling (Arendt *et al.*, 2007).

Acidification is the most evident effect of LAB metabolism in the dough. The pH of a ripe sourdough ranges from 3.8 to 4.5, depending on endogenous factors (microbial composition and the nature of the flour) and exogenous factors (temperature and time of fermentation and also dough yield). The acidification of bread dough affects the activity of microbial and cereal enzymes, the rheology of the dough, and the flavor of the bread. However, its perception in

bread is not well accepted by many consumers, who prefer mild acidity. Indeed, because sourdough bread can reach a pH of 4.0, control of the acidity level in sourdough wheat bread is essential for bread acceptability.

The texture of wheat bread depends greatly on the formation of a gluten network, a viscoelastic structure that entraps the CO_2 produced during fermentation and allows expansion of the dough. Biological acidification of dough is important in improving loaf volume, crumb softness, and delayed starch retrogradation of bread. A gradual decrease of pH during fermentation supports the activity of several enzymes, such as amylases and proteinases, that are active at low pH. Therefore, the acidity acts on the gluten network, improving the softness and extensibility of the dough and favoring the retention of CO_2 produced in the fermentation process (Clarke *et al.*, 2002). Excessive acidity and hydrolysis of gluten proteins—for instance, as a consequence of a long fermentation time—results in a softer, less elastic dough, reduces the loaf volume, and increases staling and bread firmness, as demonstrated by the addition of bacterial proteases to the sourdough (Gocmen *et al.*, 2007; Katina, Heiniö, *et al.*, 2006). Acidification of dough due to microbial fermentation has a positive effect, especially in high-fiber breads, in which the addition of cereal bran decreases the bread volume and crumb elasticity, causing severe problems in bread quality (Katina, Salmenkallio-Marttila, *et al.*, 2006).

Straight yeasted bread is subject to microbial spoilage by molds and *Bacillus* microorganisms. Mold growth is due to contamination after baking, whereas *Bacillus* spores are in the raw material and germinate after baking. Chemical additives, such as sorbate, propionate, and benzoate, are commonly mixed in the dough to control the growth of contaminants, but an increase in resistant strains and public demand to reduce chemical additives in food have stimulated research on natural antimicrobial compounds. Sourdough contains microorganisms recognized as playing a fundamental role in the preservation and microbial safety of fermented foods. The antimicrobial activity has usually been attributed to the production of organic acids, especially acetic acid. However, an antimicrobial compound produced by sourdough LAB, namely phenyllactic acid (PLA), has been discovered. Sourdough bread manufactured with PLA-producing strains has been proved to delay the growth of a few mold species, such as *Aspergillus*, *Fusarium*, and *Penicillium*, although the growth of *Penicillium roqueforti* was only delayed when PLA was used in combination with calcium propionate (Ryan *et al.*, 2008). Inhibitory activity of sourdough bread against rope-forming *Bacillus* has been observed (Katina *et al.*, 2002); in this case, the antimicrobial mechanism is supposed to be achieved by a combination of low pH, organic acids, and other substances, such as bacteriocins.

Consumer appreciation of sourdough bread has increased in recent years not only because of better knowledge of its nutritional properties but also mainly because consumers enjoy the stronger taste and flavor of sourdough bread compared to yeasted bread. The effectiveness of sourdough in enhancing bread flavor has been established by many authors, and bread from unfermented dough has been observed to have a much smaller amount of volatile compounds than fermented dough. Bread flavor is influenced by numerous factors, including the type of cereal flours, the parameters of sourdough preparation, the baking conditions, and the metabolism of fermenting microorganisms. However, the enhanced proteolysis of dough due to the fermenting microorganisms in sourdough is the major factor leading to the formation of amino acids, the precursors of aromatic substances. The weaker flavor of yeasted bread is most likely due to the fact that yeast utilizes a high amount of amino acids for its metabolism. Factors improving the formation of flavors, such as a long fermentation time or whole flours, seem to contrast with the enhancement of bread texture and volume (Katina, Heiniö, *et al.*, 2006). Rye flour is a particularly flavoring substrate because it contains amino acid flavor precursors, fatty acids, and phenolic compounds that are converted during the baking process.

HEALTHY PROPERTIES OF SOURDOUGH BREAD

Consumer interest in the health aspects of food continues to increase, and traditional food is quite often perceived as having nutritional properties that favor health and well-being. In the past decade, sourdough fermentation has been associated with the health-promoting properties of bread, and numerous beneficial effects have been reported (Figure 4.5). Protective compounds in bread have been identified, especially in wholemeal bread, which contains dietary fiber, minerals, vitamins, and complex carbohydrates. Conversely, wholemeal bread contains a significant amount of phytate, which interferes with the absorption of essential minerals such as calcium, zinc, and iron. The degradation of phytate by sourdough microorganisms has been extensively established; in the past few years, it has been reported that the activity of wheat phytase is predominant over that of sourdough microorganisms, but the lowering of pH in sourdough, due to the fermentative metabolism of bacteria, is fundamental in providing favorable conditions for the endogenous cereal phytase (Reale *et al.*, 2007).

The production of organic acids is also quite important in reducing the postprandial glycemic response in human blood. Starch bread is usually rapidly digested and absorbed, leading to hyperglycemia in people suffering from insulin-resistance syndrome. Organic acids produced in sourdough are responsible for a reduction of the glycemic index; this seems to be associated with a delay in gastric empting in the case of acetic acid, whereas lactic acid induces interactions between starch and gluten during dough baking and reduces starch availability (Björck and Liljeberg Elmståhl, 2003).

Cereals are widely used in human diets throughout the world. Prolamin proteins of cereals are responsible for an autoimmune disorder known as celiac disease or gluten intolerance, which is activated by the intake of prolamin-containing foods. Although numerous studies are under way to find a pharmacological treatment, the only solution at the moment is a diet free of gluten from wheat, rye, barley, and other cereals. Several attempts have been made to reduce the toxic proteins in bread by enhancing their hydrolysis via microbial enzymes or native enzymes of cereals. The use of selected lactobacilli, able to extensively

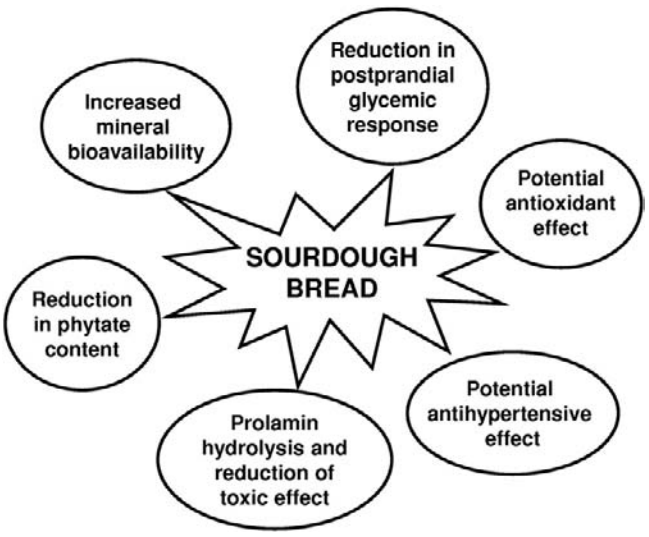

FIGURE 4.5
Influence of sourdough on the nutritional properties of bread. Sourdough fermentation can significantly improve the nutritional properties of bread because it increases the mineral bioavailability (especially in wholemeal bread) and reduces the glycemic index in human blood. New applications are being studied, involving the hydrolysis of prolamin proteins to prevent gluten intolerance, as well as the antioxidant and antihypertensive activities of some bread components.

hydrolyze prolamin proteins, has been proposed in a complex fermentation process of wheat sourdough (30%) mixed with nontoxic flours. The resulting bread was comparable to a common wheat sourdough bread, and clinical tests showed that this bread was tolerated by gluten-intolerant patients (Di Cagno *et al.*, 2004). Another approach to prolamin hydrolysis was sourdough fermentation of germinated wheat and rye grains (Loponen *et al.*, 2009). In this case, the activity of lactobacilli did not lead to hydrolysis, but prolamins were extensively hydrolyzed by the native enzymes of the germinated cereals, most probably favored by the lowering of pH caused by the lactobacilli. No evidence of clinical safety has been produced for these products. Furthermore, the hydrolysis of gluten has been found to be exploitable in rye flour, whereas in wheat flour it prevents the rising of the dough.

In addition to the nutritional aspects of sourdough breads already studied for many years, research has shown the antioxidant and antihypertensive activity of bread components. An antioxidant named pronyl-L-lysine produced via the Maillard reaction during baking has been identified in bread crust. It acts as a monofunctional inducer of glutathione S-transferase, which serves as a functional parameter of an antioxidant chemopreventive activity *in vitro*. The amount of this antioxidant was found to be higher in sourdough bread than in bread obtained by yeast fermentation, being highly dependent on the pH value (Lindenmeier and Hofmann, 2004).

ACE is an angiotensin I-converting enzyme responsible for an increase in blood pressure in humans. Several biopeptides with an ACE inhibitory effect have been found, especially in cheese or dairy products. Rizzello *et al.* (2008) showed that selected LAB used during sourdough fermentation of mainly wholemeal flour can synthesize ACE inhibitory peptides and γ-aminobutyric acid with a potential antihypertensive effect.

SUMMARY POINTS

- Sourdough bread refers to a leavened bread obtained using dough fermented by lactic acid bacteria and yeast.
- Sourdough consists of a spontaneous fermentation process and can undoubtedly be considered the primordial form of bread leavening. Its use developed in ancient Egypt.
- Lactic acid bacteria are mainly responsible for the production of lactic and/or acetic acid, whereas yeasts are mainly responsible for the production of CO_2; both are responsible for the production of aromatic precursors of bread.
- The most representative species of lactic acid bacteria and of yeasts are respectively *Lactobacillus sanfranciscensis* and *Saccharomyces exiguus*.
- In the past century, sourdough was replaced by baker's yeast for bread leavening, although consumer demand for sourdough bread has increased in recent years.
- San Francisco bread is the most famous sourdough bread currently produced in the United States.
- In northern Europe, sourdough is employed mainly in the baking process of rye flour.
- In the Mediterranean area, sourdough bread is mainly produced in artisanal bakeries producing traditional breads—for example, PDO breads such as Pane di Altamura and Pagnotta del Dittaino.
- Durum wheat flour is used in southern Italy, the Middle East, and Arab countries to produce sourdough for loaf breads and leavened flat breads.
- The use of sourdough in bread making improves loaf volume and flavor, delays staling, and inhibits the growth of spoilage fungi and bacteria.
- Sourdough fermentation has been associated with the health-promoting properties of bread, such as a reduction of the postprandial glycemic response in human blood.

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CHAPTER 5

Focaccia Italian Flat Fatty Bread*

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CHAPTER OUTLINE

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LIST OF ABBREVIATIONS

$C_{18:1\ trans}$ *trans* oleic acid
DG Diglycerides
FFA Free fatty acids
HPSEC High-performance size-exclusion chromatography
MRPs Maillard reaction products
ox-TG Oxidized triglycerides
P/*L* Tenacity/extensibility alveographic ratio
p-AV *p*-Anisidine value
PV Peroxide value
TGP Triglyceride oligopolymers
TOTOX (2 PV + *p*-AV) Measure of total oxidation that takes into account the contribution of peroxide value, an expression of primary oxidation, and of *p*-anisidine value, an index of secondary oxidation
W Alveographic measure of gluten strength

ORIGINS OF FOCACCIA

The word "focaccia" derives from the Latin *focus*, meaning fireplace. The *De Agricoltura* by Marco Porcio Catone (234–149 BC) is the first Latin written source that mentions the *Libum farrem*, the ancient Roman focaccia, offered in sacrifices and during weddings (Bordo and Surrasca, 2002). In the Middle Ages, focaccia was considered a poor food, made with remnants of the dough destined to bread making and baked to test the temperature of wood-fired ovens before introducing bread. Once made, it was consumed by bread makers.

Today, focaccia is largely appreciated for its sensory properties and has its own market. It is usually consumed, still hot, as a "street food," immediately after its production. If not consumed very fresh, it loses flavor and its consistency tends to harden. It is common in Italy to see people waiting to buy fresh-baked focaccia at bread makers' shops. The smell of hot focaccia spreads in the streets and induces people to buy it. It is usually baked two or three times per day—once or twice in the morning and once in the late afternoon.

Focaccia is made of a few simple ingredients—flour, water, fatty substances (oil or lard), yeast, and salt—but a myriad of nuanced differences are obtainable by topping it, prior to cooking, with fresh tomato, onions, potatoes, olives, cheese, etc. or flavoring it with herbs (rosemary, sage, oregano, etc.). It usually appears as a circular flat bread, single layered, oily, and variously topped.

Especially in its "red version," topped with tomato, focaccia may appear similar to Italian pizza, but actually there are many differences between these two food categories regarding both the ingredients and the way in which they are consumed. Focaccia is characterized by a high content of fatty substances in the dough, whereas in pizza only a very small amount of oil is added on the surface. This contributes to a different flavor and also makes the consistency of these two products different. In addition, focaccia is generally thicker and, above all, less humid than pizza. The latter, being always topped with mozzarella cheese, is characterized by the presence of some liquid on the surface.

These characteristics make a piece of focaccia a perfect street food to be eaten as a snack or an appetizer, whereas in Italy pizza is usually consumed while sitting at a table, using dishes, typically at dinner or, in recent years, also at lunch.

TYPES OF FOCACCIA PRODUCED IN THE ITALIAN REGIONS

Many types of focaccia are produced in Italy, varying by region (Figure 5.1). They are prepared according to well-established traditional processing methods, which are homogeneous

FIGURE 5.1
Geographical distribution of focaccia flat fatty breads in Italy. The map identifies the locations of the productive areas of different types of focaccia according to the various Italian regions.

throughout the areas concerned. In some cases, this regional food has been officially recognized as a Traditional Agri-food Product according to Italian Legislative Decree of April 30, 1998, No. 173.

Piedmont

FOCACCIA NOVESE (NOVI-STYLE FOCACCIA)

Originally prepared without salt, focaccia novese is a "white" (i.e., without tomato on the surface) focaccia containing lard and extra-virgin olive oil. After kneading the ingredients, the dough is left to rise and then is stretched in baking trays, making cuts and dimples on the surface. After a final proofing, it is baked at 230°C for 20 min (Bordo and Surrasca, 2002). Focaccia novese was officially recognized in 2002 as a Traditional Agri-food Product.

Lombardy

SCHIACCIATA

This focaccia, the name of which means "flattened," is a square white focaccia obtained from soft wheat flour, water, lard, yeast, and salt. After kneading the ingredients, the dough is left to rise and then flattened, cut in squares, proofed, and baked at 270°C for 20 min (Bordo and Surrasca, 2002).

Liguria

FOCACCIA GENOVESE (GENOAN-STYLE FOCACCIA)

This white focaccia, called *fugassa* in Genoan dialect, is similar to the French *fougasse* produced in the neighboring French region of Provence. It is made of flour—sometimes a blend of soft

and durum wheat flour (INSOR, 2000)—mixed with water, extra-virgin olive oil, mashed boiled potatoes, yeast, and salt. The ingredients are kneaded and the dough is left to rest for approximately 45 min. Then, it is flattened in an oiled circular baking pan and sprinkled with coarse salt and oil. After a final proofing, baking occurs at 230°C, possibly in a wood-fired oven, until the surface appears golden brown (18—20 min). Final thickness is approximately 2 cm. Focaccia from Genoa has been included in the Italian list of Traditional Agri-food Products and is classified as a Slow Food Product (Bordo and Surrasca, 2002).

FOCACCIA DI RECCO (RECCO-STYLE FOCACCIA)

Although single-layered focaccias represent the more usual type, focaccia di Recco is a double-layered filled focaccia. For the dough, durum wheat flour, water, extra-virgin olive oil, yeast, and salt are used, whereas the filling is made of stracchino soft cheese. Flour is mixed with warm water, oil, yeast, and salt and then kneaded. The dough is left to rise for 45 min, and then it is rolled out into two very thin sheets. One sheet is put on an oiled circular baking tray, covered with small flakes of cheese, and then the second sheet of dough is placed on top. The edges of the sheets are closed, and finally the surface is drizzled with oil. It is baked at 280°C for 10 min until the color is golden brown and the cheese is melted (Bordo and Surrasca, 2002).

FOCACCIA DI VOLTRI (VOLTRI-STYLE FOCACCIA)

This is similar to focaccia from Genoa, but the consistency of its dough, before baking, is softer because of the use of a higher amount of water. After sheeting, this focaccia is left to leaven on surfaces sprinkled with yellow corn flour, which confers a peculiar flavor and color to the product. Finally, it is baked directly on the lower surface of the oven (Bordo and Surrasca, 2002).

SARDENAIRA

This is a "red" focaccia whose dough is made of flour, water, olive oil, yeast, and salt. After kneading and leavening, it is flattened, topped with tomato sauce, olives, and anchovies, and then baked. The final product is approximately 4 cm thick (INSOR, 2000).

Emilia-Romagna

GNOCCO INGRASSATO

This white focaccia (literally "fatty" *gnocco*), also called *spianata* ("flatted one"), is obtained from soft wheat flour, water, lard, small cubes of fatty ham, yeast, and salt. After leavening, flatting in a baking tray, and sprinkling the surface with coarse salt, it is baked at 200°C for approximately 30 min (Bordo and Surrasca, 2002). The final product is 5—8 cm thick (INSOR, 2000).

STRIA

The dough of this white, 3-cm-thick focaccia is made by kneading soft wheat flour, water, olive oil, lard, and yeast. After resting, the dough is flattened, and the surface is dimpled, sprinkled with coarse salt, and rubbed with lard. Finally, it is baked in a wood-fired oven (Bordo and Surrasca, 2002).

Tuscany

SCHIACCIA MAREMMANA

Its dough is made of flour, water, and yeast. After leavening, it is sheeted upon the paddle at a thickness of 3 or 4 cm, drizzled with extra-virgin olive oil, and baked at 280°C for approximately 1 h. The final product is rectangular with a brown surface (Bordo and Surrasca, 2002).

Umbria

TORTA AL TESTO OR CRESCIA

It is obtained from soft wheat flour, water, extra-virgin olive oil or lard, baking soda, and salt. After kneading and resting, the dough is flattened and baked on a hot metal plate (Bordo and Surrasca, 2002; INSOR, 2000).

Lazio

PIZZA BIANCA DI ROMA (ROME-STYLE PIZZA BIANCA)

The local name of this focaccia, literally meaning "white pizza," is misleading because it is not a pizza. It is obtained from soft wheat flour, water, oil, malt, yeast, and salt. After kneading, the dough is left to rise, and then it is flattened and baked. The final product is 2 or 3 cm thick and has an oily, golden brown surface (Bordo and Surrasca, 2002).

Marches

CRESCIA MACERATESE

It is prepared with soft wheat flour, water, extra-virgin olive oil, yeast, and salt. This circular white focaccia can be topped with rosemary or onions (Bordo and Surrasca, 2002).

CROSTOLO DEL MONTEFELTRO

It is a white and nutritious circular focaccia obtained from soft wheat flour, water, lard, eggs, baking soda, salt and pepper, and, sometimes, some whey remaining from cheese making. The dough is left to rest for 1 h, and after sheeting it is baked on a hot clay plate (Bordo and Surrasca, 2002).

Campania

TORTANO

The tortano or focaccia chiena (literally "filled focaccia") is actually a 6-cm-thick pie stuffed with small pieces of boiled eggs, cheese, and salami, usually prepared at Easter. The dough is made of flour, lard, water, yeast, salt, and pepper. After leavening, it is sheeted, filled, rolled, and baked. This kind of focaccia is also produced in the neighboring Basilicata region under the name pzzetto chieno (Bordo and Surrasca, 2002). In a variant named casatiello, one to three entire eggs (with the shell) are fixed by thin dough strips on the surface before baking, without including eggs in the filling.

Basilicata

RUCCOLO

It is made of soft wheat flour, water, oil, yeast, and salt. After kneading, leavening, and flattening, it is topped with oil, garlic, oregano, and hot red pepper (INSOR, 2000).

Apulia

FOCACCIA BARESE (BARI-STYLE FOCACCIA)

This "red" focaccia (Figure 5.2) is obtained by kneading a blend of soft and durum wheat flour with water, mashed potatoes, extra-virgin olive oil, yeast, and salt. After leavening, it is flattened in a circular baking tray and topped with fresh cherry tomatoes and a few olives, which are pressed slightly into the dough. The surface is then sprinkled with dried oregano and a small amount of additional oil. Final baking is done at 200°C for 20 min. This 3- or 4-cm-thick focaccia can be varied by changing toppings (tomatoes can be substituted with potatoes, onions, or rosemary).

FIGURE 5.2
Tomato-topped Bari-style focaccia. The picture shows a piece of Bari-style focaccia almost totally covered by fresh cherry tomatoes.

Calabria

PITTA MANIATA

This very soft 5-cm-thick focaccia is made of soft wheat flour, water, lard, yeast, and salt. Swine entrails, previously cooked apart with pepper, are added to the basic dough. All is kneaded again and finally baked. A variant filled with cheese and eggs is similar to tortano produced in the Campania region.

Sicily

SFINCIONE

It is made of durum wheat flour, water, yeast, and salt. After leavening, the dough is flattened and seasoned with tomato sauce, anchovies, onions, and cheese. It is an extremely soft focaccia up to 5 cm thick.

THE BASIC INGREDIENTS OF FOCACCIA

Flour

To obtain a good yield and a high specific volume, flour characteristics have to fulfill the requirements of the processing technology of the focaccia type considered. The major issue is the overall duration of the process. In the past, only the sourdough-based prolonged process was performed. Today, to accomplish faster production rhythms, fresh compress baker's yeast is used in the majority of bakeries.

According to the voluntary Italian wheat grading standard, superior bread-making wheat, suitable for the production of focaccia by prolonged process, is defined by (1) protein content comprised between 13.5 and 14.5% dry matter, (2) tenacity/extensibility ratio (alveograph P/L) lower than 0.6, (3) gluten strength (alveograph W) higher than 220×10^{-4} J, and (4) farinographic stability from 10 to 15 min. Protein content between 11.5 and 13.5%, W values within the range of $160-200 \times 10^{-4}$ J, and stability from 5 to 10 min are sufficient for ordinary bread making (Pagani *et al.*, 2006).

Interestingly, durum wheat is used in some types of focaccia. P/L values higher than 1.0 can be tolerated in durum wheat flours, but to avoid a too compact structure, a blend with soft wheat flour and the addition of mashed potatoes are essential. Analysis of samples of durum wheat re-milled semolina from southern Italy (Figure 5.3), the area where the majority of Italian

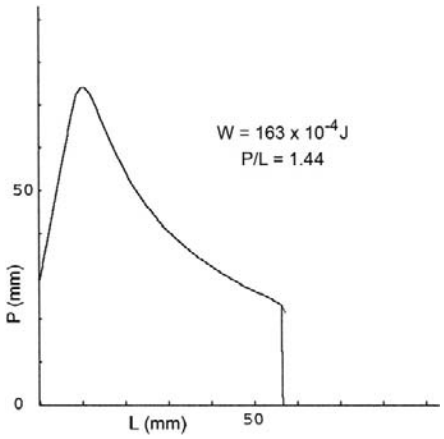

FIGURE 5.3
Alveograph curve of durum wheat re-milled semolina used in the production of Bari-style focaccia. The curve shows the values of gluten strength and the tenacity/extensibility ratio for durum wheat re-milled semolina commonly used in focaccia making in the Apulia region.

durum wheat milling capability is concentrated, indicated that a tenacious gluten is usually present, with mean alveograph *P*/*L* of 1.13 (Pasqualone *et al.*, 2004).

Fatty substances

Many fatty substances can be used in bakery: butter, lard, hydrogenated vegetable oils, margarines, refined seed oils, olive oil, and olive-pomace oil. Their content may range from 5—15% for some bread substitutes, such as crackers, breadsticks, and focaccia, to 20—30% in biscuits and cakes. The choice of the most suitable lipid is closely related to the desired dough workability, the product's rheological and sensory properties, shelf life, and consumers' needs. The demand for low-calorie foods has led to the marketing of bakery products with low lipid content, commonly called "light." To not jeopardize the product quality in terms of stability and sensory properties, lipids are partially or completely substituted by substances with similar functional properties but less calories (polysaccharides, proteins, and modified lipids) (Nicoli, 2003). In addition, by substituting animal fats (lard, butter, etc.) with lipids of vegetable origin, it is possible to obtain bakery products low in cholesterol content.

Olive oil, notably extra-virgin olive oil, is an essential ingredient in the preparation of many types of focaccia. It makes focaccia pleasant and palatable, and it provides a characteristic smell and taste. From a nutritional and health standpoint, extra-virgin olive oil supplies nutrients that pomace oil or lard cannot supply. It contains a wide range of substances, constituting the unsaponifiable fraction (sterols, aliphatic and triterpene alcohols, polyphenols, and tocopherols), involved in many biochemical and physiological processes. Moreover, oleic acid, the most represented fatty acid in olive oil (the optimal value being not less than 73%), is the most digestible monounsaturated fatty acid for humans. Because its melting point is below the human body temperature, olive oil stimulates pancreatic lipase secretion and enhances its hydrolytic activity. Moreover, the high absorption coefficient of oleic acid, due to the stimulation on biliary secretion (cholecystokinetic effect), favors the absorption of the liposoluble vitamins contained in food (Conte, 2004).

TECHNOLOGICAL ISSUES: EFFECT OF BAKING ON THE LIPID FRACTION OF FOCACCIA

Although extra-virgin olive oil has many appreciable properties, it may undergo oxidative alterations during thermal treatments, such as baking, that may decrease its quality and

healthy features. Little research has focused on the impact of baking on the oxidative state of the lipid fraction of baked products, with the exception of investigations regarding *trans* fatty acids (van Erp-Baart *et al.*, 1998). Oxidative phenomena regarding lipids have been studied for other cooking methods, such as frying (Arroyo *et al.*, 1992; Naz *et al.*, 2005).

Delcuratolo *et al.* (2008) evaluated the impact of baking on the lipid fraction of focaccia, focusing on hydrolytic and oxidative changes. Four types of focaccia—Bari-style focaccia (tomato-topped focaccia) and three white variants of it (potato-topped focaccia, onion-topped focaccia, and rosemary-topped focaccia)—were studied. Approximately 400 g of dough was seasoned with 200–250 g of toppings (except for the last type, which was topped with 2 g of dried rosemary), flavored with extra-virgin olive oil (150 g/kg flour), and baked at 220°C for 20 min.

Level of *trans* isomers

The obtained results (Table 5.1) showed that the uncooked oil contained 0.02% $C_{18:1\ trans}$, which was considerably lower than the allowed maximum limit (0.05%) foreseen by the current rules (EC Regulation 2568/91, 1991) for the extra-virgin olive oil category. Moreover, it contained only trace levels of *trans* isomers of linoleic and linolenic acids.

The oils extracted from the baked focaccias showed amounts of $C_{18:1\ trans}$ four or five times greater than that of the uncooked oil, and levels of *trans* isomers of linoleic and linolenic acids ranged from 0.01 to 0.02%. The oil from the rosemary-topped focaccia showed lower levels of $C_{18:1\ trans}$ and *trans* isomers of linoleic and linolenic acids than did the oils from the other types of focaccia examined. The amounts of *trans* isomers of fatty acids found in focaccias were smaller than those generally reported for other baked products (van Erp-Baart *et al.*, 1998).

The metabolic effects of *trans* isomers are today a matter of controversy, but many studies have reported that their consumption increases the risk of cardiovascular disease (Pizzoferrato *et al.*, 1999).

Oxidative and hydrolytic degradation

Results of analyses of peroxide value (PV) and *p*-anisidine value (*p*-AV) provided initial information about the oxidative degradation that affects olive oil during focaccia baking. Total oxidation (TOTOX) of the oils from focaccias, calculated as 2 PV + *p*-AV, was two- or threefold greater than in the unbaked oil, with particularly high values in focaccias topped with onions and rosemary (Table 5.2).

High-performance size-exclusion chromatography (HPSEC) of the polar compounds (PC) provided more detailed information about both the oxidative and the hydrolytic degradation

TABLE 5.1 Levels of Fatty Acid *trans* Isomers of Each Oil Sample Examined[a]

		Oil Extracted From			
Sample	Extra-Virgin Olive Oil	Potato-Topped Focaccia	Tomato-Topped Focaccia	Onion-Topped Focaccia	Rosemary-Topped Focaccia
$C_{18:1t}$[b]	0.022 ± 0.002[a]	0.110 ± 0.018[b,c]	0.105 ± 0.006[b,c]	0.125 ± 0.007[b]	0.085 ± 0.008[c]
$C_{18:2t} + C_{18:3t}$[b]	Tr[a]	0.023 ± 0.003[b]	0.012 ± 0.001[c]	0.010 ± 0.001[c]	0.008 ± 0.004[c]

Source: Modified with permission from Delcuratolo, D., Gomes, T., Paradiso, V. M., and Nasti, R. (2008). Changes in the oxidative state of extra-virgin olive oil used in baked Italian focaccia topped with different ingredients. *Food Chemistry*, 106, 222–226.

[a]*This table shows the results of analyses performed on unbaked oil and on oil extracted from four focaccia types, after baking, to assess the contents of fatty acid* trans *isomers.*

[b]*Results of statistical analysis at* $p < 0.05$. *Mean values of three independent repetitions ± SD; one common letter following an entry indicates no significant difference.*

[c]$_{18:1t}$, trans *oleic acid;* $C_{18:2t}$, trans *linoleic acid;* $C_{18:3t}$, trans *linolenic acid; Tr, traces (not integrated).*

TABLE 5.2 Mean Results of the Indices of Oxidative and Hydrolytic Degradation Determined in Each Oil Sample[a]

		Oil Extracted From			
Sample	Extra-Virgin Olive Oil	Potato-Topped Focaccia	Tomato-Topped Focaccia	Onion-Topped Focaccia	Rosemary-Topped Focaccia
TOTOX[b]	38.7	78.0	85.4	125.8	127.2
PC[c]	3.34 ± 0.10^{a}	3.68 ± 0.06^{b}	5.44 ± 0.10^{c}	4.78 ± 0.12^{d}	5.55 ± 0.12^{c}
TGP[c]	0.08 ± 0.01^{a}	0.13 ± 0.03^{b}	0.22 ± 0.03^{c}	0.27 ± 0.02^{d}	0.25 ± 0.03^{c}
ox-TG[c]	0.75 ± 0.04^{a}	0.73 ± 0.05^{a}	1.06 ± 0.13^{b}	1.69 ± 0.19^{c}	2.08 ± 0.32^{d}
DG[c]	1.63 ± 0.16^{a}	1.55 ± 0.01^{a}	1.95 ± 0.05^{b}	1.83 ± 0.06^{b}	1.85 ± 0.05^{b}
2 TGP% + ox-TG%[c]	0.91 ± 0.04^{a}	0.99 ± 0.06^{b}	1.50 ± 0.12^{c}	2.23 ± 0.15^{d}	2.58 ± 0.31^{d}

Source: Modified with permission from Delcuratolo, D., Gomes, T., Paradiso, V. M., and Nasti, R. (2008). Changes in the oxidative state of extra-virgin olive oil used in baked Italian focaccia topped with different ingredients. *Food Chemistry*, 106, 222–226.

[a]*This table shows the results of analyses performed on unbaked oil and on oil extracted from four focaccia types, after baking, to assess the degree of oxidative and hydrolytic degradation.*

[b]*Mean values of two independent repetitions.*

[c]*Results of statistical analysis at* $p < 0.05$. *Mean values of three independent repetitions ± SD; one common letter following an entry indicates no significant difference.*

DG, diglycerides; ox-TG, oxidized triglycerides; p-*AV,* p-*anisidine value; PC, polar compounds; PV, peroxide value; TGP, triglyceride polymers; TOTOX = 2 PV +* p-*AV.*

of oil via the determination of the following classes of compounds: triglyceride oligopolymers (TGP), oxidized triglycerides (ox-TG), and diglycerides (DG).

The HPSEC chromatograms of PC detected in the uncooked oil and in the oil extracted from onion-topped focaccia are shown in Figure 5.4. The uncooked oil already contained detectable amounts of TGP that were indicative of the inception of the oxidative process, but the same oil, extracted from the baked focaccia, contained substantially higher TGP and ox-TG levels.

The amount of PC in the oils from all the baked focaccias was significantly greater than that in the uncooked oil. The smallest difference was observed in the potato-topped focaccia (10% increase over the uncooked oil). In the oil from the other three types of focaccia, increases in PC ranged from 43 to 66%.

The amount of ox-TG measured in the uncooked oil (0.75%) was not statistically different from the amount measured in the oil extracted from the potato-topped focaccia. By contrast, significant differences were registered in the other types of focaccia, with ox-TG values ranging from 1.4 to 2.7 times the amount measured in the uncooked oil. These findings confirm that the oils from the focaccias topped with tomatoes, onions, and rosemary had undergone more intense oxidation, as highlighted by the TOTOX values.

Baking led to the formation of oligopolymers in all the considered focaccias. The amount of TGP in the uncooked oil was 0.08%, whereas in the potato-topped focaccia it was 0.13%, namely 1.5 times higher. In the other three cases, the amount of TGP was almost threefold that of the starting oil. The evaluation of the oligopolymers provided further evidence that the oil extracted from the potato-topped focaccia presented a less intense degradation than the other baked oils.

The amount of DG in the uncooked oil was 1.63%. Significantly higher values were found in the baked focaccias, except for the potato-topped type.

Table 5.2 shows the values of the sum of 2 TGP% + ox-TG%, a parameter that provides a better evaluation of the overall oxidation (Gomes *et al.*, 2003). Substantial increases in overall oxidative degradation were found in oils extracted from focaccias topped with tomato, onion, or rosemary. Again, the oil from the potato-topped focaccia seemed to be less affected by the baking process, with an overall oxidation index (2 TGP% + ox-TG%) of 0.99 compared to 0.91

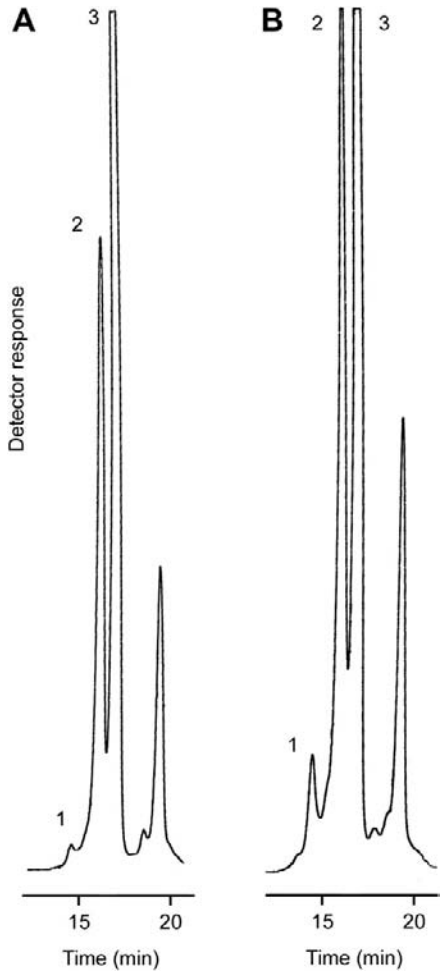

FIGURE 5.4
HPSEC chromatograms of the polar compounds in unbaked extra-virgin olive oil (A) and in the same oil extracted from onion-topped focaccia after baking (B). The chromatogram shows the trend of the polar compound classes: 1, triglyceride oligopolymers; 2, oxidized triglycerides; 3, diglycerides. *Source: Reprinted with permission from Delcuratolo, D., Gomes, T., Paradiso, V. M., and Nasti, R. (2008). Changes in the oxidative state of extra-virgin olive oil used in baked Italian focaccia topped with different ingredients.* Food Chem. *106, 222–226.*

for the uncooked oil. As already shown by the TOTOX index, the oils from the focaccias topped with onions and rosemary proved to be the most oxidized. TOTOX and (2 TGP% + ox-TG%) for all samples showed a substantial agreement and a positive correlation ($p < 0.05$).

Influence of the toppings

The different levels of oxidation found in the oils extracted from focaccias seem to be ascribable to the type of toppings. When toppings were humid and covered the entire surface, the rise of temperature during baking was mitigated as a consequence of the evaporation, thus exposing the oil to a less severe heat stress. Seasoning with sliced potatoes seemed to better protect the lipid fraction from oxidation, probably due to the formation of Maillard reaction products (MRPs) (Mottram *et al.*, 2002). MRPs have a strong antioxidant activity and effectively slow down the oxidative degradation of lipids (Severini and Lerici, 1995).

The highest oxidative degradation level was detected in focaccia topped with dry rosemary, probably due to the absence of the protective effect of evaporation during baking. Furthermore, the drying process and storage of the herb may have negatively affected the rosemary

antioxidant substances, carnoxic acid and carnosol (Offord *et al.*, 1997). Moreover, the stability of the antioxidant power is little known in relation to cooking time and temperatures.

It is interesting to note that the overall level of oxidation, expressed in terms of (2 TGP% + ox-TG%), of extra-virgin olive oil extracted from baked focaccias was lower than that in uncooked refined oils previously examined (Gomes and Caponio, 1997; Gomes *et al.*, 2003). Moreover, it was considerably lower than that of fried oils (Arroyo *et al.*, 1992).

Thus, the oxidation involving seasoning oil during focaccia baking is moderate when employing extra-virgin olive oil, which, after baking, still shows lower oxidation levels than uncooked refined seed oils.

CONCLUSIONS

To produce focaccia, it is advisable to use extra-virgin olive oil because this oil is particularly resistant to thermal oxidation. This is due to the presence of highly antioxidant micronutrients and a polyunsaturated/monounsaturated/saturated fatty acid ratio equal to 0.5:5:1 (Conte, 2004). Regardless of the toppings used, the modifications induced by baking do not compromise the final quality of focaccia.

SUMMARY POINTS

- Focaccia is a typical bakery product of many Italian regions, and it shows variants depending on the productive area.
- The productive steps of the various focaccia types are similar: mixing, kneading, leavening, flattening, proofing, and baking.
- Whereas in the past only a sourdough-based prolonged productive process was performed, today fresh compress baker's yeast is used in the majority of bakeries to prepare focaccia.
- Durum wheat re-milled semolina is used in the production of some types of focaccia.
- Olive oil, notably extra-virgin olive oil, is an essential ingredient in many types of focaccia.
- Extra-virgin olive oil is characterized by well-known healthy features due to the absence of cholesterol and the high content of antioxidant micronutrients.
- The partial or total substitution of extra-virgin olive oil by refined seed or pomace-oil negatively affects the focaccia's sensory properties, digestibility, shelf life, and nutritional value.
- The level of degradation of the seasoning oil is influenced by the amount and percentage of moisture of the toppings used in focaccia preparation.
- Regardless of the toppings used, the modifications induced by baking on extra-virgin olive oil extracted from focaccia are moderate and do not compromise the quality of the final product.
- The oxidation phenomena involving extra-virgin olive oil during focaccia baking lead to levels of oxidized substances that are lower than those found in uncooked refined seed oils.

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CHAPTER 6

Flour and Bread from Black-, Purple-, and Blue-Colored Wheats

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

BGW 76 Black-grained wheat 76
DPPH• 2,2-Diphenyl-1-picryhydrazyl free radical
HMW-glu High-molecular-weight glutenin
IVPD *In vitro* protein digestibility
MPT Midline peak time
ORAC Oxygen radical absorbance capacity
PWB Purple wheat bread
SDS Sodium dodecyl sulfate
WWB Whole wheat bread
WFB White flour bread

INTRODUCTION

Wheat is one of the most important grains in our daily diets. In recent years, bioactive compounds in wheat have attracted increasingly more interest from both researchers and food manufacturers because of their benefits in promoting health and preventing disease. For example, wheat bran extracts significantly inhibited lipid peroxidation in human low-density lipoprotein *in vitro* (Yu *et al.*, 2005). Wheat with high levels of antioxidant activity has the potential for value-added use, particularly in the formulation of functional foods.

Flour and Breads and their Fortification in Health and Disease Prevention. DOI: 10.1016/B978-0-12-380886-8.10006-6

Unlike red and white wheats, blue-, purple-, and black-colored wheats contain natural anthocyanin compounds. The role of anthocyanin pigments as medical agents has been well-accepted dogma in folk medicine throughout the world, and in fact these pigments are linked to an amazingly broad-based range of health benefits (Lila, 2004). Based on the potential of these colorful-grained wheats, several functional foods have been developed from these wheats in recent years, including purple wheat bran muffin (Li *et al.*, 2007a) and antho-beer made from purple-grained wheat (Li *et al.*, 2007b), soy sauce (Li *et al.*, 2004), vinegar, breakfast cereal and instant noodles produced from black-grained wheat, and fine dried noodles made from blue-grained wheat (Pei *et al.*, 2002).

Neither blue- nor purple-colored wheat pigmentation originated in common wheat (Knievel *et al.*, 2009). Knott (1958) found the blue seed color of blue-colored wheat to be inherited from *Agropyron* chromosome additions or substitutions into common wheat rather than a natural occurrence among common wheat species. Zeven (1991) reported that the blue aleurone trait was introgressed into common wheat from blue pigmented *Triticum boeoticum*, *Agropyron tricholphorum*, *Agropyron glaucum*, and, most frequently, from *Agropyron elongatum*. For example, blue-colored wheat cultivar *Leymus dasystachys*, which is related to the *Agropyron* genus, was crossed with common wheat to produce blue-colored wheat (Zeven, 1991). Purple-colored wheat was discovered in tetraploid durum, *Triticum dicoccum*, in east African areas such as Ethiopia, and it was introgressed into common wheat (Zeven, 1991). Copp (1965) reported that a stable hexaploid wheat with purple grain color was obtained from the cross *Triticum dicoccum* var. *Arraseita* Perc. × *Triticum aestivum* L., and its purple grain color was as intense as that in the original tetraploid purple-colored wheat. Above "*Triticum dicoccum* var. *Arraseita* Perc." was purple tetraploid wheat from Abyssinia, with the purple pericarp color being inherited as a monofactorial dominant character in tetraploid wheats (Sharman, 1958). Above "*Triticum aestivum* L." was commercial hexaploid wheat (Copp, 1965). The breeding process of black-grained wheat cultivar was as follows (Sun *et al.*, 1999). A blue-grained hexaploid wheat (allo substitution line "blue 1") was first bred by scientists from Shanxi Academy of Agricultural Science in the 1970s, and then through the breeding effort of 20 years, the scientists bred a purple-grained hexaploid wheat ("purple 12-1"), a blue—purple-grained hexaploid wheat ("blue—purple 114"), and a black-grained hexaploid wheat ("black 76"). The blue 1 was bred by crossing common hexaploid wheat (*Triticum aesticum*) with *Agropyron glaucum*. Purple 12-1 was bred by crossing common hexaploid wheat (*T. aesticum*) with *Elymus dasystachys*. However, blue—purple 114 was bred by crossing blue 1 with purple-grained tetraploid wheat. Finally, black 76 was bred by crossing blue—purple 114 used as the female parent with purple 12-1 as the male parent. Another purple-grained wheat (*T. aestivum*) cultivar was UM 606a (Hard Federation//Chinese Sping/Nero/3/3* Pitic 62) (Dedio *et al.*, 1972), derived from the crosses of "Chinese Spring" with the purple *T. durum* cultivar "Nero" (Piech and Evans, 1979).

In bread wheats (*T. aesticum*), white and red wheats are common, but purple- and blue-colored wheats are rare (Zeven, 1991). Researchers reported on purple-colored bread wheat accessions that had cultivars K-49990, K-55583, and K-59158, derived from the crosses of common bread wheats by "*T. aethiopicum*" (purple-colored tetraploid wheat from Ethiopia) (Zeven, 1991). Although pigments exist in wheat grains at very low concentrations, they substantially influence the quality of wheat products such as bread, pasta, and noodles (Abdel-Aal and Hucl, 2003). Because of the colorful appearance of black, blue, and purple wheat grains, they are currently produced only in small amounts for making specialty foods (Abdel-Aal *et al.*, 2006). However, it is useful to understand the antioxidant properties, qualities, and traits of these colored wheat grains in order to increase their production and use.

QUALITY AND TRAITS

Sun *et al.* (1999) reported on the nutritive composition of black-grained wheat 76 (BGW 76) and the common wheat, Jinchun 9. For example, their nutrient composition was as follows:

crude protein, 20.50% in BGW 76 and 12.90% in Jinchun 9; lipid, 1.60% in BGW 76 and Jinchun 9; carbohydrate, 62.10% in BGW 76 and 71.90% in Jinchun 9; crude fiber, 2.40% in BGW 76 and 2.10% in Jinchun 9; ash, 1.90% in BGW 76 and Jinchun 9; vitamin K, 11.47 mg/kg in BGW 76 and 7.01 mg/kg in Jinchun 9; calcium, 0.56 g/kg in BGW 76 and 0.14 g/kg in Jinchun 9; phosphorus, 4.10 g/kg in BGW 76 and 2.41 g/kg in Jinchun 9; and selenium, 1.04 mg/kg in BGW 76 and 0.26 mg/kg in Jinchun 9. Compared with Jinchun 9, levels of crude protein, crude fiber, vitamin K, calcium, phosphorus, and selenium in BGW 76 were increased by 58.91, 14.29, 63.62, 300, 70.12, and 300%, respectively (Sun *et al.*, 1999). In comparison with common bread wheats, Klasic (hard white wheat), Yecora Rojo (red grain wheat), and Glenlea (Canadian hard red spring wheat), crude protein, ash, and lipid were 17.71, 2.29, and 2.59% in the wholemeal of black-grained wheat; 14.07, 1.62, and 2.52% in the wholemeal of bread wheat Klasic; 13.67, 1.64, and 2.03% in the wholemeal of bread wheat Yecora Rojo; and 14.52, 1.88, and 2.89% in the wholemeal of bread wheat Glenlea, respectively (Li *et al.*, 2004). The flour produced from black-grained wheat also showed the highest crude protein content in comparison with flours prepared from bread wheats Klasic, Yecora Rojo, or Glenlea. The nutritive composition of flour from black-grained wheat and bread wheats Klasic, Yecora Rojo, and Glenlea, respectively, was as follows: crude protein, 18.26, 13.76, 12.59, and 14.23%; ash, 0.95, 0.74, 0.70, and 0.96%; lipid, 1.56, 1.84, 1.31, and 2.18%; and carbohydrate, 75.03, 77.73, 79.32, and 76.03% (Li *et al.*, 2004). Bean *et al.* (1990) reported that protein content ranged from 8.1 to 14.0% in the wholemeal of bread wheat Klasic and averaged 9.5% in its flour. The protein and ash contents were 14.2–15.7 and 1.55–1.64%, respectively, in the wholemeal of bread wheat Yecora Rojo, and they were 12.9–15.1 and 0.64–0.68% in its flour, respectively (Al-Mashhadi *et al.*, 1989).

Campbell (1970) reported that high protein content in grain seed was associated with dark seed color and shattering. Color determination indicated that black-grained wheat had low L^* value compared with bread wheats Klasic, Yecora Rojo, or Glenlea (Li *et al.*, 2004). The L^* value is an indicator of the degree of whiteness; for example, the L^* value was 98.04 for the white body reference and 34.51 for the black body reference. The L^* values of seed, bran, wholemeal, and flour were 38.04, 62.66, 81.74, and 97.34 for black-grained wheat; 64.46, 71.95, 94.69, and 100.77 for bread wheat Klasic; 57.94, 71.95, 90.99, and 100.77 for bread wheat Yecora Rojo; and 55.77, 71.99, 89.39, and 97.90 for bread wheat Glenlea, respectively (Li *et al.*, 2004). Because the L^* value (38.04) of black-grained wheat was very similar to the L^* value (34.51) of the black body reference, the seed color of black-grained wheat was also visually black. The high crude protein content of black-grained wheat appears to be correlated to its black (deep purple) seed color.

The bread making quality of wheat is largely determined by the quantity and quality of the storage proteins of the grain endosperm or prolamins (Wall, 1979). Prolamins consist of glutenins and gliadins. Payne *et al.* (1987) reported on the effects of glutenin subunits and gliadins on bread making quality. It is important to fully understand protein properties of blue-, purple-, and black-colored wheats in order to predict their potential uses. Li *et al.* (2006) reported protein characteristics of black-grained wheat, three common bread wheats—Klasic, Yecora Rojo, and Glenlea—and the common steamed-bread wheat Taifeng. For example, sodium dodecyl sulfate (SDS) sedimentation values of their whole meals decreased in the order bread wheat Glenlea (16.9 ml/g) > bread wheat Klasic (16.5 ml/g) > bread wheat Yecora Rojo (15.0 ml/g) > black-grained wheat (13.3 ml/g) > steamed-bread wheat Taifeng (9.9 ml/g) (Li *et al.*, 2006). The high SDS sedimentation value was associated with strong gluten strength because the SDS sedimentation value had a positive correlation with gluten strength (Dick and Quick, 1983). Gluten index decreased in the order Glenlea (99.37%) > Yecora Rojo (98.88%) > Klasic (98.66%) > black-grained wheat (69.74%) > Taifeng (50.09%) (Li *et al.*, 2006). Therefore, low gluten index value indicated poor strength of wet gluten dough because there was a positive correlation coefficient ($R = 0.9606$) between gluten

index and SDS sedimentation value (Li *et al.*, 1998). For bread making, the optimum gluten index range is between 60 and 90 (Perten, 1990), and the gluten index value (69.74%) of black-grained wheat fell in the optimum gluten index range. Mixograph curves and parameters derived from the curves are also useful tools for indicating good or poor gluten strength of different wheat cultivars. For example, the mixograph parameter midline peak time (MPT) shows the best correlation with gluten strength. A low MPT value is an indication of weak gluten strength. The MPT values of wheat flours in water decreased in the order Glenlea (6.27 min) > Yecora Rojo (6.11 min) > Klasic (5.41 min) > black-grained wheat (2.93 min) > Taifeng (2.26 min) (Li *et al.*, 2006). The order of their MPT values was similar to that of their gluten index. MPT values also indicated that gluten strength of black-grained wheat flour was stronger than that of Taifeng wheat flour, but obviously it was weaker than that of bread wheat flours Klasic, Yecora Rojo, and Glenlea.

In the case of pepsin, *in vitro* protein digestibility (IVPD) of whole meal and gluten at 24 h was 82.42 and 96.45% for black-grained wheat, 82.94 and 95.07% for steamed-bread wheat Taifeng, 87.23 and 95.42% for bread wheat Klasic, 84.43 and 95.37% for bread wheat Recora Rojo, and 84.04 and 94.62% for bread wheat Glenlea, respectively (Li *et al.*, 2006). The wholemeal of Klasic had significantly higher IVPD ($p < 0.05$) than that of black-grained wheat, Taifeng, Yecora Rojo, and Glenlea, but there were no significant differences ($p < 0.05$) in IVPD among their glutens. Total essential amino acid and total amino acid were 4.23 and 15.54% in the flour of black-grained wheat, 3.48 and 13.13% in Taifeng flour, 3.10 and 11.63% in Klasic flour, 2.74 and 10.17% in Yecora Rojo flour, and 3.26 and 12.10% in Glenlea flour, respectively (Li *et al.*, 2006). The increase in total essential amino acid in the flour of black-grained wheat was up to 21.55, 36.45, 54.38, and 29.75% higher than that in the flours of Taifeng, Klasic, Yecora Rojo, and Glenlea, respectively. Similarly, the increase in total amino acid in the flour of black-grained wheat was up to 18.35, 33.62, 52.80, and 28.43% higher than that in the flours of Taifeng, Klasic, Yecora Rojo, and Glenlea, respectively. High total amino acid content in the flour of black-grained wheat was associated with its high crude protein content.

Dough stickiness values of flours decreased in the order steamed-bread wheat Taifeng (392.75 g) > bread wheat Yecora Rojo (313.05 g) > black-grained wheat (223.76 g) > bread wheat Klasic (186.01 g) > bread wheat Glenlea (182.67 g) (Li *et al.*, 2006). A high dough stickiness value indicates stickier dough. Bakery characteristics are poor if the dough is too sticky. The flour from black-grained wheat had better baking properties than that obtained from Taifeng and Yecora Rojo, but its dough stickiness was somewhat stickier than that of flours from Klasic and Glenlea. The baking quality of wheat cultivars can be predicted according to their high-molecular-weight glutenin (HMW-glu) subunits. For example, Taifeng has HMW-glu subunits similar to Anza (2 + 12 and 7 + 8 subunits), Klasic has HMW-glu subunits similar to Yecora Rojo (1, 17 + 18, and 5 + 10 subunits), and black-grained wheat has HMW-glu subunits similar to Glenlea (2^*, 7 + 8, and 5 + 10 subunits) (Li *et al.*, 2006). Because good baking quality is strongly correlated with the presence of 1 and 5 + 10 or 2^* and 5 + 10 HMW-glu subunits and poor baking quality is usually associated with 2 + 12 HMW-glu subunits, the HMW-glu subunits (2^* and 5 + 10) in black-grained wheat predict that black-grained wheat can be classified as bread wheat (Li *et al.*, 2006).

ANTIOXIDANT PROPERTIES

Purple wheat flour bread (PWB) and two bread controls, whole wheat meal bread (WWB) and white flour bread (WFB), were prepared according to the method described by Gélinas and McKinnon (2006), and their antioxidant properties were evaluated. Their 2,2-diphenyl-1-picryhydrazyl free radical (DPPH•) scavenging activity and kinetics are shown in Table 6.1 and Figure 6.1, respectively. Purple wheat bread PWB had the highest DPPH• scavenging activity (47.58%) at 60 min compared to wholemeal bread WWB (34.06%) and white

TABLE 6.1 Free Radical Scavenging Activity of Bread Extracts Reacting with DPPH• (at 60 min)[a]

Bread	DPPH• Scavenging (%)
Whole wheat meal bread	34.06
Wheat white flour bread	32.20
Purple wheat flour bread	47.58

Source: Reprinted from our unpublished data.
[a]*Bread (3 g) was extracted in 10 ml 95% ethanol:1 N HCl (85/15, v/v) at 25°C for 20 h. DPPH• scavenging activity was determined according Li* et al. *(2007a). Results are expressed as mean,* n = *2.*

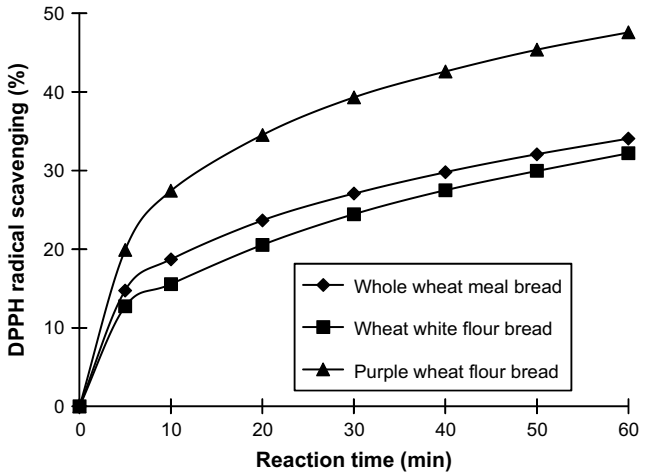

FIGURE 6.1
Radical scavenging activity kinetics of bread extracts reacting with DPPH• for 60 min. Bread (3 g) was extracted in 10 ml 95% ethanol:1 N HCl (85/15, v/v) at 25°C for 20 h. DPPH• scavenging activity was determined according to Li *et al.* (2007a). Results are expressed as mean, $n = 2$. *Source*: Reprinted from our unpublished data.

bread WFB (32.20%). The DPPH• scavenging activity from kinetics curve was also in the order PWB > WWB > WFB (see Figure 6.1). The DPPH• scavenging activity of whole meal in several wheat genotypes was 33.51% for black-grained wheat, 25.57% for purple-grained wheat, 23.66% for blue-grained wheat, and 25.40 % for white-grained wheat (Li *et al.*, 2005). The oxygen radical absorbance capacity (ORAC) of three bread extracts decreased in the same order PWB (12.09 g/kg) > WWB (10.64 g/kg) > WFB (8.88 g/kg) as that of DPPH• scavenging activity (Table 6.2). A high ORAC value is an indication of high antioxidant capacity in sample extract.

Total anthocyanin content in PWB was 78 mg/kg (Table 6.3). Anthocyanin was not detectable in WWB and WFB. Anthocyanins are members of the bioflavonoid phytochemicals, which have been recognized to have health-enhancing benefits due to their antioxidant activity and anti-inflammatory and anticancer effects (Abdel-Aal *et al.*, 2006). The total anthocyanin content of bran, wholemeal, and flour ranged between 415.9–479.7, 139.3–163.9, and 18.5–23.1 mg/kg in blue-grained wheat; 156.7–383.2, 61.3–153.3, and 3.1–14.3 mg/kg in purple-grained wheat; and 9.9–10.3, 4.9–5.3, and 1.5–1.7 mg/kg in red-grained wheat, respectively (Abdel-Aal and Hucl, 2003). Siebenhandl *et al.* (2007) reported total anthocyanin contents of 225.8 and 17.0 mg/kg in the bran and flour of blue-grained wheat and 34.0 and 8.2 mg/kg in the wholemeal and flour of purple-grained wheat, respectively. Hosseinian *et al.* (2008) reported total anthocyanin contents of 500.6 mg/kg in normal purple-grained wheat and 526.0 mg/kg in heat-stressed purple-grained

TABLE 6.2 ORAC Values of Bread Extracts Reacting with 2,2′-Azobis (2-Methylpropionamide) Dihydrochloride[a]

Bread	Equivalent of Trolox (g/kg)
Whole wheat meal bread	10.64
Wheat white flour bread	8.88
Purple wheat flour bread	12.09

Source: Reprinted from our unpublished data.
[a]*Bread (3 g) was extracted in 10 ml 95% ethanol:1 N HCl (85/15, v/v) at 25°C for 20 h. ORAC was determined according to Li et al. (2007a). Results are expressed as mean, n = 2.*

TABLE 6.3 Total Anthocyanin Content of Bread Extracts Determined Using the pH Differential Method[a]

Bread	Equivalent of Cyanidin 3-Glucoside (mg/kg)
Whole wheat meal bread	Not detectable
Wheat white flour bread	Not detectable
Purple wheat flour bread	78

Source: Reprinted from our unpublished data.
[a]*Bread (3 g) was extracted in 10 ml 95% ethanol:1 N HCl (85/15, v/v) at 25°C for 20 h. Total anthocyanin content was determined according to Li et al. (2007a). Results are expressed as mean, n = 2.*

wheat, and they confirmed that cyanidin 3-glucoside was the predominant anthocyanin (103.0 mg/kg) in normal purple-grained wheat. The major anthocyanins were delphinidin 3-glucoside (56.5 mg/kg), delphinidin 3-rutinoside (49.6 mg/kg), cyanidin 3-glucoside (20.3 mg/kg), and cyaniding 3-rutinoside (16.8 mg/kg) in blue-grained wheat (Abdel-Aal *et al.*, 2006) and cyanidin 3-glucoside (19.73−46.44 mg/kg) in purple-grained wheat (Abdel-Aal and Hucl, 2003). Anthocyanins make an important contribution to the antioxidant capacity of black-, purple-, and blue-colored wheats in comparison with that of red and white wheats, which contain only very low levels of anthocyanin (Abdel-Aal and Hucl, 2003).

Total phenolic contents of PWB, WWB, and WFB are shown in Table 6.4. PWB showed the highest total phenolic content, and total phenolic contents decreased in the same order of PWB (1111 mg/kg) > WWB (1005 mg/kg) > WFB (515 mg/kg) as that of DPPH• scavenging activity and ORAC. Gélinas and McKinnon (2006) reported that total phenolic contents (gallic acid equivalent), which ranged from 522 to 866 mg/kg for the wholemeal of organic white wheat varieties, were up to more than 1000 mg/kg for their wholemeal bread and approximately 400 mg/kg for their white bread. Baking slightly increased the level of total phenolic content in bread crust (Gélinas and McKinnon, 2006), likely due to the Maillard reaction. Total phenolic contents (ferulic acid equivalent) were 1973.5 and 811.6 mg/kg in the wholemeal and flour of purple-grained wheat and 7616.4 and 646.5 mg/kg in the bran and flour of blue-grained wheat, respectively (Siebenhandl *et al.*, 2007). The total phenolic contents (ferulic acid equivalent) of bran and wholemeal were, respectively, 2415 and 1108 mg/kg in black-grained wheat, 2290 and 929 mg/kg in purple-grained wheat, 1416 and 706 mg/kg in blue-grained wheat, and 2215 and 817 mg/kg in white-grained wheat (Li *et al.*, 2005). A high correlation ($R = 0.96$) was found between total phenolic content and DPPH• scavenging activity for wheat wholemeals (Li *et al.*, 2005). High total phenolic levels are also an indication of high antioxidant capacity. Because there are differences in phenolic content among wheat genotypes, the level of phenolics in bread will be affected by the raw wheat material ingredients used.

TABLE 6.4 Total Phenolic Content of Bread Extracts Determined Using the Folin–Ciocalteau Method[a]

Bread	Equivalent of Ferulic Acid (mg/kg)
Whole wheat meal bread	1005
Wheat white flour bread	515
Purple wheat flour bread	1111

Source: Reprinted from our unpublished data.
[a]*Bread (3 g) was extracted in 10 ml 95% ethanol:1 N HCl (85/15, v/v) at 25°C for 20 h. Total phenolic content was determined according to Li et al. (2007a). Results are expressed as mean,* n = *2.*

TABLE 6.5 Phenolic Acid Composition of Breads Determined Using the HPLC Method[a]

Phenolic acid	WWB (mg/kg)	WFB (mg/kg)	PWB (mg/kg)
Gallic acid	12	12	13
Protocatechuic acid	Not detectable	Not detectable	20
p-Hydroxybenzoic acid	5	4	7
Vanillic acid	12	8	18
Syringic acid	4	8	7
m-Coumaric acid	2	Not detectable	2
Caffeic acid	8	3	15
p-Coumaric acid	12	9	84
Ferulic acid	250	65	228
Sinapinic acid	8	2	9
Total phenolic acids	313	111	403

Source: Reprinted from our unpublished data.
[a]*Bread (2 g) was hydrolyzed in 60 ml of 4 M NaOH solution at 25°C for 4 h. Phenolic acid composition was determined according to Li et al. (2005f). Results are expressed as mean,* n = *2.*
PWB, purple wheat bread; WFB, white flour bread; WWB, whole wheat bread.

The phenolic acid composition of PWB, WWB, and WFB after hydrolysis is shown in Table 6.5. Ten types of phenolic acid were detected in PWB, 9 types of phenolic acid in WWB, and 8 types of phenolic acid in WFB. Major phenolic acids (>50 mg/kg) were ferulic acid (228 mg/kg) and *p*-coumaric acid (84 mg/kg) in PWB, ferulic acid (250 mg/kg) in WWB, and ferulic acid (65 mg/kg) in WFB. Total phenolic acids decreased in the same order of PWB (403 mg/kg) > WWB (313 mg/kg) > WFB (111 mg/kg) as that of total phenolic content. PWB had 3.63 and 1.29 times higher total phenolic acids than WFB and WWB, respectively. Siebenhandl *et al.* (2007) reported that ferulic acid was 851.7 and 180.1 mg/kg in the wholemeal and flour of purple-grained wheat and 3503.3 and 151.1 mg/kg in the bran and flour of blue-grained wheat, vanillic acid 35.1 and 9.9 mg/kg in the wholemeal and flour of purple-grained wheat and 99.8 and 10.1 mg/kg in the bran and flour of blue-grained wheat, and *p*-coumaric acid 24.3 and 4.6 mg/kg in the wholemeal and flour of purple-grained wheat and 456.6 and 6.3 mg/kg in the bran and flour of blue-grained wheat, respectively. The level of phenolic acids in the wholemeal of soft wheat cultivars ranged from 455.92 to 621.47 mg/kg for ferulic acid, 8.44 to 12.68 mg/kg for vanillic acid, 8.86 to 17.77 mg/kg for syringic acid, and 10.40 to 14.10 mg/kg for *p*-coumaric acid (Moore *et al.*, 2005). Potential health benefits have been demonstrated for phenolic compounds because of their ability to act as antioxidants (Siebenhandl *et al.*, 2007).

TECHNOLOGICAL ISSUES

Problems of quality and technical processes

Protein, flour, and dough quality of purple- and blue-colored wheats need to be evaluated for their potential in making bread products. It is important to breed black-, purple-, and

blue-colored wheats as bread wheat in the future. It is valuable to investigate chemical transformations of phenolic acids and anthocyanins during baking.

Adverse reactions

Although very little is known about adverse reactions to black-, purple-, and blue-colored wheats and their food products, known adverse reactions to wheat include allergies and celiac disease. Different clinical forms of wheat allergy include baker's asthma, atopic eczema/dermatitis syndrome, urticaria, and wheat-dependent, exercise-induced anaphylaxis.

SUMMARY POINTS

- Black-grained wheat contains a high crude protein content compared to that of common bread wheats Klasic, Yecora Rojo, and Glenlea.
- Black-grained wheat can be used as bread wheat for making bread because its HMW-glu subunits (2^*, $7 + 8$, and $5 + 10$) are similar to those found in the bread wheat Glenlea.
- Protein quality of black-grained wheat needs to be improved through breeding technology to increase its gluten strength, which is weak compared with that of common bread wheats Klasic, Yecora Rojo, and Glenlea.
- In addition to containing phenolic acids, black-, purple-, and blue-colored wheats also contain natural anthocyanins, whereas red and white wheats do not.
- Anthocyanins are important antioxidants and therefore likely to be beneficial in improving health and preventing some diseases.
- Anthocyanins and phenolic compounds make a major contribution to the antioxidant capacity of black-, purple-, and blue-colored wheats.
- Purple wheat bread showed the highest antioxidant capacity, with the antioxidant capacity of three bread products decreasing in the order purple wheat bread > whole meal bread > white bread.
- There is potential to use black-, purple-, and blue-colored wheats as novel ingredient resources for the development of value-added products.

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CHAPTER 7

Emmer (*Triticum turgidum* spp. *dicoccum*) Flour and Breads

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CHAPTER OUTLINE

INTRODUCTION

Wheat is one of the more abundant sources of protein and energy and is consumed as bread—a major staple food—in most countries throughout the world. The importance of wheat originates from the properties of wheat gluten, a cohesive network of strong endosperm proteins that stretch with the expansion of fermenting dough yet congeal and hold together when heated to produce a "risen" loaf of bread. The stretchable mass of gluten, with its ability to deform, expand, recover shape, and trap gases, is critical in the production of bread and all fermented products. Of all the cereals, wheat is almost unique in this respect. Throughout the centuries, traditional bread varieties have been developed using the accumulated knowledge of craft bakers regarding how to make the best use of their available raw materials to achieve the desired bread quality. In some countries, the nature of bread making has been preserved in its traditional form, whereas in others it has changed considerably. The flat breads of the Middle East and the steamed breads of China are examples of traditional bread varieties that are still a vital part of the culture of the countries in which they were originally produced and are still baked in large quantities. In contrast, in North America, the arrival of wheat accompanying the influx of settlers and farmers from Western Europe eventually led to the production of new wheat varieties and the rapid industrialization of bread making practices where the maize-based products of the Indians had previously been the main cereal-based foods.

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Evidence indicates that the number of people and the ratio of the global population suffering from micronutrient malnutrition have increased during approximately the past four decades. This alarming trend is believed to be caused by replacement of ancient crop varieties. Modern plant breeding has been historically directed toward high agronomic yield rather than nutritional quality. Increased grain yield may have resulted in a lower density of minerals in grain, although evidence for the traditional bread varieties that still retain is contradictory. Biofortification aimed at enhancement of micronutrient concentrations and its bioavailability in plant foods through genetic improvement is considered to be a vital approach to overcoming the micronutrient malnutrition problem.

The need for crop diversification and the increasing demand for nutritionally healthy food products and their alleged therapeutic properties have led to a renewed interest in ancient wheats such as emmer (*Triticum turgidum* L. spp. *dicoccum* Schrank ex Schübler) and einkorn (*T. monococcum* L.). Wheat (*Triticum* spp.), the basic food to civilization, is a complex, living, dynamic species with many mysteries yet to be uncovered. Its most important food use is in making flour for a variety of products, such as leavened and nonleavened breads, cakes, biscuits, pasta, and noodles. The unique potential of wheat flour dough for bread making is due primarily to the physicochemical properties of the ingredient gluten proteins. Hulled wheats, possessing nonfragile spikes and hulled kernels, comprise species that bridge between cultivated (bread and durum) and wild wheats. Moreover, the hulled kernel types were the earliest to be domesticated almost 10,000 year ago (i.e., dating as far back in time as agriculture itself in the Neolithic Period) and played an important role in the phylogenesis of modern wheats (Nesbitt and Samuel, 1996). All possible wheat polyploidy levels of diploid (2×), tetraploid (4×), and hexaploid (6×) contain hulled wheats. At the tetraploid level, *T. turgidum* spp. *dicoccum* (emmer wheat) is the domesticated form derived from *T. turgidum* spp. *dicoccoides* (wild emmer wheat), from which the *T. turgidum* ssp. *durum* (Desf) Husn. (durum wheat) originated (Figure 7.1). Emmer wheat (*T. turgidum* spp. *dicoccum*, 2n = 4x = 28), einkorn (*T. monococcum* L., 2n = 2x = 14), and spelt (*T. spelta* L., 2n = 6x = 42) represent the three cultivated species belonging to the group of hulled wheats.

The free-threshing hexaploid bread wheat (*T. aestivum* L., AABBDD) in common use today was most likely derived from a hulled hexaploid progenitor that originated from hybridization between the tetraploid emmer wheat (*T. turgidum* spp. *dicoccum*, AABB) and the diploid goat grass (*Aegilops tauschii*, DD) (Arzani *et al.*, 2005). The loss of strong glumes, converting hulled wheat into the free-threshing one, is considered to be an important trait for wheat

FIGURE 7.1
Spikes, spikelets, and kernels of emmer wheat.

domestication. The major genetic determinants of the free-threshing habit are recessive mutations at the *Tg* (tenacious glume) loci, accompanied by modifying effects of the dominant mutation at the *Q* locus and mutations at several other loci (Jantasuriyarat *et al.*, 2004). This characteristic causes the greatest morphological difference between emmer and durum wheats.

Emmer wheat (*T. turgidum* ssp. *dicoccum*) originated more eastward, in the mountains of the Fertile Crescent, an area in the Middle East stretching from Palestine, Jordan, and Lebanon to Syria, Iraq, and Iran (Figure 7.2) where its wild progenitor (*T. dicoccoides* Koern. ex Schweinf.) still thrives (Harlan and Zohary, 1966). As the main wheat in the Old World during the Neolithic and Bronze ages, it played a strategically important role as part of the human diet among the ancient nations, including those of the Babylonians, the Assyrians, and the Egyptians (Figure 7.3). Emmer is still grown in some areas of the Balkans, Italy, Turkey, Iran, Ethiopia, Caucasia, and India. It also spread later in the course of history to Ethiopia on the Abyssinian plateau, where it is still being cultivated and appreciated. However, emmer has never been subjected to breeding programs, and only its landraces and wild forms are currently available.

Consumers are becoming increasingly aware of the benefits of including a variety of cereal grains in their diets. Increased consumption of cereals should spawn consumer interest to seek out breads and products made from cereal grains other than common bread wheat cultivars. The key factor in producing light-texture breads is the gluten quality of the flour. Dough properties and baking performance of wheat are determined by the structure and quantity of gluten proteins, which strongly depend on genotype. Whereas desirable gluten traits have been successfully obtained in common bread wheat, little effort has been applied to other cereal crops.

Increasing interest in natural and organic products has led to the "rediscovery" of emmer on the following grounds:

1. Its food characteristics, which make it especially suitable for preparing many different dishes using whole, pearled, and broken kernels and using flour and semolina to make bread, biscuits, and pasta

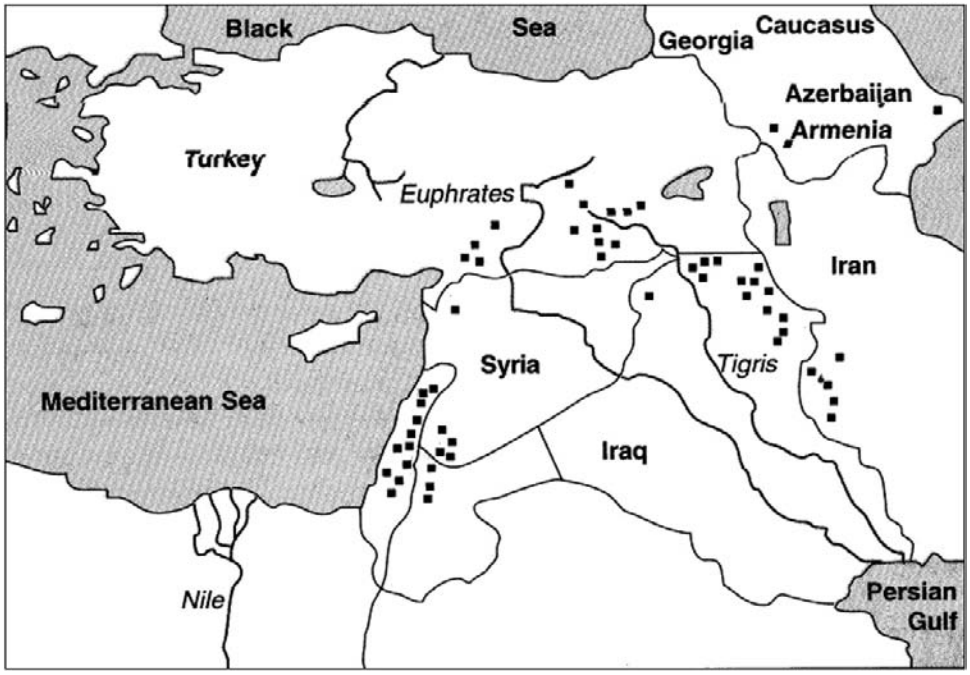

FIGURE 7.2
Growth sites of *Triticum turgidum* ssp. *dicoccoides*, the progenitor of *T. turgidum* ssp. *dicoccum*. *Source: Reproduced with permission from Harlan, J. R., and Zohary, D. (1966). Distribution of wild wheats and barley.* Science *153, 1074–1080.*

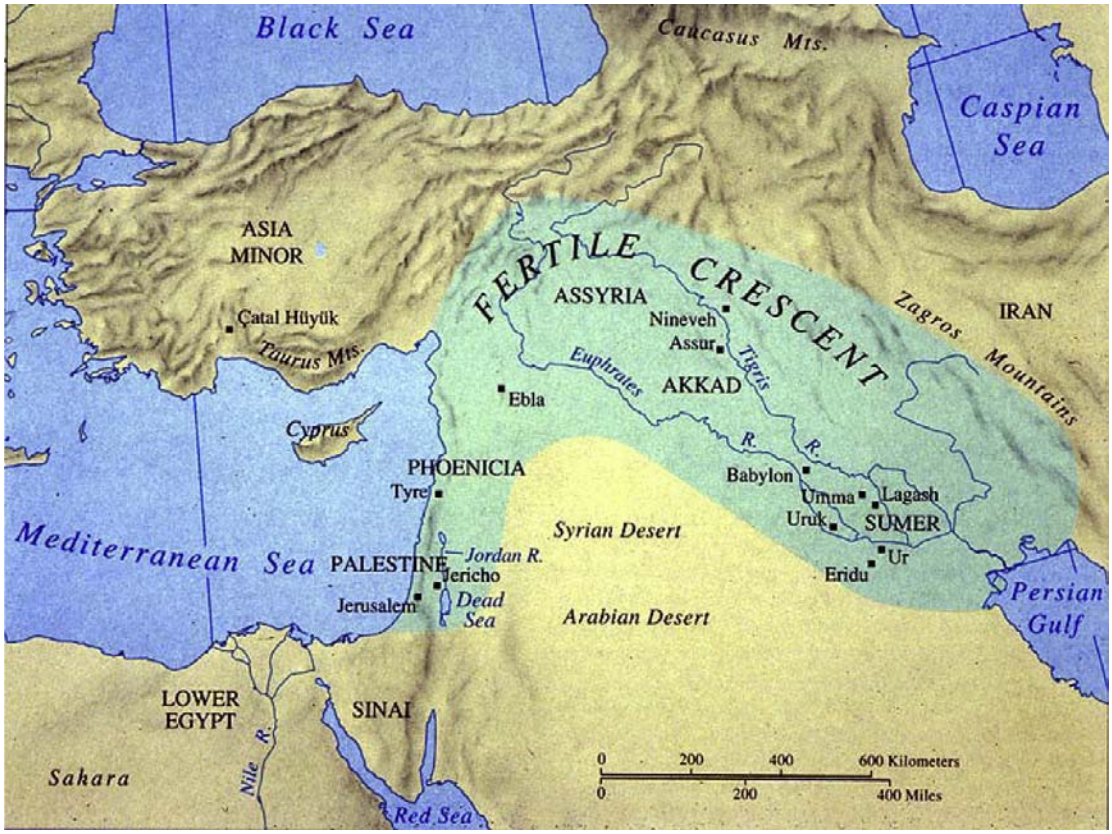

FIGURE 7.3
The Fertile Crescent (shaded area) is known as the domestication site of wheat and includes the Levant and Mesopotamia. It also includes Sumer, the birthplace of the first civilization, and Bronze Age Mesopotamia containing the Sumerian, Akkadian, Babylonian, and Assyrian empires.

2. Its high starch-resistant content and its nutritional and healing effects in the treatment of such diseases as high blood cholesterol, colitis, and allergies
3. Its ability to grow in soils with conventional, low input, and organic crop systems; its superiority for tolerance to both abiotic and biotic stresses such as pest, cold, heat, drought, and salinity
4. It being a potential source of genes for economically important traits in wheat breeding programs

Its cultivation is of significance particularly in the marginal areas of high altitude, where its low input requirements and cold resistance make the crop economically viable.

TYPE OF UTILIZATION

Like einkorn and spelt, emmer is a hulled wheat. In other words, it has tough glumes (husks) that enclose the grains, and it has a semibrittle rachis. On threshing, a hulled wheat spike breaks up into spikelets. Thus, hulled wheats are mainly characterized by the fact that they maintain their glumes adhered to the grain after threshing and by semibrittle rachis. Milling or pounding is required to release the grains from glumes. Cultivation of the crop is associated with complications and challenges related to the harvesting techniques used and the need to dehisce the spikelets to obtain the grain for human consumption.

Emmer is mainly used for human food, but it is also used for animal feed. Although it is principally used in bread making, it is also used in making various dishes, particularly in rural areas. It may be ground into flour and baked into unfermented bread (pancake). It can also be

crushed and milk or water can be added to make soft porridge. Ethnographic analyses based on the taste and texture standards of traditional bread in some emmer-growing areas suggest that emmer makes good bread, and this is supported by evidence of its widespread consumption as bread in ancient civilizations. Emmer bread is widely available in Switzerland and found as pane di farro in bakeries in some areas of Italy. Its use for making pasta is a recent response to the health food market, whereas some judge that emmer pasta has an unattractive texture.

Bulgur (cracked grain) of emmer is also mixed with boiling water and butter to produce hard porridge. Emmer has traditionally been consumed as bulgur whole grains in different kinds of soups worldwide. In some rural areas in Iran and Italy, emmer is also used like rice.

Because it is rich in fiber, protein, minerals, carotenoids, antioxidant compounds, and vitamins, emmer is a complete protein source when combined with legumes, making emmer bread and pasta ideal for vegetarians or for anyone simply wanting a plant-based high-quality protein food source.

BREAD MAKING HISTORY

Bread has a long history and, indeed, will have a long future. Bread is a nourishing food that can be stored and eaten later—a desirable attribute that enabled civilizations throughout history to survive. After ancient citizens initiated farming, they strived to develop tools to process the harvested crops and procedures to cook the grains. The first bread was a type of flat bread and dates back to Neolithic times (New Stone Age), which began in approximately 8000 to 10,000 BC. At that time, bread was produced from emmer and einkorn wheat grains. It consisted of hand-crushed grain mixed with water, which was then laid on heated stone and covered with hot ash. People from Sumeria, in southern Mesopotamia, were the first to bake leavened bread. In approximately 6000 BC, they started to mix sourdough with unleavened dough. Sourdough is generated during the natural yeasting process of flour and water, during which carbon dioxide is formed, which in turn causes the dough to rise.

The Sumerians passed on their style of preparing bread to the Egyptians in approximately 3000 BC. The Egyptians refined the system and added yeast to the flour. Moreover, they developed a baking oven in which it was possible to bake several bread loaves simultaneously. The Egyptians experimented with adding yeasts, which made the dough rise, and the leavened dough created bread that was lighter yet bigger. The successful achievement of wheat loaf bread production by the Egyptians, the Greeks, and the Romans was considered by them as a sign of a high degree of civilization. Nowadays, many different forms of bread are produced throughout the world. Hence, the term "bread" is used to describe a wide range of products with different shapes, sizes, textures, crusts, colors, elasticity, eating qualities, and flavors.

The ancient civilizations initially consumed emmer as a porridge before bread making was developed. With a long history of bread production in diverse cultures, many different types of breads have evolved, and new variations continue to be developed to meet consumer demands for more varied and nutritious foods.

FLOUR AND BREAD FORTIFICATION WITH EMMER

Some of the ancient wheats have a unique composition in secondary components, such as carotenoids and starch, which may play a role as functional food ingredients. Emmer is particularly appreciated for its content of resistant starch, fiber, carotenoids, and antioxidant compounds (D'Antuono *et al.*, 1998; Galterio *et al.*, 2003; Serpen *et al.*, 2008). Although

emmer flour does produce a satisfactory loaf of bread, the quality is not as good as that of bread made with common wheat. Galterio *et al*. (1994) reported a high lysine content (3.1%) for emmer grains. In contrast, it is known that common wheat protein is deficient in lysine. The poor gluten quality of emmer is confirmed by its low gluten index value (Cubadda and Marconi, 1996; Galterio *et al*., 1994). Konvalina *et al*. (2008) reported that emmer has a high grain protein content, whereas the quality of its gluten is inferior to that of bread wheat as determined by the gluten index and the Zeleny test. It is assumed that the poor gluten quality of emmer is due to its storage protein composition, which is dominated by high concentrations of the intermediate-molecular-weight glutenin group (78−50 kDa), poor synthesis of low-molecular-weight glutenin subunits (45−30 kDa), and the absence of gliadin fractions γ-42 and γ-45 (Galterio *et al*., 1994, 2000). The potential use of emmer flour in bread making could be more promising if it is used in blends with bread wheat flour. Consequently, the high lysine and low gluten content of emmer wheat could complement those of wheat flour, which is poor in lysine but rich in gluten content.

During the past few decades, increasing attention has been paid to phytonutrients, which have been shown to significantly reduce the incidence of aging-related and chronic human diseases. Among the numerous antioxidant compounds present in foods, lipid-soluble antioxidants play a vital role in disease prevention. Interestingly, the natural antioxidant activity of these compounds might complement their positive functional characteristics in maintaining freshness and shelf life of the food products. Wheat, as the major staple food for humans, is not only a source of energy and protein but also a valuable source of such antioxidant compounds. In bread wheat, however, the concentration of carotenoids and other antioxidants is low, but they are more abundant in emmer wheat.

In wheat, whole grain flour and its bran fraction are a reliable source of fiber, especially the water-insoluble type (Ranhotra, 1994). In contrast, white flour is not rich in total fiber, but it is relatively rich in soluble fiber. Epidemiological studies reveal a strong relationship between low fiber intake and many disease conditions, particularly those of the gastrointestinal tract (Birdsall, 1985). In developing countries, it is believed that the large amount of plant fibers consumed by people from rural areas protect them against many diseases common to people from urban areas, such as cardiovascular diseases, colon cancer, diverticulae, appendicitis, hemorrhoids, and varicose veins of the legs. Research relates the high-fiber diets to decreased blood pressure in normal as well as in hypertensive subjects (Birdsall, 1985). For elevated blood serum lipids, dietary recommendations include increasing carbohydrate consumption to make up 65% of total daily calories, emphasizing complex carbohydrates from natural sources because they influence the absorption of fat-soluble substances from the digestive tract, and the reabsorption of bile acids and neutral sterols. These recommendations are also given to diabetics because cardiovascular diseases are their most likely cause of death (Anderson *et al*., 1990). Therefore, there is increasing evidence that high-fiber diets, especially those containing cereal fibers, have definite health benefits in reducing the risk of chronic diseases such as diabetes, cancer, and coronary heart disease. A diet rich in complex carbohydrates improves glucose metabolism in diabetic subjects by increasing their sensitivity to insulin, resulting in reduced dosage requirements (Birdsall, 1985). Moreover, a high-fiber diet is positively associated with the control of obesity and physical gastrointestinal tract disorders. Accordingly, consumers will be interested in utilizing functional cereal products that will enhance their heath and help them to avoid becoming overweight. High-fiber cereal products will thus be asked for and undoubtedly consumed. However, as always with food items, the major criteria for consumer acceptability are good flavor and texture in cereal products. In other words, consumers expect functional cereal products such as high-fiber breads to have at least similar good quality features as those in standard wheat bread. Emmer flour can thus fully or partially substitute wheat flour in bread products to exploit the advantages of the higher fiber content of emmer wheat.

IMPACT OF EMMER ON COMBATING MALNUTRITION

Considering the increasing requirements for richness, diversity, and good quality of food products, interest in emmer wheat is greater than ever (Marconi and Cubadda, 2005). Perrino *et al*. (1993) found high mean values of protein (17.1%) in grains of 50 emmer accessions. It is also believed that the gluten structure of emmer differs from that of modern wheat so that people with gluten allergies can safely use it without any adverse effects.

Improvement in dietary quality may be the ultimate solution to micronutrient malnutrition in developing countries, which affects billions of people and is basically the consequence of extensive consumption of staple cereals with low quantity of available micronutrients (Bouis, 2003; Cakmak, 2008). Micronutrient malnutrition is a great concern worldwide. Currently, enrichment of staple food crops with mineral nutrients is a high-priority research area as a temporary solution, and the major strategy to improve the level of mineral nutrients is to exploit the natural genetic variation in grain concentration of micronutrients in food crops. With the exception of calcium, modern wheat cultivars with a greater yield potential possess lower grain concentrations of mineral nutrients than the old cultivars with a lower yield (Murphy *et al*., 2008). Hence, modern wheat germplasm cannot contribute the genetic potential to the development of new genotypes with higher levels of mineral nutrients. On the other hand, current literature indicates that primitive wheats, such as *T. monococum* (einkorn wheat) and *T. turgidum* spp. *dicoccum* (emmer wheat), contain the germplasm for improving grain micronutrient concentrations (Genc and McDonald, 2008; Ortiz-Monasterio and Graham, 2000).

FUTURE DIRECTION OF RESEARCH

The danger of genetic erosion of crop plants and the potential consequences for agriculture are evident when their wild and primitive progenitors are considered throughout plant domestication and subsequent breeding. The challenge is to exploit the mostly unrealized potential of ancestral species as a component of sustainable crop production, particularly under less favorable environmental conditions. Therefore, the major task of modern breeders is not only to identify, in the primitive ancestors of crop plants, valuable and outstanding traits and introduce them into cultivated crops but also to undertake genetic improvement projects that address the crops, particularly the domesticated ones. Lage *et al*. (2006) demonstrated that genetic variation for quality in tetraploid emmer wheat could be transferred to synthetic hexaploid wheats and combined with plump grains and high grain weight to be used for bread wheat improvement.

Like other ancient kinds of wheat, emmer is high in protein, fiber, minerals, and phytochemicals. It is also considered to be very valuable in breeding programs for improving wheat cultivars for a higher concentration and a better composition of health-beneficial phytochemicals. In particular, research on the genetic diversity of nutritional and health-beneficial properties of emmer should be carried out to exploit its potential in breeding programs and to improve the quality of both emmer and bread wheats. By collaborating with the private sector (millers), modern emmer products that suit the tastes of urban consumers can be developed either by blending flour of emmer and common wheats or by utilizing the flour of emmer *per se*. Diversification in emmer products in the flour industry can be promoted through models in countries such as Italy that successfully produce emmer products.

Emmer, ancient hulled wheat, was one of the first cereals ever domesticated in the Fertile Crescent. Emmer grain holds the characteristics of two wild wheats (including wild Einkorn) and is known to have been the primary wheat grown in Asia, Africa, and Europe through the first 5000 years of recorded agriculture. Throughout the centuries, however, emmer was gradually abandoned in favor of hull-less varieties of durum and bread wheats. By the beginning of the twentieth century, higher yielding wheat cultivars had replaced emmer almost everywhere.

TABLE 7.1 Whole Grain Flour Composition of Emmer (*Triticum turgidum* spp. *dicoccum*), Durum (*T. turgidum* spp. *durum*), and Hard and Soft Common (*T. aestivum*) Wheats[a]

	Emmer	Durum	Hard Common	Soft Common
Protein (g)	12.5	12.8	14.8	13.9
Fat (g)	2.4	1.6	1.7	1.6
Total carbohydrate (including fiber) (g)	71	69.5	69.7	70.7
Fiber (g)	2.7	2.4	2.6	2.5
Ash (g)	1.8	2.1	1.6	1.5
Calcium (g)	38	48	55	54
Phosphorus (g)	360	300	317	275
Iron (mg)	4.7	—	8.2	6.5
Moisture (g)	12.3	14	12.2	12.3

Source: Food and Agriculture Organization, Corporate Document Repository (http://www.fao.org/documents).
[a]*All values expressed as per 100 g of whole grain flour.*

Wheat is the most widely grown crop and has traditionally been selected for its technological functionality, resulting in the selection of hard bread wheat (*T. aestivum* L.) cultivars with a high level of strong gluten proteins or of durum wheat (*T. turgidum* ssp. *durum*) with the yellow-colored pasta products. However, little interest has been devoted to the nutritional and favorable health properties of grains and improvement through breeding programs (Leenhardt *et al.*, 2006). Table 7.1 compares the whole grain flour composition of four groups of wheat—emmer, durum, and bread (hard and soft) wheat—based on the means of the tested genotypes within each group. Despite the great interspecific variations observed for the nutritional values of the grain in *Triticum* spp., large intraspecific variations are also observed for the traits in this genus. Evidence from clinical and epidemiological studies implies that a diet high in whole grains may have a protective role in reducing the risk of coronary heart disease (Behall *et al.*, 2006; Jacobs *et al.*, 2002), type 2 diabetes (Fung *et al.*, 2002; Montonen *et al.*, 2003), age-related eye diseases, and certain types of cancer (Chatenoud *et al.*, 1998; Kasum *et al.*, 2001). Health-advantageous properties of whole grain flour of wheat have been attributed to the levels of natural antioxidants, including flavonoids, phenolic acids, phytic acids, tocopherols, and carotenoids (Moore *et al.*, 2005; Mpofu *et al.*, 2006).

Limited information is available on the use of emmer flour as a partial substitute for wheat flour in bread production based on physicochemical and rheological properties of dough and bread, and further research is required to address this issue.

SUMMARY POINTS

- The danger of genetic erosion in wild species and primitive forms of crop plants and the associated likely consequences for agriculture reinforce the need for future exploitation of the unrealized potential of ancestral species such as *T. turgidum* spp. *dicoccum* (emmer wheat) for the improvement of grain protein, fiber, minerals, and phytochemicals in durum and bread wheats.
- Increasing interest in natural and organic products has led to an emmer "rediscovery" not only for its nutritional and health properties but also because it is amenable to low input and organic farming systems.
- Emmer flour can fully or partially substitute wheat flour in most bakery products, such as bread and pasta. Modern cooks are rediscovering the full flavors, textures, and nutrition of whole grain emmer pasta and bread, while they are also exploring new ideas such as adding emmer grains to dishes such as soups.

- Further research is needed to elucidate the physical, chemical, and nutritional properties of emmer grain and to address its beneficial health effects.
- Emmer's superiority for tolerance to environmental and biotic stresses such as pests, pollution, cold, heat, drought, and salinity could help farmers to sustainably manage harsh environments and to meet their subsistence needs without depending on mechanization, chemical fertilizers, pesticides, or modern agricultural technologies.

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CHAPTER 8

Einkorn (*Triticum monococcum*) Flour and Bread

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

d.m. Dry matter
SDS Sodium dodecyl sulfate

INTRODUCTION

Einkorn (*Triticum monococcum* L. subsp. *monococcum*), a close relative of durum and bread wheats, is a diploid ($2n = 2x = 14$) hulled wheat domesticated approximately 10,000 years ago in the Karacadağ region of Turkey. Einkorn, one of the founder crops of agriculture along with barley and emmer, spread to Europe during the Neolithic Revolution. For several thousand years, it was the staple food of European farmers, as confirmed by archeological remains and by the colon content analysis of Ötzi (a.k.a. the Similaun Iceman), a frozen Copper Age human body found in the Alps in 1991. Starting with the Bronze Age, its cultivation lost momentum because of the new availability of higher yielding, free-threshing tetraploid and hexaploid wheats. However, it was still consumed in Roman times, as well as in the ensuing "Dark Ages."

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Nowadays, traditional einkorn crops may still be found in marginal mountain areas of the Mediterranean region, Turkey, Balkan countries, southern Italy, southern France, Spain, and Morocco, whereas its wild progenitor, *T. monococcum* subsp. *boeoticum*, thrives in the central and eastern parts of the Fertile Crescent. The grains are often used for animal feeding, and the straw is employed in the construction of thatched roofs. However, in recent years the trend toward low-impact and sustainable agriculture, coupled with a stronger interest in the nutritional aspects of food, led to the rediscovery of several 'ancient' cereals, including einkorn. New research projects are assessing *T. monococcum's* potential for human consumption in terms of both nutritional and technological quality; meanwhile, breeding programs for the constitution of new einkorn lines with high yielding ability, free threshing, and that are suitable for modern cropping practices are ongoing in several countries, including Italy, Germany, and Canada.

FLOUR COMPOSITION

Wholemeal flour

Seed size has a marked influence on many compositional and qualitative traits because big, heavy kernels have a higher proportion of starchy endosperm and smaller amounts of the external pericarp and aleurone layers. Einkorn germ proportion is only marginally superior to that of bread wheat (3.1 vs. 2.9%, respectively); sharp differences are instead observed for bran (22.9 vs. 16%) and endosperm (74.0 vs. 81%) (Hidalgo and Brandolini, 2008a). The higher bran fraction of einkorn is related to its smaller seeds (Borghi *et al.*, 1996; Løje *et al.*, 2003), whose average weight is 25–28 g/1000 kernels (Brandolini *et al.*, 2008); however, some genotypes reach 34.9 g/1000 kernels (Brandolini *et al.*, 2008), a value in the lower end range of bread wheat (Gebruers *et al.*, 2008). Einkorn spikes, hulled seeds, and threshed seeds are depicted in Figure 8.1.

PROTEINS

Triticum monococcum kernels show a protein content sharply superior to that of bread wheat (Borghi *et al.*, 1996; Corbellini *et al.*, 1999), averaging 18 g/100 g dry matter (d.m.) and often exceeding 20 g/100 g d.m. (Table 8.1) (Brandolini *et al.*, 2008). Although part of this superiority is linked to reduced seed size (and the related higher proportion of aleurone), the endosperm is also a good source of protein. Genes play a significant role, as evidenced by the broad within-einkorn variation (15.5–22.8 g/100 g; Brandolini *et al.*, 2008) as well as by the identification of two quantitative trait loci controlling total protein content (Taenzler *et al.*, 2002). The amino acid composition of *T. monococcum* seed proteins and the nutritional

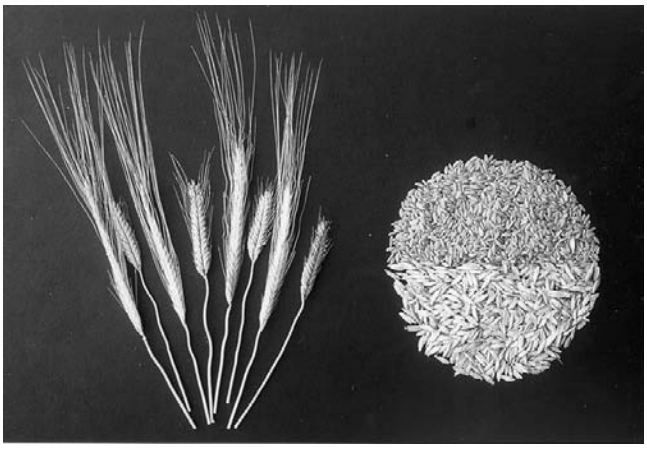

FIGURE 8.1
Einkorn spikes, hulled seeds, and threshed kernels.

TABLE 8.1 Einkorn Composition[a]

Compound	Mean	Range
Protein (g/100 g)[b]	18.2	15.5–22.8
Lipid (g/100 g)[c]	4.2	4.0–4.4
Starch (g/100 g)[b]	65.5	60.6–71.4
Amylose (g/100 g starch)[b]	25.7	23.2–28.6
Carotenoids (mg/kg)[d]	8.4	5.3–13.6
Tocols (mg/kg)[d]	78.0	61.5–115.9
Ash (g/100 g)[b]	2.3	2.1–2.8
Zinc (mg/kg)[e]	54.8	42.7–71.1
Iron (mg/kg)[e]	47.0	37.2–62.6
Manganese (mg/kg)[e]	49.3	34.4–68.2
Copper (mg/kg)[e]	6.4	4.9–8.3

[a]*Principal nutritional characteristics of einkorn wholemeal flour.*
[b]*Modified data from Brandolini et al. (2008); 65 einkorn accessions tested.*
[c]*Modified data from Hidalgo, Brandolini, and Ratti (2009); 5 einkorn accessions tested.*
[d]*Modified data from Hidalgo et al. (2006); 57 einkorn accessions tested.*
[e]*Modified data from Özkan et al. (2007) and Özkan (personal communication); 54 einkorn accessions tested.*

adequacy are very similar to those of polyploid wheats; nevertheless, when adjusted to a common protein level (16.7%), the essential amino acid content is slightly superior in einkorn than in bread wheat cv. Centauro (on average, 32.2 vs. 29.1% of total protein, respectively) (Acquistucci *et al.*, 1995).

LIPIDS

Although the relative proportions of einkorn and common wheat germs are similar, einkorn has a lipid content 50% higher than that of bread wheat (4.2 vs. 2.8 g/100 g d.m., respectively) (Hidalgo, Brandolini, and Ratti, 2009). The analysis of fatty acid composition distinguishes up to 14 different compounds (Table 8.2). Linoleic (C18:2*n*6), oleic (C18:1*n*9 + C18:1*n*7), and

TABLE 8.2 Fatty Acids of Einkorn Wheat

Fatty Acid		Mean[a]	Range
Myristic	C14:0	0.53	0.49–0.61
	C15:0	0.12	0.11–0.13
Palmitic	C16:0	16.65	16.07–17.73
	C16:1*n*9	0.09	0.09–0.10
Palmitoleic	C16:1*n*7	0.18	0.16–0.20
Margaric	C17:0	0.11	0.10–0.13
Stearic	C18:0	1.18	1.10–1.26
Oleic	C18:1*n*9[b]	24.77	23.23–26.51
Linoleic	C18:2*n*6	50.86	49.89–51.47
Arachidic	C20:0	0.18	0.15–0.19
Gadoleic	C20:1*n*11	2.75	2.47–3.04
Linolenic	C18:3*n*3	1.95	1.82–2.08
Behenic	C22:0	0.23	0.19–0.25
Lignoceric	C24:0	0.41	0.37–0.45
SFA		19.4	18.7–20.6
MUFA		27.8	26.3–29.2
PUFA		52.8	51.9–53.3

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.
[a]*Mean (and range) relative percentages of fatty acids in wholemeal flour of five einkorn genotypes.*
[b]*(C18:1n9+ C18:1n7).*

palmitic (C16:0) acids are by far the most abundant compounds, with mean relative percentages of 50.9, 24.8, and 16.7%, respectively. In bread wheat, linoleic acid is also the prevalent fatty acid, but palmitic acid is more abundant than oleic acid, whereas the levels of other fatty acids are similar between species. Consequently, einkorn lipids have higher monounsaturated fatty acids and lower polyunsaturated fatty acids and saturated fatty acids than bread wheat (Hidalgo, Brandolini, and Ratti, 2009).

VITAMINS AND ANTIOXIDANTS

The concentration of folate, a water-soluble form of vitamin B_9 important also in the prevention of neural tube defects in the fetus, was 429−678 ng/g d.m. in a group of five einkorns—that is, in the same range of 150 bread wheat cultivars (323−774 ng/g d.m.) (Piironen *et al.*, 2008).

Lipophilic (carotenoids and tocols) and hydrophilic (phenols, flavonoids, and lignans) antioxidants from fruits, vegetables, and grains contribute to lowering the frequency of aging-related and chronic diseases.

Several researchers, noticing the yellow tinge of einkorn flour, analyzed pigment contents and observed carotenoid values highly superior to those of the polyploid wheats (Borghi *et al.*, 1996; Corbellini *et al.*, 1999; D'Egidio *et al.*, 1993). More detailed study of the yellow pigment, performed by high-performance liquid chromatography, found that it consisted mostly of the lutein (>90%) in all *Triticum* species (Abdel-Aal *et al.*, 2002). In wholemeal einkorn flours, the lutein content average is approximately 8.4−8.5 mg/kg (Abdel-Aal *et al.*, 2002; Hidalgo *et al.*, 2006); this value is approximately four to eight times higher than that of bread wheat and twice that of durum wheat, in which the yellow color of the semolina, and the derived pasta, is perceived as an important quality trait. Interestingly, Hidalgo *et al.* (2006) found several accessions with carotenoid values greater than 10 mg/kg, up to a maximum of 13.64 mg/k d.m. (see Table 8.1). Furthermore, several accessions showed significant amounts of carotenes (>25% of total carotenoids), sometimes together with high lutein content.

Tocols (vitamin E) consist of two classes, tocopherols and tocotrienols, each including four derivatives (α, β, γ, and δ). The total tocol content of einkorn is higher than that of bread and durum wheats, with an average concentration of 77.96 mg/kg d.m. and a maximum value of 115.85 mg/kg d.m. (see Table 8.1); in the same study, tocol content of several *Triticum aestivum* and *Triticum turgidum* samples was approximately 62.75 and 52.91 mg/kg d.m., respectively (Hidalgo *et al.*, 2006). In *T. monococcum*, β-tocotrienol (61.9% of total) is the most abundant compound, followed by α-tocotrienol (16.4%), α-tocopherol (15.6%), and β-tocopherol (6.1%). The mean tocotrienol:tocopherol ratio is 3.68, which is higher than those of *T. aestivum* and *T. turgidum* (2.97 and 1.79, respectively).

Phenolic acids are present in soluble free, soluble bound, and insoluble bound forms. Insoluble bound phenolics, linked to cell wall structural components such as cellulose, lignin, and proteins, are more abundant than the soluble forms. In wheat, ferulic acid is the main phenolic component of both the soluble fraction and the insoluble fraction. The information on phenolic acids in einkorn is scant but points to a lesser concentration than in bread wheat. According to Serpen *et al.* (2008), *T. monococcum* shows a total phenolics content inferior to *T. aestivum* (3.37 and 4.36 μmol/g, respectively). This finding is corroborated by the results of Lavelli *et al.* (2009): the soluble phenolics in wholemeal extracts vary in the ranges 212−453 and 331−488 mg/kg d.m. (as gallic acid equivalents) for *T. monococcum* and *T. aestivum*, respectively. Flavonoid content in six einkorn accessions, instead, averaged 1.13 ± 0.28 μmol/g (range, 0.80−1.59 μmol/g) versus 1.32 ± 0.04 μmol/g for bread wheat (Serpen *et al.*, 2008).

As a result of its peculiar antioxidant content and composition, radical scavenging activity of einkorn wholemeal flour is superior to that of bread wheat wholemeal flour, as observed by Lavelli *et al.* (2009) and Serpen *et al.* (2008).

STARCH AND DIETARY FIBER

Cereal endosperm, which accumulates storage products, is mostly starchy. The total starch content of *T. monococcum* is 65.5% (range, 60.6–71.4; see Table 8.1), whereas in the endosperm-richer *T. aestivum* the value is higher (68.5%) (Brandolini *et al.*, 2008). The amylose fraction, which has a special role with regard to the pasting properties of the flour and the shelf life of bread, is approximately 26% (range, 15–35%) (Brandolini *et al.*, 2008; Mohammadkhani *et al.*, 1998).

Not all starch is rapidly assimilated during digestion; the fraction that resists digestion and absorption in the human small intestine has been defined as resistant starch and has physiological functions similar to those of dietary fiber. *Triticum* species in general have low resistant starch contents, and einkorn has approximately half that of bread wheat (Brandolini *et al.*, in press).

Arabinoxylans and β-glucans, the principal components of endospermatic cell walls, are found in limited quantities in einkorn. Gebruers *et al.* (2008) sampled five einkorns and 151 bread wheats and observed a total arabinoxylan content of 1.45–2.35 versus 1.35–2.75% and a β-glucan content of 0.25–0.35 versus 0.50–0.95%, respectively. Similar results for β-glucan were also obtained by Grausgruber *et al.* (2004) and Løje *et al.* (2003). On the other hand, fructan content was significantly higher in *T. monococcum* (1.90 g/100 g) than in *T. aestivum* (1.29 g/100 g; Brandolini *et al.*, unpublished results). Although such values might seem quite low, in fact wheat already provides approximately 70% of fructans in the U.S. diet, indicating that even minimal changes could induce noticeable improvements in wheat-consuming countries.

Overall, *T. monococcum* is reported to have little total dietary fiber. Values inferior to 10% are reported by Grausgruber *et al.* (2004) and Løje *et al.* (2003), whereas Gebruers *et al.* (2008) describe a variation from 9.3 to 12.8% in einkorn and from 11.5 to 18.3% in bread wheat.

MICROELEMENTS AND ASH

Cereals and cereal-based foods are extensively consumed worldwide, and as such they represent the primary source of iron and zinc for humans in many developing countries. However, cereals are inherently poor in both concentration and bioavailability of zinc and iron, leading to a low intake of these micronutrients: Approximately half of the world population suffers from micronutrient deficiencies. Iron and zinc deficiencies are responsible for health problems such as impairment of the immune system; disturbed physical growth and mental and cognitive development; and increased rates of anemia, morbidity, and mortality. Some researchers have evaluated microelement variation in the cultivated wheat gene pool as a way to improve the nutritional value of cereals. Özkan *et al.* (2007) analyzed seeds of 54 accessions of einkorn and detected a large genotypic variation, with mean values of 47.04 mg/kg for iron, 54.81 mg/kg for zinc, 49.29 mg/kg for manganese, and 6.40 mg/kg for copper. Zhao *et al.* (2009) screened 5 einkorns and 150 bread wheats; they observed broad variability for all traits and found that *T. monococcum* averaged better content than *T. aestivum* for iron (45.9 vs. 38.2 mg/kg, respectively) and selenium (279 vs. 99 μg/kg, respectively). Not surprisingly, the total ash content of einkorn wholemeal is high, ranging from 2.1 to 2.8% (Brandolini *et al.*, 2008; D'Egidio *et al.*, 1993; Løje *et al.*, 2003); in comparison, the mean ash content of bread wheat is less than 2.0%.

White flour

The distribution of nutrients varies considerably in wheat fractions. The bran has high levels of minerals, proteins, and some antioxidants such as tocotrienols and phenolics. The germ is particularly rich in proteins, lipids, and tocopherols, whereas the starchy endosperm is abundant in storage proteins (mostly gliadins and glutenins) and carbohydrates. Notwithstanding the lower concentrations, the endosperm contributes the greatest amount of protein (66.4%) and lutein (76.6%), as well as one-fourth of total tocols, to the whole kernel (Hidalgo and Brandolini, 2008a), thus indicating that for these traits the white flour still retains most of

the nutritional value of the whole kernel. For example, protein content in white flour of 24 einkorn accessions cropped in three different locations ranged from 15.4 to 25.2%; of 62 samples examined, 17 presented a protein content greater than 20% (Corbellini *et al.*, 1999). Similar results have been reported by Borghi *et al.* (1996). An additional way to retain the high nutrient levels of wholemeal flour in white flour is the use of seed parboilization before milling because parboilization inactivates enzymes and favors the diffusion of vitamins and minerals throughout the grain, altering their concentration among its fractions. In einkorn, the steaming process induced migration of lutein and tocopherols from the bran and germ fractions to the kernel endosperm (Hidalgo *et al.*, 2008).

Several factors influence the post-milling nutritional value of wholemeal and white flours, including storage conditions and technological transformations. Low conservation temperatures preserve over long periods the antioxidant properties of freshly milled flours, which are instead quickly lost under more exacting conditions, such as storage at higher than 20°C (Hidalgo and Brandolini, 2008b; Hidalgo, Brandolini, and Pompei, 2009).

TECHNOLOGICAL ISSUES

During its heyday, *T. monococcum* was mainly eaten as porridge or plainly cooked; this type of use did not require leavening, a processing step discovered and employed for bread making by the Egyptians. In more recent times, einkorn was preferentially fed to animals, keeping for humans the easily threshable durum and common wheats. Therefore, during the prehistory no selection whatsoever favoring bread making attitude was exerted on einkorn, and a similar trend was probably maintained even in more recent times. As a result, until recently einkorn was deemed unsuitable for bakery products because of its sticky dough and poor rheological properties. Nevertheless, and despite dough handling difficulties, D'Egidio and Vallega (1994) were able to obtain some breads with loaf volumes and characteristics similar to those of bread wheat. Afterwards, the screening of a broad collection (>1000 accessions) of *T. monococcum* ssp. *monococcum* and ssp. *boeoticum* accessions led to the identification of several wholemeal samples (approximately 16% of the total) having sodium dodecyl sulfate (SDS) sedimentation values greater than 60 ml (Borghi *et al.*, 1996), which is the threshold value for bread making potential. SDS sedimentation is a small-scale test for evaluating the baking attitude of wheat flours. Further analysis of the most promising genotypes showed alveograph values and farinograph stability indices similar to those of bread wheat (Borghi *et al.*, 1996; Corbellini *et al.*, 1999), thus opening new horizons for the use of this ancient crop. The breads prepared from einkorn white flour following standard micro-baking tests showed a broad volume variation, ranging from very poor to outstanding (Figure 8.2); the finest samples compared favorably with the best bread wheats (Borghi *et al.*, 1996; Corbellini *et al.*, 1999). The characteristic that differentiates the breads prepared with *T. monococcum* from those prepared with *T. turgidum* or *T. aestivum* is the appealing, deep-yellow color of einkorn crumb. Whereas only some einkorn genotypes are suitable for bread making, all the accessions show excellent attitude for the preparation of other bakery products, such as biscuits and pastry (Figure 8.3).

FIGURE 8.2
Einkorn breads. Bread loaves prepared from white flour of einkorn (from left, cv. Monlis, accessions ID1395 and ID331, and advanced lines SAL 98-38 and SAL 98-32) and bread wheat (cv. Blasco, right).

FIGURE 8.3
Einkorn bakery products. Bakery products prepared from einkorn flour. From left to right: einkorn flour and seeds (foreground), bread and cake (center), and biscuits and pasta (background).

Bread making quality is strictly related to storage protein composition. Electrophoretic analysis of 668 *T. monococcum* ssp. *monococcum* accessions detected 39 glutenin bands (6 *x* subunits and 8 *y* subunits in the high-molecular-weight fraction and 25 in the low-molecular-weight region) and 44 gliadin bands (20 in the ω region, 10 in the γ region, and 14 in the α/β region) (Brandolini *et al.*, 2003). Eight glutenin and 8 gliadin bands correlated significantly with an increase in bread making quality, as assessed by the SDS sedimentation test. Particularly relevant was the effect of three linked glutenin fragments that, coupled with reduced or absent ω gliadins (Figure 8.4), improved the SDS sedimentation volume of wholemeal from 24 to 45 ml. A molecular map, integrating restriction fragment length polymorphism, acid polyacrylamide gel electrophoresis, and storage protein information, positioned the loci for the three glutenin and the ω bands on the short arm of chromosome 1 in a region rich in glutenin and gliadin coding genes (Taenzler *et al.*, 2002).

Dough stickiness and leavening as well as end product and staling kinetics are also influenced by the pasting properties of the flour. Einkorn amylographic viscosity values are superior to those of spelt and common wheat and, in most instances, also emmer (Løje *et al.*, 2003). A study of the rapid viscosity analyzer pasting properties of 65 einkorn accessions of different geographical origin showed that einkorn has higher peak viscosity and final viscosity than *T. turgidum* and *T. aestivum* (Brandolini *et al.*, 2008). The differences are probably related to the smaller size and different grading of einkorn starch granules as well as to the lower amylose percentage of einkorn flour.

Some external factors show a relevant influence on bread making. Parboilization leads to gelatinization of starch granules and denaturation of proteins, thus inducing major changes in the technological properties of the flour. The limited information available for einkorn describes a steep decline, after low-moisture parboilization, in SDS sedimentation values and thus in bread making quality, as well as a decrease in viscosity; the changes are stronger under more drastic steaming conditions (Hidalgo *et al.*, 2008). Storage conditions also modify the

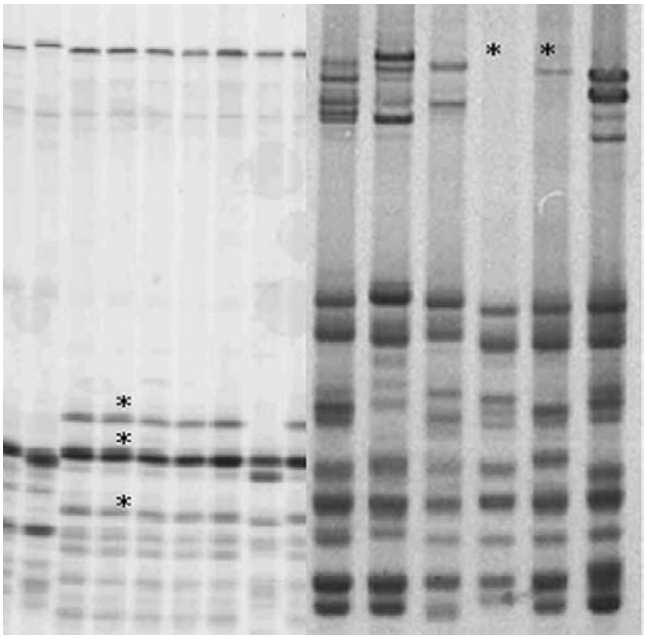

FIGURE 8.4
Einkorn storage proteins. Fingerprinting of einkorn storage proteins. (Left) Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the glutenin fraction: The asterisks indicate the three bands correlated to bread making quality. (Right) Acid polyacrylamide gel electrophoresis of the gliadin fraction: The asterisks indicate the scarce or absent ω-gliadin bands correlated to bread making quality.

technological characteristics of the flours: SDS sedimentation values and viscosity of einkorn flour change during conservation, particularly at high temperatures (30° and 38°C) (Brandolini *et al.*, 2009).

ADVERSE REACTIONS

Wheat is a major diet constituent for much of humankind, but wheat consumption often leads to fastidious or even life-threatening problems. Two different types of adverse reactions are linked to wheat consumption: allergies and celiac disease.

Allergies, such as baker's asthma, are abnormal immune system reactions to one or more wheat proteins. They may result in a wide range of symptoms, including rash, difficultly breathing, and nausea, and may sometimes cause a life-threatening reaction (anaphylaxis). Very little is known about einkorn allergenicity. Although the inhibitory activity toward human α-amylase of einkorn salt extracts is nonexistent, their IgE binding action is similar to that of *T. aestivum* and *T. turgidum* (Sánchez-Monge *et al.*, 1996).

Celiac disease is an inflammatory immunomediated condition triggered by the gluten prolamins of several cereals (including *Triticum* spp.) in predisposed individuals. The main causal agent is the gliadin fraction of gluten. All three structural types of gliadins (α/β, γ, and ω) are active; nevertheless, glutenin components can exacerbate celiac disease. Two different pathological effects can be distinguished: a rapid cytotoxic effect on the intestinal epithelium and an immune response involving T cells that recognize specific prolamin epitopes.

Up to 35 immunologically active sequences have been detected. The majority of these epitopes are immunogenic, stimulating specific T cell lines and clones derived from jejunal mucosa or peripheral blood of celiac patients; however, a few of them are toxic and induce mucosal damage. Studies by Pizzuti *et al.* (2006) and Vicentini *et al.* (2007) suggest a reduced or absent

toxicity of *T. monococcum*, which lacks a highly immunoreactive α/β-gliadin peptide (LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF) encoded by genes located on chromosome 6D (Mølberg *et al.*, 2005): Genome D is absent in einkorn. Furthermore, *in vitro* studies show that einkorn prolamines do not agglutinate human myelogenous leukemia K562(S) cells and do not induce lesions in intestinal cell cultures of celiac patients—properties attributed to the reaction between a toxic peptide and a "protective" peptide that inhibits the agglutinating activity in K562(S) cells. However, the sequencing of einkorn cDNA clones related to storage protein genes of *T. monococcum* revealed the presence of 4 toxic peptides and 13 immunogenic peptides (Vaccino *et al.*, 2009) belonging to all the storage protein classes, therefore indicating that einkorn has the full potential to induce the celiac disease syndrome. This finding stresses the necessity for more data before it can be acknowledged that *T. monococcum*-derived products are less toxic for celiac patients.

SUMMARY POINTS

- Einkorn is a hulled wheat—that is, after harvesting, its seeds are tightly enclosed by the glumes.
- Domesticated in the Fertile Crescent approximately 12,000 years ago, einkorn was instrumental in the diffusion of agriculture.
- Broadly cropped and eaten in Europe and the Near East for several thousand years, einkorn was replaced by the more productive durum and bread wheats during the Bronze Age.
- Einkorn kernels have higher protein and antioxidant (carotenoids and tocols) content than other wheats.
- The lipidic fraction of einkorn is rich in monounsaturated fatty acids.
- Some genotypes show very good bread making attitude, producing outstanding bread loaves with an appealing deep yellow crumb.
- All einkorns are suitable for bakery product preparation.
- Einkorn flour apparently elicits weaker toxic reactions than other wheats in celiac patients, but more research is needed.

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CHAPTER 9

Maize: Composition, Bioactive Constituents, and Unleavened Bread

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

G' Elastic modulus
G'' Viscous modulus
GI Glycemic index
RDS Rapidly digestible starch
RS Resistant starch
SDS Slowly digestible starch

INTRODUCTION

Maize (*Zea mays*), also known as corn, is grown throughout the world, and approximately 8 million tons of corn is produced worldwide. The United States, China, Brazil, Argentina, India, France, and Indonesia are the main corn-producing countries. Corn of different types (flour corn, flint corn, dent corn, sweet corn, popcorn, waxy corn, and amylomaize) and color (ranging from white to yellow, red, and purple) is grown. Floury corn (*Z. mays* var. *amylacea*), also known as "soft" corn, has mainly white-colored grains with rounded or flat crown, consisting almost entirely of soft endosperm and a small portion of hard endosperm. Flint corn (*Z. mays* var. *indurata*), also known as Indian corn, has soft starch in the middle surrounded by a hard shell, and its color ranges from white to red. Dent corn (*Z. mays* var.

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indentata) is either yellow or white in color with a depressed crown. Sweet corn (*Z. mays* var. s*accharata* and *Z. mays* var. *rugosa*) has higher sugar content than other corn types and is consumed in different forms (boiled, roasted, frozen, or canned). Popcorn (*Z. mays* var. *everta*) is mainly used for popping and has greater ability to pop, which is linked to dense starch filling in the endosperm. Waxy corn (*Z. mays* var. *ceratina*) has starch mainly consisting of amylopectin (approximately 99%), and amylose is present in very small amounts. Waxy corn starch produces paste that has a low tendency toward retrogradation with high transmittance and characteristics resembling potato starch. Waxy corn starch has many food (fruit pies, canned foods, frozen foods, and dairy products) and nonfood applications (gummed tapes). White corn has white-colored endosperm containing higher amounts of vitreous endosperm relative to floury endosperm and is preferred for nixtamalized products such as tortillas. Blue-, purple-, and red-pigmented corn kernels are rich in anthocyanins with well-established antioxidant and bioactive properties (Adom and Liu, 2002). Interest in pigmented corn rich in anthocyanins or carotenoids and phenolic compounds having antioxidant and bioactive properties has increased due to their health benefits.

STRUCTURE AND COMPOSITION

Corn grain is composed of endosperm (82–83%), germ (10–11%), pericarp (5–6%), and tip cap (0.8–1.0%). The pericarp is the outermost layer that is characterized by high crude fiber content, mainly consisting of hemicellulose, cellulose, and lignin. Hemicellulose is present in the highest concentration in the crude fiber. Pericarp thickness varies in different corn types and extends to the base of the kernel joining the tip cap. The pericarp and tip cap contribute a negligible amount to total kernel lipids. The endosperm is composed of a large number of cells, each packed with starch granules embedded in a continuous matrix of protein. The cell walls consist of nonstarch polysaccharides (β-glucan and arabinoxylan), proteins, and phenolic acids. Corn grain has two types of endosperm—floury and horny endosperm. Floury endosperm contains loosely packed starch granules surrounding the central fissure, whereas horny endosperm has tightly packed, smaller starch granules toward the periphery. Dry milling of corn is done to produce grits of different size and corn types, with a higher proportion of horny endosperm to mealy endosperm being preferred. The storage proteins of endosperm are located within subcellular bodies, simply known as protein bodies, and comprise the protein matrix. Protein bodies are composed almost entirely of a prolamine-rich protein fraction known as zein, which is extremely low in lysine. At least four major fractions have been identified within the zein storage protein: α-zein (21–25 kDa), β-zein (17–18 kDa), γ-zein (27–28 kDa), and δ-zein (9–10 kDa). The individual zeins vary in composition and concentration based on the corn genotype (Esen, 1987). The β- and δ-zeins are soluble in aqueous alcohols in the presence of reducing agents, and γ-zeins are soluble in both aqueous and alcoholic solvents in the presence of salt and reducing agents (Wilson, 1991). The crude fat content of the endosperm is relatively low (approximately 1%). The lipids present in endosperm contain more saturated fatty acids compared to germ lipids. The germ is composed of embryo, the living organ of the grain, and the scutellum that nourishes the embryo. The germ is characterized by a high lipid (approximately 33%) and protein (approximately 18%) content. Germ oil is low in saturated fatty acids and high in polyunsaturated fatty acids. Germ oil is relatively stable due to the presence of high levels of natural antioxidants, and it is considered good for health because of its fatty acid composition, mainly consisting of oleic and linoleic acid.

Endosperm constitutes approximately 82–83% of grain and contains approximately 87–88% of starch that consists of amylose and amylopectin. Amylose is a linear polymer composed of glucopyranose units linked through α-D-(1–4) glycosidic linkages, whereas

the amylopectin is a branched polymer. The packing of amylose and amylopectin within the granules has been reported to vary among the starches from different species. The branches of the amylopectin molecule form double helices that are arranged in crystalline regions. X-ray diffractometry is used to reveal the presence and characteristics of the crystalline structure of the starch granules. The "A-," "B-," and "C-type" patterns are different polymeric forms of the starch, which differ in the packing of the amylopectin double helices. Corn and other cereal starches exhibit a typical A-type pattern, in which double helices comprising the crystallites are densely packed with low water content. Tuber starches show the B-type pattern, in which crystallites are less densely packed and have more open structure containing a hydrated helical core (Tester *et al.*, 2004). Waxy corn starch has higher crystallinity than normal and sugary corn starch (Singh *et al.*, 2006). Sugary corn starch has been reported to have a larger amount of amylose–lipid complex (Singh *et al.*, 2006). The difference in crystallinity in different corn starches has been attributed to the difference in the proportion of short, medium, and long branch chains of amylopectin and amylose content.

Scanning electron micrograms of normal corn, waxy corn, and sugary corn starches are shown in Figure 9.1. Normal corn and waxy corn starch granules display spherical or angular shape, whereas sugary corn starch displays irregular-shaped granules consisting of lobes (Sandhu *et al.*, 2007). Irregular-shaped granules with average granule size of 36 μm for white corn starch and defined round shape with average granule size of 20 and 40 μm for black and blue corn starch, respectively, have been reported (Agama-Acevedo *et al.*, 2008).

When starch is heated in excess water, the granules swell several times to their original size. This results from the absorption of water and loss of crystalline order. The changes in starch slurries during heating and cooling are measured with a Rapid Visco Analyzer and

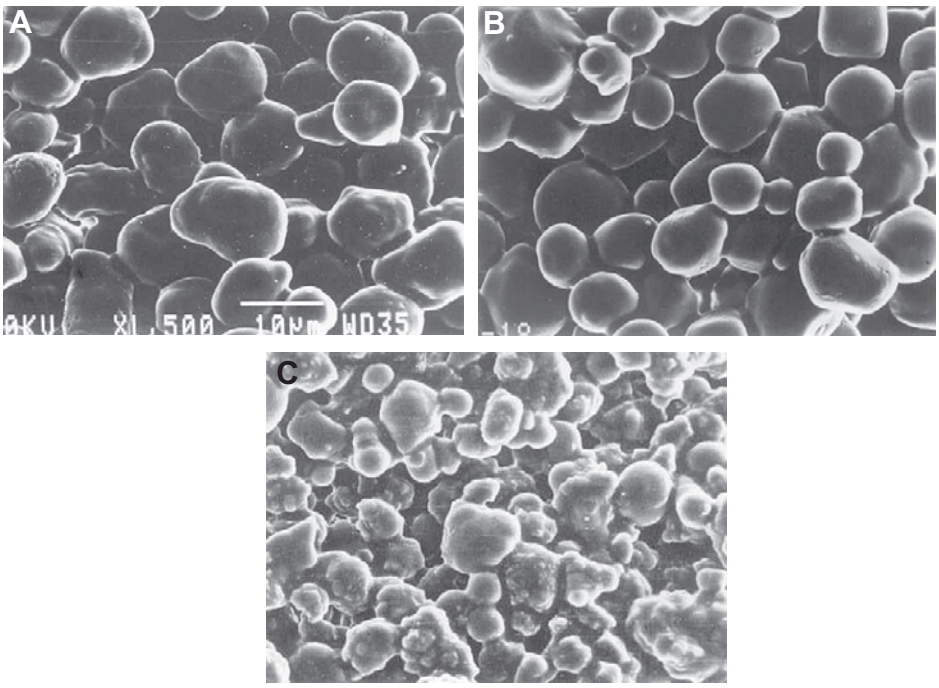

FIGURE 9.1
Scanning electron micrograms of (A) normal corn, (B) waxy corn, and (C) sugary corn starch granules. Source: *Reprinted with permission from Sandhu, K. S., Singh, N., and Lim, S.T. (2007). Functional properties of normal, waxy and sugary corn starches.* J. Food Sci. Tech. *44, 565–571.*

Brabender Viscoamylograph. These instruments measure changes in viscosity of starch pastes during heating and cooling with continuous stirring. During heating, the viscosity increases with increase in temperature due to swelling of the starch granules. This is followed by a decrease in viscosity caused by rupturing and fragmentation of granules. During cooling, the starch molecules re-associate to form gel, wherein amylose molecules aggregate and result in a network, embedding remnants of starch granules. The pasting properties of starch are influenced by the constituents that leached out from the granules during heating and the interactions between the chains. Sugary corn starch has pasting curves with a flatter peak, whereas normal and waxy corn show sharper peaks. Waxy corn starches have higher peak viscosity and lower breakdown viscosity compared to normal and sugary corn starches. The higher peak viscosity of waxy corn starches has been related to the absence of amylose–lipid complex because these starches have negligible amylose (Sandhu *et al.*, 2007). The lipids and phospholipids form a complex with the amylose and long branch chains of amylopectin in the cereal starches, which results in a reduction in swelling and inhibited amylose leaching (Singh *et al.*, 2003). Waxy starches have negligible amounts of lipids and give pastes with higher peak viscosity. Sugary and normal corn starch have a higher amylose content, which restricts swelling and limits the increase in viscosity by developing aggregated structure. Waxy corn starches have lower pasting temperature than the normal corn starches (Sandhu *et al.*, 2007). Black corn starch had higher peak viscosity, followed by white and blue corn starch; this difference was attributed to the difference in starch organization and damaged starch content (Agama-Acevedo *et al.*, 2008).

DRY AND WET MILLING

Dry milling

The main objective of dry milling is to get the maximum amount of grit with minimum contamination of hull, germ, and tip cap. The grains are cleaned to remove impurities, conditioned with cold or hot water or steam, and then tempered for a varying amount of time depending on the product required. The conditioning makes the hull and germ tough and endosperm mellow. The grains are then passed through either a Beall degerminator or fluted roller mills to separate germ and hull. After degermination, the material is dried to approximately 15% moisture content, cooled, and graded to get fractions of different particle size ranging from large hominy grits to fine flour. The products obtained from dry milling include flaking grits; coarse, medium, and fine grits; coarse or granulated meal; and fine meal. In India, traditionally the corn is milled in stone mills to get wholemeal or "atta," which is used in the preparation of unleavened flat bread or "roti."

Wet milling

Throughout the world, large quantities of corn are wet milled to produce starch and other valuable by-products, such as gluten, germ, and bran. The first step in the wet milling of corn is steeping in water (30–40 h at 50°C) in the presence of SO_2 (0.02%) under carefully controlled conditions to soften the kernels. SO_2 prevents fermentation and facilitates the separation of starch from protein. After steeping, the steep water is drained, and grains are coarsely ground to free the germ from endosperm and hull. The germ portion is then separated, dried, and expelled for oil extraction. The fiber and starch suspension is then fine milled and separated by screening, centrifugation, and washing. The starch is dried and converted into a number of valuable products, such as dextrose, fructose syrups, dextrins, modified starches, and sorbitol, by chemical and/or enzymatic processes. α-Amylase, β-amylase, glucoamylase, and pullulanase enzymes from bacterial and fungal sources are used for starch hydrolysis.

BIOACTIVE COMPOUNDS AND RESISTANT STARCH

Bioactive compounds

Various bioactive constituents, such as carotenoids, anthocyanins, and phenolic compounds, which are associated with health promotion and disease prevention properties, are present in fruits and vegetables and have also been reported in corn. These bioactive compounds are present mainly in whole grains. Total phenolic and anthocyanin contents of different corn types are shown in Table 9.1. The anthocyanins present in blue corn come from cyanidin and malvidin (mainly from derivatives of the former), whereas in red corn they come from pelargonidin, cyanidin, and malvidin. The carotenoids with molecules containing oxygen are also known as xanthophylls, which are the source of yellow color in corn. The carotenoids vary in corn according to type and genotype. Yellow corn has more carotenoids than floury corn. Lutein and zeaxanthin are the major carotenoids in corn, and to a lesser extent, α- and β-cryptoxanthin and α- and β-carotene are present. Blue and white corn are low in lutein and zeaxanthin content, whereas yellow and high-carotenoid corn have a higher content of these (de la Parra *et al.*, 2007). Several desirable health-related properties of lutein and zeaxanthin have been identified; for example, lutein has been shown to have anti-tumor-promoting activity and suppression of tumor growth in mice (Park, 1998). Lutein and zeaxanthin have also been associated with the prevention of age-related macular degeneration, a human disorder similar to cataracts that causes early blindness. Bioactivities of purple, blue, and red pigmented corn have been associated with the presence of anthocyanins. The antimutagenic and radical scavenging activities of anthocyanins are well documented.

The carotenoid content of raw corn and its processed products is always different because certain amounts of carotenoids are lost during processing. The losses in carotenoid content are dependent on the processing methods of foods, such as canning, freezing, and extrusion (Table 9.2). The carotenoids are sensitive to heat, light, air, and pH; however, lutein has better heat stability. High-carotenoid corn genotypes have the best overall phytochemical profile, followed by yellow, blue, and red corn. White corn genotypes have lower antioxidant activity due to lower amounts of anthocyanins and carotenoids. The utilization of yellow and pigmented corn instead of white corn can provide nutritionally better products. However, in many industrially manufactured products, white corn is preferred (e.g., nixtamalized products).

TABLE 9.1 Phenolic and Anthocyanin Content in Different Corn Types

Corn Type	Total Phenolics (mg/100 g Dry wt)[a]			Anthocyanin Content (mg/100 g Dry wt)[b]
	Free	Bound	Total	
White[c]	34.7 ± 0.4	226.0 ± 6.3	260.7 ± 6.1	1.33 ± 0.02
Yellow[c]	43.6 ± 1.8	242.2 ± 13.1	285.8 ± 14.0	0.57 ± 0.01
Red[c]	38.2 ± 0.4	205.6 ± 4.5	243.8 ± 4.6	9.75 ± 0.44
Blue[c]	45.5 ± 0.5	220.7 ± 0.5	266.2 ± 0.7	36.87 ± 0.71
High carotenoid[c]	50.0 ± 2.5	270.1 ± 9.4	320.1 ± 7.6	4.63 ± 0.06
Black[d]	103 ± 2.6	354 ± 3.1	457 ± 7.4	76.2 ± 2.2
Purple[d]	83.7 ± 2.5	381 ± 4.4	465 ± 9.8	93.2 ± 1.1
Red[d]	82.3 ± 0.8	384 ± 7.1	465 ± 4.4	85.2 ± 2.2
Blue[d]	73.1 ± 1.4	271 ± 4.5	343 ± 8.6	99.5 ± 1.8
Orange[d]	40.3 ± 1.4	175 ± 2.3	215 ± 5.1	30.6 ± 0.9
Yellow[d]	104 ± 2.2	447 ± 4.3	551 ± 3.8	70.2 ± 0.9
White[d]	33.4 ± 1.5	136 ± 3.2	170 ± 1.1	1.54 ± 0.9

[a]*Expressed as gallic acid.*
[b]*Expressed as cyanidin-3-glucoside.*
[c]*Data from de la Parra et al. (2007).*
[d]*Data from Lopez-Martinez et al. (2009).*

TABLE 9.2 Carotenoid Content of Different Corn Types and Processed Corn

Corn Type	Carotenoid Content (μg/100 g Dry wt)			
	Lutein	Zeaxanthin	β-Cryptoxanthin	β-Carotene
White[a]	5.73 ± 0.18	6.01 ± 0.06	1.27 ± 0.06	4.92 ± 0.18
Yellow[a]	406.2 ± 4.9	353.2 ± 23.1	19.1 ± 1.2	33.6 ± 1.2
Red[a]	121.7 ± 12.1	111.9 ± 9.2	13.1 ± 1.8	20.2 ± 1.9
Blue[a]	5.17 ± 0.49	14.3 ± 1.0	3.41 ± 0.39	23.1 ± 2.1
High carotenoid[a]	245.6 ± 9.4	322.3 ± 10.7	23.1 ± 1.0	45.8 ± 3.9
White corn (fresh)[b]	5.5 ± 1.2	28.5 ± 5.2	0.4 ± 0.1	0.82 ± 0.08
White corn (canned)[b,c]	6.6 ± 0.5	30.5 ± 3.4	0.5 ± 0.2	0.68 ± 0.17
White corn (frozen)[b]	6.3 ± 1.1	47.7 ± 10.2	0.9 ± 0.2	2.37 ± 0.42
Golden corn (fresh)[b]	330 ± 19.8	209.0 ± 12.0	31.6 ± 15.5	15.69 ± 0.60
Golden corn (canned)[b,c]	336.4 ± 67.5	215.9 ± 42.2	42.8 ± 10.0	11.66 ± 2.47
Golden corn (frozen)[b]	361.6 ± 34.2	212.3 ± 36.0	33.1 ± 3.9	16.68 ± 1.83
Yellow corn oil[d,f]	10.4 ± 0.4	16.2 ± 0.7	15.5 ± 0.3	18.6 ± 0.8
Yellow corn oil[d,e]	80.4 ± 5.5	140.5 ± 11.7	68.2 ± 2.2	35.4 ± 1.3
Yellow corn germ oil[d,f]	1.4 ± 0.2	0.9 ± 0.1	Nil	Nil
Yellow corn germ oil[d,e]	2.1 ± 0.3	2.9 ± 0.4	Nil	Nil
Yellow corn fiber oil[d,f]	6.3 ± 0.6	5.7 ± 0.4	6.8 ± 1.2	5.6 ± 0.2
Yellow corn fiber oil[d,e]	25.1 ± 1.4	26.4 ± 1.9	17.9 ± 0.4	10.7 ± 0.2

[a]*Data from de la Parra et al. (2007).*
[b]*Data from Scott and Elridge (2005).*
[c]*Corn and brine were analyzed in all canned samples, with brine content factored out of final calculations.*
[d]*Data from Moreau et al. (2007).*
[e]*Ethanol extracted.*
[f]*Hexane extracted.*

The phenolic compounds, including ferulic acid, are unique to the grains. Ferulic acid is an important phytochemical in corn and other cereal grains. Ferulic acid is present in different forms (free, conjugated, and bound), and the concentration of each form varies in different corn types (Table 9.3). The high-carotenoid corn contains higher amount of total ferulic acid compared to white, yellow, red, and blue corn. Most of the ferulic acid (approximately 94–96%) is present in bound form in corn (Adom and Liu, 2002). It has been demonstrated that thermal processing increases the free phytochemical content and antioxidant activity of

TABLE 9.3 Ferulic Acid Content in Different Corn Types

Corn Type	Ferulic Acid (mg/100 g Dry wt)			
	Free	Soluble Conjugated	Bound	Total
White[a]	0.50 ± 0.02	0.76 ± 73	119.20 ± 11.2	120
Yellow[a]	0.65 ± 0.01	1.47 ± 102	100.84 ± 5.0	102
Red[a]	0.58 ± 0.02	1.26 ± 48	128.45 ± 11.5	130
Blue[a]	0.68 ± 0.05	1.45 ± 27	127.85 ± 8.1	130
High carotenoid[a]	0.97 ± 0.08	1.96 ± 33	150.08 ± 12.4	153
Black[b]	1.87 ± 0.3	—	150 ± 1.4	151
Purple[b]	1.97 ± 0.1	—	152 ± 1.5	154
Red[b]	2.02 ± 0.5	—	151 ± 2.9	153
Blue[b]	2.02 ± 0.4	—	149 ± 2.2	152
Orange[b]	2.42 ± 0.2	—	161 ± 3.3	164
Yellow[b]	2.01 ± 0.1	—	138 ± 1.1	140
White[b]	1.57 ± 0.6	—	146 ± 2.3	148

[a]*Data from de la Parra et al. (2007).*
[b]*Data from Lopez-Martinez et al. (2009).*

corn. Processing methods such as lime cooking, tortilla baking, and tortilla chip frying increase the amount of free and soluble conjugated ferulic acid (de la Parra *et al.*, 2007). de la Parra *et al.* explained that bound ferulic acid survives stomach and intestinal digestion to reach the colon and helps in preventing colon, breast, and prostate cancer. Ferulic acid acts as an antioxidant and is used as an ingredient in various supplements that claim to slow the aging process. Ferulic acid has been approved in certain countries as a food additive to prevent lipid oxidation. Ferulic acid is also suggested to be useful in alleviating oxidative stress and attenuating the hyperglycemic response associated with diabetes. A wide range of therapeutic properties of ferulic acid have been reported, such as anti-inflammatory, anti-atherogenic, anti-diabetic, anti-aging, neuroprotective, radioprotective, and hepatoprotective properties (Srinivasan *et al.*, 2007). Ferulic acid can readily form a resonance stabilized phenoxyl radical, which accounts for its potent antioxidant potential. Corn has a higher antioxidant capacity compared to wheat, oat, and rice (Adom and Liu, 2002). Antioxidant activity of bound phytochemicals in corn grains has been reported to be 157.68 μmol/g compared to 68.74, 43.60, and 39.76 μmol/g in grains of wheat, oat, and rice, respectively (Adom and Liu, 2002).

Efforts are being made in different countries to develop varieties of corn high in carotenoid content to derive the health benefits from its dietary consumption. New varieties of corn with high β-carotene content have been bred to combat vitamin A deficiency in African countries (Harjes *et al.*, 2008). Knowledge of the carotenoid synthesis pathway, which involves certain enzymes regulating the proportion of different carotenoids, has been used to develop new corn lines with increased concentrations of specific carotenoids (Harjes *et al.*, 2008). The health implications of yellow and pigmented corns in different processed products need to be studied in-depth.

Resistant starch

During heating, starch is gelatinized and the semicrystalline structure of starch disintegrates. Cooling of the gelatinized starch pastes leads to recrystallization of the starch chains, known as retrogradation. The retrogradation rate and its extent vary with starch properties (molecular and crystalline structure) and storage conditions (temperature, duration, water content, etc.). The starches with higher amylose content have higher retrogradation rates (Singh *et al.*, 2006). Changes in the elastic modulus (G') among the cooked pastes of corn starches with different amylose content measured using a Haake dynamic rheometer at 10°C during a 10-h period are compared in Figure 9.2. Sugary corn starch with higher amylose has a higher retrogradation rate than normal corn starch (Singh *et al.*, 2006). The retrogradation makes the starch resistant toward breakdown by digestive enzymes and consequently reduces the glycemic index (GI) (Fredriksson *et al.*, 2000). GI characterizes the carbohydrates consumed in the form of different foods on the basis of the postprandial level of blood glucose (Jenkins, 2007). Carbohydrates that break down quickly during digestion and release glucose rapidly into the bloodstream are considered to have a high GI. Starch is classified into three groups according to the rate of glucose release and its absorption in the gastrointestinal tract: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS is the group of starches that can be rapidly hydrolyzed by digestive enzymes, SDS is the group that is digested at a relatively slow rate (Englyst *et al.*, 1992), and RS is not digested by digestive enzymes and consequently is transferred into the colon. Waxy corn starch is more rapidly digested than high-amylose starch, possibly due to more surface area per molecule of the amylopectin than amylose. RS has been associated with health benefits such as improved cholesterol metabolism and reduced risk of type II diabetes and colon cancer (Hoebler *et al.*, 1999).

Normal corn starch is more susceptible to amylolysis compared to high-amylose corn starch, possibly due to the presence of surface pores and channels that facilitate enzymatic diffusion (Zhang *et al.*, 2006). The association between amylose chains and their potential for amylose–lipid complex formation (Morita *et al.*, 2007), higher crystalline lamella thickness, and a thicker peripheral layer (Jenkins and Donald, 1995) are the factors that make the high-amylose

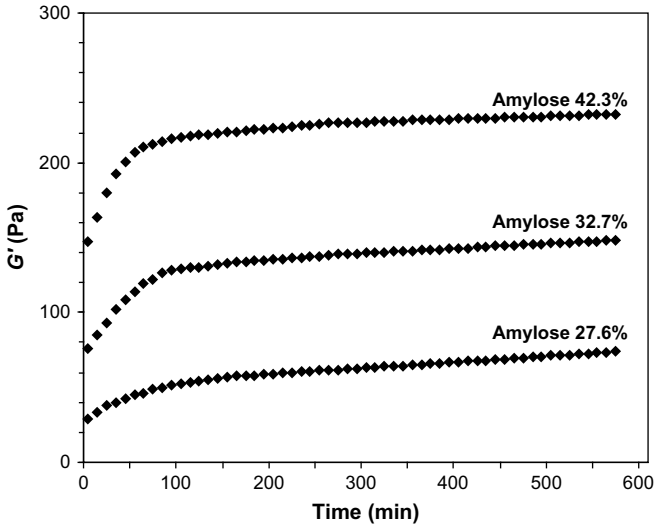

FIGURE 9.2
Changes in G′ at 10°C during 10 hr holding of cooked paste from corn starches varying in amylose content (measured using a dynamic rheometer; Haake RheoStress 6000).

corn starch granules resistant to amylolysis. High-amylose corn starch granules are hydrolyzed predominantly by exocorrosion, whereas normal corn starch is internally hydrolyzed in an "inside-out" pattern (Zhang *et al.*, 2006).

Mechanical and thermal treatments change the structure and digestibility of starch. Waxy starches and low-amylose corn starches are readily damaged by milling, rendering them more vulnerable to amylolysis (Tester and Morrison, 1994). Thermal treatments such as autoclaving, baking, steam cooking, and parboiling affect the gelatinization and retrogradation processes and, consequently, the formation of RS in foods. Hi-maize containing more than 80% amylose and 42% RS is a commercial source of RS that is widely used in various baked goods. The chemical modifications of starches are done to change the functional properties that also change the susceptibility toward the action of enzymes. Esterification, etherification, and cross-linking of starch make it resistant to α-amylase (Hood and Arneson, 1976). Chung *et al.* (2008) reported that substitution and oxidation of corn starch increase RS, whereas cross-linking does not affect starch digestibility considerably.

RDS, SDS, and RS of the modified corn starches in the prime and gelatinized states using enzymatic hydrolysis were studied by Chung *et al.* (2008). These authors reported that the oxidized starch had a higher amount of RDS (33.5%) compared to the hydroxypropylated starch (27.9%), cross-linked and acetylated starches (approximately 24%), and unmodified starch (25.6%). They observed that the oxidized starch was rapidly hydrolyzed during the early stage of digestion (up to 60 min) because the chain degradation of starch occurred during oxidation. Therefore, the fast rate of hydrolysis resulted in the greatest amount of RDS. These authors reported that the exceptionally higher swelling ability of hydroxypropylated starch enhanced the access of digestive enzymes inside the granules, thereby increasing the RDS content. Although the acetylated starch swelled more readily than unmodified starch, the acetyl groups could hinder the enzymatic action during hydrolysis.

UNLEAVENED BREAD MAKING

The different size fractions of grit that are produced during dry milling of corn vary in composition and end-use suitability. Flaking grits are used for making "cornflakes." Coarse and medium grits are used in the processing of breakfast cereals and snack foods. Fine grits are

preferred in porridge making and also used as brewing adjunct, often to reduce the cost of beer. Coarse or granulated meal is used in pancakes, muffin mixes, and different bakery products. Finely ground corn is used in the production of "tortilla," a type of unleavened bread. Tortilla is an important product for the population of suburban and rural areas of Meso-American countries, such as Mexico and Guatemala. Tortillas are prepared from white, yellow, and blue corn; however, white corn is preferred in commercial processing. Similar bread made from corn in South America is called "Arepa," which has greater thickness compared to tortillas.

Wheatmeal is generally used for making unleavened bread called chapattis (roti) in India and Pakistan. However, cornmeal is also used to prepare roti in winters and is very popular in north Indian states. Yellow cornmeal is preferred in the preparation of roti. Roti made from cornmeal is less pliable compared to that from wheatmeal due to the differences in composition and rheological properties. Dough is generally characterized by empirical (farinograph) and fundamental rheological measurements. Farinograph is extensively used to characterize dough mixing properties (water absorption, development time, stability, and mixing tolerance) of wheat flour/meal. Dynamic oscillation measurement involves small deformation and is a fundamental approach to study dough rheology. The dynamic oscillation technique is preferred for studying the structure and fundamental properties of cereal flour dough. Mechanical spectra of cornmeal and wheatmeal dough obtained using a Haake dynamic rheometer are shown in Figure 9.3. G' of wheatmeal and cornmeal dough was greater than the viscous modulus (G''), indicating predominance of elastic character. Higher G' with lower G'' and tan δ of cornmeal dough compared to wheatmeal dough reflect its higher rigidity and stiffness. Wheatmeal dough shows lower G' and G'' compared to cornmeal dough. Also, the difference between the moduli was less, showing good balance between these. The roti prepared from wheatmeal has better textural qualities than cornmeal because of a good balance between the moduli.

Cornmeal is kneaded into soft pliable dough with water for roti making. Cornmeal dough lacks the viscoelasticity of wheat dough; therefore, warm water is used during kneading. Warm water helps in aggregation of particles due to partial gelatinization of starch. After mixing, dough is divided into small balls, rounded, flattened, and sheeted into circular disks (approximately 5 in. in diameter and ¼ in. thick) with a rolling pin or hand. The disks are roasted on a heavy iron or earthen griddle until crisp. The disks are turned several times during roasting. After roasting, the roti is coated with butter oil or butter. In Punjab states of India and Pakistan, roti is traditionally served hot with "Sarson ka saag" (mustard leaf gravy) and salad consisting of onion, radish, and lemon.

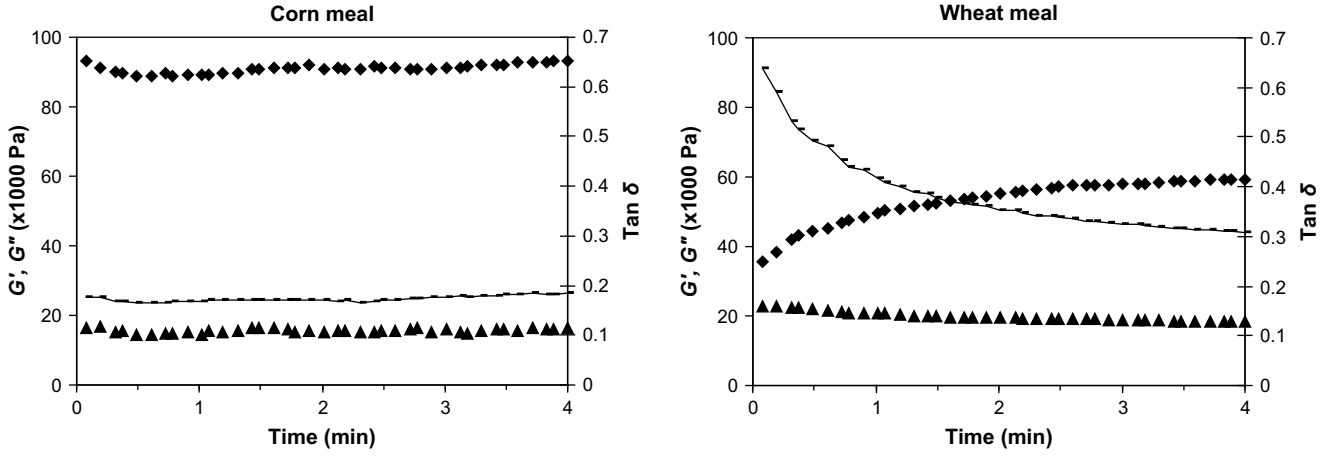

FIGURE 9.3
Mechanical spectra of dough from cornmeal and wheatmeal. ■, G'; ▲, G'' —, tan δ.

TECHNOLOGICAL ISSUES

Knowledge of the carotenoid synthesis pathway (Harjes *et al.*, 2008) could help in developing new varieties of corn with higher concentrations of specific carotenoids. These varieties could be useful in combating the problems related to vitamin A deficiency in developing countries. The processing conditions have variable effects on the bioactive constituents present in corn. Therefore, processing methods with minimum losses of bioactive constituents for different corn products need to be developed. The utilization of pigmented corn instead of white corn could provide nutritionally better products; however, white corn is preferred for many industrially manufactured products.

SUMMARY POINTS

- High-amylose corn is a good source of RS, which can be used in various food products for its health benefits. RS has been associated with improved cholesterol metabolism and reduced risk of type II diabetes and colon cancer.
- Various bioactive compounds with the health-promotion and disease-prevention properties present in fruits and vegetables have also been reported in corn.
- Ferulic acid is an important phytochemical present in higher amounts in high-carotenoid corn compared to white, yellow, red, and blue corn.
- The purple-, blue-, and red-pigmented corn inhibits colorectal carcinogenesis in male rats and possesses antimutagenic and radical scavenging activities. These bioactivities have been associated with the presence of anthocyanins.
- The health implications of pigmented corn in different processed products need to be studied in-depth.
- Corn varieties with high β-carotene content can combat vitamin A deficiency in developing countries. Knowledge of the carotenoid synthesis pathway can be used for developing such varieties.

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CHAPTER 10

Amaranth: Potential Source for Flour Enrichment

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

AmA Amaranth albumin
CaMV Cauliflower mosaic virus
GI Glycemic index
PAGE Polyacrylamide gel electrophoresis
SDS Sodium dodecyl sulfate

INTRODUCTION

Amaranthus or amaranth, a traditional Mexican plant, is a cosmopolitan genus of herbs with approximately 60 plant species, the majority of which are wild (Stallknecht and Schulz-Schaeffer, 1993). *Amaranthus* plants have inflorescences and foliage with different colors, ranging from purple to red and gold. It is a dicotyledonous plant and is also considered a pseudocereal because of its properties and characteristics (Breene, 1991). Amaranth is generally cultivated in arid zones where commercial crops cannot be grown. *Amaranthus* has a good capacity to produce high biomass and is used as grains, leafy vegetables, and ornamentals. Several species of amaranth are often considered as weeds. *Amaranthus cruentus* and *A. hypochondriacus* are the species that are primarily cultivated for grain, whereas *A. blitum*,

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A. hypocondriacus *A. caudatus*

FIGURE 10.1
Grains of different amaranth varieties

A. dubius, *A. tricolor*, *A. lividus*, and *A. spinosus* are used as vegetables. *Amaranthus tricolor* and *A. caudatus* are also grown for ornamental or decorative purposes. *Amaranthus viridis*, *A. retroflexus*, *A. hybridus*, *A. gracilis*, *A. gangeticus*, *A. paniculatus*, and *A. graecizans* are wild types. The leaves of *Amaranthus* are a potential alternative source of betalains because of their betacyanin pigments, and they also show anticancer activity.

GRAIN CHARACTERISTICS

Amaranth grain is nearly spherical, approximately 1 mm in diameter, and varies in color from creamish yellow to reddish. It also has a unique composition of protein, carbohydrates, and lipids. *Amaranthus hypochondriacus* produces creamish yellow grains, whereas the grains of *A. caudatus* are red (Figure 10.1). Hunter color L^*, a^*, and b^* values are approximately 62–68, 5.5–6.7, and 21.2–23.7, respectively, for *A. hypochondriacus*, and they are 49–51, 13–13.8, and 10.6–13.2, respectively, for grains of *A. caudatus*. Amaranth grain structure differs significantly from that of cereals such as maize and wheat. Amaranth seeds have a circular-shaped embryo or germ, which surrounds the starch-rich perisperm and, together with the seed coat, represents the bran fraction, which is relatively rich in fat and protein (Bressani, 1994). Bran fraction is proportionally higher in amaranth seeds than in common cereals, such as maize and wheat, which explains the higher levels of protein and fat present in these seeds (Bressani, 1994).

GRAIN COMPOSITION

Amaranth grain has approximately 62–65% starch, which is made up of amylose and amylopectin. Amylose is a linear chain molecule, whereas amylopectin is highly branched, consisting of a main chain of (1–4)-linked α-D-glucose along with short chains of (1–6)-α-D-glucose-linked branches. Amaranth starch has a low amylose content, ranging between 2 and 12%, that varies by genotype. Amaranth starch granules have diameters ranging between 0.5 and 2.5 μm, similar to rice but smaller than those found in starches of other cereal grains. A comparison of amaranth starch granules with those of wheat, rice, and potato is illustrated in Figure 10.2. Amaranth starch has polygonal-shaped granules and displays an A-type X-ray pattern, which is similar to those of wheat, rice, and maize starches. Amaranth starch shows greater crystallinity compared to wheat starch, with strong reflections at $2°\theta = 15.1°$, 17.2°, 18.1°, and 23.2° (Figure 10.3). An additional peak at $2°\theta = 20.0°$ is usually present, indicating the presence of amylose–lipid complexes. Amaranth starch shows intercultivar variability in crystallinity. Its starch has a pasting temperature and a gelatinization onset temperature of

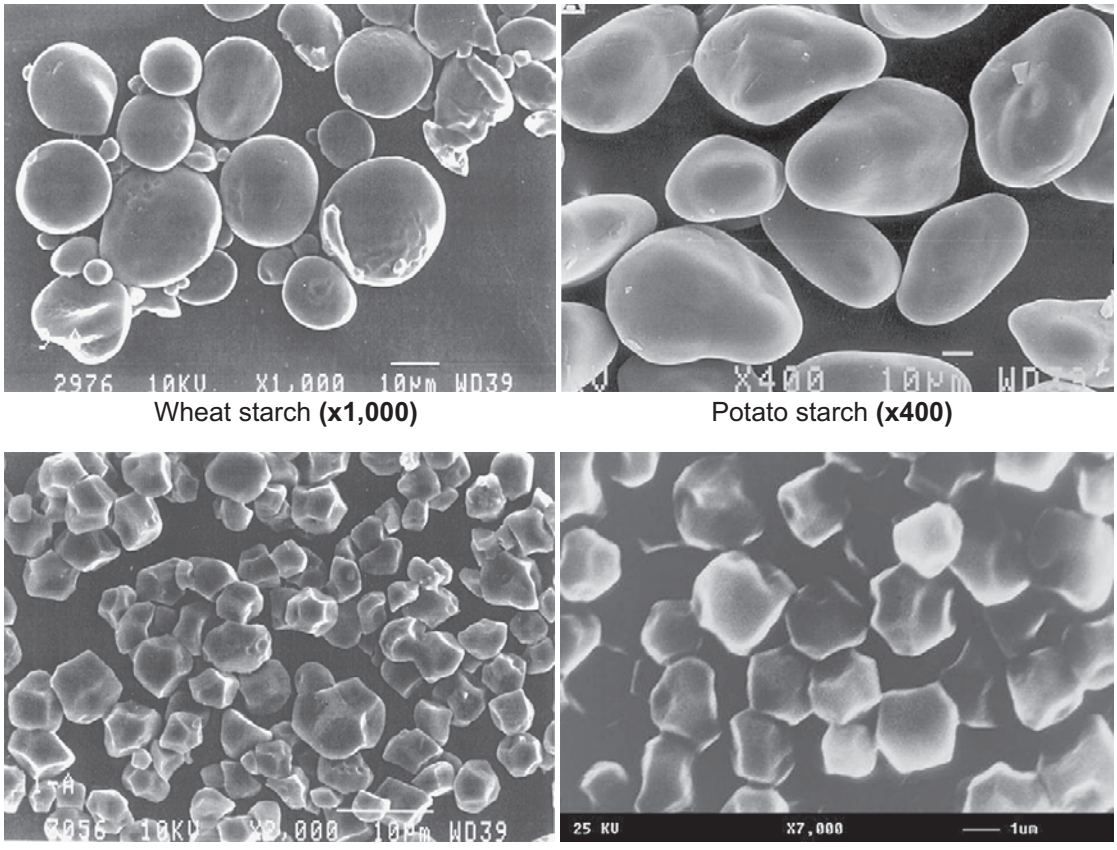

FIGURE 10.2
Scanning electron micrographs of different starches

69–72° and 60–77°C, respectively. The difference in pasting behavior among starches from different genotypes has been observed due to differences in amylose content, crystallinity, and the presence or absence of amylose–lipid complexes. The pasting curve of starch separated from two genotypes of *A. hypocondriacus* is illustrated in Figure 10.4. Its starch usually produces

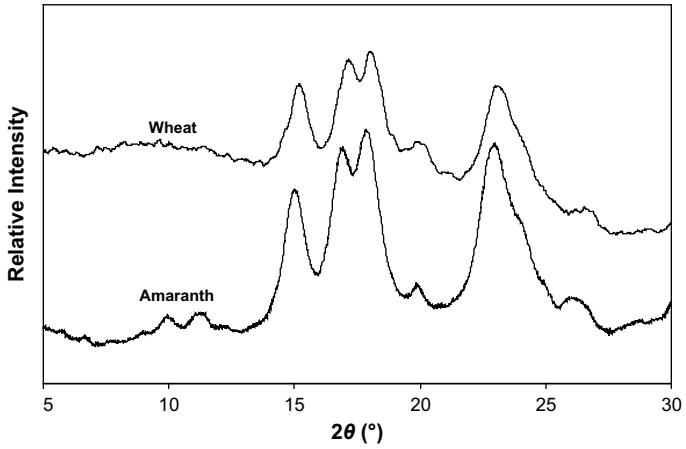

FIGURE 10.3
X-ray diffractograms of wheat and amaranth starch. *Source: N. Singh, unpublished data.*

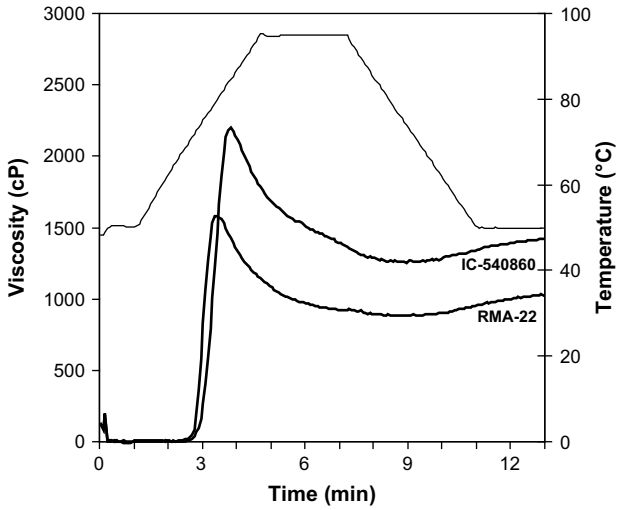

FIGURE 10.4
Pasting curves of starch separated from different amaranth genotypes. *Source: N. Singh, unpublished data.*

sharper peaks with lower setback and higher breakdown, similar to that of normal and waxy corn starches.

Amaranth grain is a high glycemic food, attributed to its small starch granule size, low amylose and resistant starch content, along with a tendency to completely lose its crystalline and granular starch structure during heating. The glycemic indexes of amaranth and other cereal grains and foods are compared in Table 10.1. The glycemic index defines carbohydrates present in different foods on the basis of the postprandial level of blood glucose (Jenkins, 2007). The relationship between the rate of *in vitro* digestion and the glycemic response to food is well-known. Raw amaranth seeds had a rapidly digestible starch content of 30.7% (dry weight basis) and predicted glycemic index of 87.2 (Capriles *et al.*, 2008). The starch digestibility of cooked, extruded, and popped *Amaranth* seeds was 92.4, 91.2, and 101.3, respectively, compared to white bread, and approximately 106 for flaked and roasted seeds.

Amaranth grains are enriched with various minerals, such as calcium, phosphorus, iron, potassium, zinc, and vitamin E and B complexes. Amaranth is a rich source of polyphenols (flavonoids) with relatively high antioxidant activity. Caffeic acid, *p*-hydroxybenzoic acid, and ferulic acid are the main phenolic compounds in amaranth grains (Klimczak *et al.*, 2002). The presence of polyphenols such as rutin (4.0–10.2 mg/g flour) and nicotiflorin (7.2–4.8 mg/g flour) in *A. hypochondriacus* varieties grown in the Mexican highlands zone has also been reported (Barba de la Rosa *et al.*, 2009).

The concentrations of calcium, magnesium, and oxalate in grains of 30 amaranth genotypes of *A. cruentus*, *A. hybrid*, and *A. hypochondriacus* have been studied (Gélinas and Seguin, 2007). Concentrations of calcium and magnesium in the grains were 134–370 and 230–387 mg/100 g, respectively, whereas the oxalate content varied from 178 to 278 mg/100 g. Although dietary oxalate is a potential risk factor for kidney stone development and lowers the availability of calcium and magnesium, most of the oxalates in the amaranth grains are in insoluble form and, thus, absorption may be low. However, this needs to be confirmed by bioavailability investigations.

The dietary fiber and lipid contents in amaranth grain were 8–17 and 3.0–10.5%, respectively. Although amaranth grain contains higher lipids than most of the cereals, the composition of its oil is quite similar to that of cereals, being high in unsaturated fatty acids (approximately 77%). Amaranth oil contains mainly linoleic acid but also tocotrienols, which are associated

TABLE 10.1 Glycemic Index (GI) of Various Foods

Food	GI
Amaranth grain (raw)[a]	87
White bread[a]	94
Amaranth grain (popped)[a]	101
Amaranth grain (roasted)[a]	106
Amaranth grain (flaked)[a]	106
Amaranth grain (extruded)[a]	91
Amaranth grain (popped)[b]	97
Pearl barley[b]	25
Sweet corn[b]	53
White rice[b]	64
Brown rice[b]	55
Parboiled rice[b]	47
Bulgur wheat[b]	48
Cornflakes[b]	84
Puffed wheat[b]	74
Wheat bread[b]	70
Wholemeal bread[b]	69
Lentils[b]	28
Soybean[b]	18
Baked beans (canned)[b]	48

[a]*Data from Capriles* et al. *(2008). Glycemic index (predicted) determined using equation = 39.71 + 0.549 (hydrolysis index) of Goni* et al. *(1997).*
[b]*Data from Foster-Powell* et al. *(2002); reference food is glucose.*

with cholesterol-lowering activity in mammalian systems (Becker, 1989). Amaranth grain oil contains a significant amount (up to 8%) of squalene (Sun *et al.*, 1997), which has important direct or indirect beneficial effects on health and is also an important ingredient in cosmetics. Therefore, amaranth oil has the potential to replace other squalene sources such as shark and whale, which are endangered species.

FOOD APPLICATIONS

The small starch granule size and composition have been suggested to be responsible for unique gelatinization and freeze/thaw characteristics that could be exploited by the food industry to develop various products (Becker *et al.*, 1981). Amaranth starch can be used in many food preparations, such as custards, pastes, and salads, and nonfood applications, such as cosmetics, biodegradable films, paper coatings, and laundry starch. Amaranth flour is used as a thickener in gravies, soups, and stews. Sprouted amaranth is used in salads. The cooking of amaranth improves its digestibility and absorption of nutrients. Amaranth flour lacks gluten proteins present in wheat; hence, it is not suitable for bread making. It is blended with wheatmeal/flour in the preparation of unleavened flat bread known as chapattis in India and tortillas in Latin America. Amaranth flour is also used in the preparation of biscuits, muffins, pancakes, pastas, flat breads, extruded products, etc. In India, the grains are most commonly used in the form of candy known as laddoos.

In comparison to amaranth grain, vegetable amaranth has received less attention by researchers. Vegetable amaranth is used as a delicacy or a food staple in many areas of the world. *Amaranthus* leaves are used as a vegetable in the northern states of India. However, its use is limited to "Sag," which is prepared by cooking with mustard leaves along with garlic, ginger, green chilies, and salt. Vegetable amaranth is better tasting than spinach and is substantially higher in calcium, iron, and phosphorous.

GRAIN PROTEIN CHARACTERISTICS

Cereals are normally deficient in lysine and tryptophan, whereas legume proteins show deficiency of sulfur-containing amino acids, namely cysteine and methionine. Amaranth proteins, on the contrary, contain significant amounts of both sulfur amino acids and lysine. Amaranth grains have higher protein (11–17%) than most of the cereal grains. Amaranth is an appropriate grain for people who are allergic to gluten. The germ and endosperm of amaranth grain contain 65 and 35%, respectively, of protein compared to an average of 15 and 85%, respectively, in most of the cereals. The amino acid composition of different amaranth protein fractions is given in Table 10.2. Albumins and globulins are relatively rich in lysine and valine, essential amino acids, whereas glutenins are high in leucine, threonine, and histidine. In addition to amino acid composition, the protein quality also depends on bioavailability or digestibility. Protein digestibility, available lysine, net protein utilization, and protein efficiency ratio, which are indicators of protein nutritional quality, are substantially higher for amaranth proteins compared to cereal grains (Guzmán-Maldonado and Paredes-López, 1999). Therefore, amaranth proteins are a promising food ingredient, capable of complementing and supplementing cereal or legume proteins (Guzmán-Maldonado and Paredes-

TABLE 10.2 Amino Acid Composition of Amaranth (*Amaranthus hypochondriacus* L.) Protein Fractions

		Protein Fractions			
Amino Acid	**Meal**	**Albumins**	**Globulins**	**Prolamins**	**Glutelins**
Isoleucine[a]	—	3.7	4.2	6.2	5.8
Leucine[a]	—	5.7	5.7	5.7	10.5
Lysine[a]	—	7.6	6.7	4.2	4.6
Methionine[a]	—	4.1	3.4	7.4	3.1
Cysteine[a]	—	5.9	3.9	6.5	6.2
Phenylalanine[a]	—	5.1	5.0	9.0	6.8
Tyrosine[a]	—	3.3	4.3	4.0	3.8
Threonine[a]	—	3.9	4.1	3.2	8.6
Valine[a]	—	4.5	4.7	2.7	3.8
Histidine[a]	—	2.5	1.1	1.1	4.7
Alanine[a]	—	5.1	4.0	4.7	3.6
Arginine[a]	—	8.1	9.5	9.4	2.7
Aspartic acid[a]	—	6.2	8.7	6.2	6.1
Glutamic acid[a]	—	17.5	17.3	13.4	13.2
Glycine[a]	—	6.2	6.6	4.4	4.9
Proline[a]	—	3.7	3.9	4.7	4.6
Serine[a]	—	4.8	4.9	5.1	5.3
Serine[b]	7.3	6.4	7.7	8.0	9.0
Glycine[b]	10.7	10.5	13.9	10.7	10.3
Histidine[b]	3.0	2.3	2.3	1.8	2.4
Arginine[b]	7.3	8.9	9.3	6.8	8.5
Threonine[b]	5.1	3.4	4.0	7.2	5.4
Alanine[b]	6.6	6.2	5.4	8.6	6.3
Proline[b]	5.7	5.0	4.0	4.5	5.9
Tyrosine[b]	1.9	2.9	2.8	3.0	3.0
Valine[b]	5.9	4.0	5.0	4.5	5.0
Isoleucine[b]	3.9	3.5	4.0	4.5	5.0
Leucine[b]	6.2	5.5	6.0	10.0	8.0
Phenylalanine[b]	3.4	3.0	2.0	3.9	4.3
Lysine[b]	5.7	6.6	7.0	6.7	4.2

[a]*Expressed as grams of amino acids/100 g of crude protein (Barba de la Rosa* et al., *1992).*
[b]*Expressed as molar percentage (Segura-Nieto* et al., *1992).*

López, 1999). The protein digestibility corrected amino acid score of amaranth whole flour is higher (0.64) than those of wheat (0.40) and oat (0.57) (Bejosano and Corke, 1998). An average protein digestibility of 74.2% for raw amaranth wholemeal flour was reported (Bejosano and Corke, 1998). Thermal processing improves protein digestibility due to opening of carbohydrate–protein complexes and/or the inactivation of antinutritional factors such as trypsin inhibitors (Bejosano and Corke, 1998).

Contrary to legumes and cereals, in which the grain proteins generally serve as storage molecules for the growing plantlets, the amaranth grain consists of albumins, which are usually biologically active, in the highest amount. According to the Osborne classification (Osborne, 1924), the amaranth grain consists of three major fractions—albumins (51%), globulins (16%), and glutelins (24%)—and a minor fraction—that is, alcohol-soluble fraction or prolamine between 1.4 and 2.0% (Gorinstein, Moshe, *et al.*, 1991; Martinez *et al.*, 1997), whereas legume grain contains salt-soluble globulins as the major storage protein fraction. Cereals such as maize and wheat, on the contrary, contain alcohol-soluble prolamins as the major storage proteins (Gorinstein, Denue, *et al.*, 1991). The characterization of grain proteins of amaranth has been carried out using different techniques of extraction and electrophoresis (Barbra de la Rosa, Gueguen, *et al.*,1992; Barbra de la Rosa, Paredes-Lopez, *et al.*,1992; Gorinstein, Denue, et al., 1991; Gorinstein, Moshe, *et al.*, 1991). On the basis of differential extraction, the amaranth albumin was classified as albumin 1 and 2 (Konishi *et al.*, 1991). Albumin 1 is extractable with water and/or saline solution, whereas albumin 2 is extractable with water after the removal of albumin 1 and globulin with saline solution. Albumin 2 consists of amarantin as the major protein component (Martinez *et al.*, 1997). The subunit size of albumin proteins varied from 10 to 37 kDa (Barbara de la Rosa, Gueguen, *et al.*, 1992; Gorinstein, Moshe, *et al.*, 1991), with low-molecular-weight subunits being more abundant (Segura-Nieto *et al.*, 1992). Barbara de la Rosa *et al.* (2009), however, differentiated the albumin fraction into two groups of proteins corresponding to approximately 18 kDa and between 40 and 80 kDa. The proteins of approximately 18 kDa were termed as methionine-rich proteins due to their high methionine content (16–18%) (Segura-Nieto *et al.*, 1994).

Determination of sedimentation coefficient by centrifugation has also been widely used to characterize the proteins. On the basis of sedimentation coefficients, the amaranth seed globulins are categorized into 10S and 12.7S compared to 7/8S and 11/12S for the legume seed globulins. The electrophoretic behavior of 10S and 12.7S amaranth globulin fractions on denaturing gel was observed to be similar to that of 7S and 11S storage proteins of legumes and hence referred to as 7S and 11S, respectively (Barba de la Rosa, Moshe, *et al.*, 1992). The higher sedimentation coefficients of amaranth globulins, as observed on linear sucrose gradients, suggested that these proteins contain polypeptides of higher molecular weight than those present in 7S and 11S from pea globulins (Segura-Nieto *et al.*, 1992). The 7S and 11S amaranth seed globulins also differed in their solubility in salt solution, with the former being extractable with 0.1 M and the latter with 0.8 M NaCl (Barbara de la Rosa *et al.*, 2009). The 7S globulin fraction of amaranth grain was characterized by the presence of a main band of 38 kDa and lacked disulfide bridges, whereas the 11S-like globulins consisted of both acidic (35–38 kDa) and the basic polypeptides (22–25 kDa). These results are in agreement with previous studies that found that globulins consisted of polypeptides of heterogeneous sizes, as demonstrated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analyses (Segura-Nieto *et al.*, 1992). However, these observations contradicted the findings of Gorinstein, Moshe, *et al.* (1991), who reported that the globulin was composed of polypeptides of only 14–18 kDa. Martinez *et al.* (1997) proposed that both 7S and 11S globulins correspond to one type of globulin, whereas polymerized globulins (albumin 2) and glutelins correspond to two other types of globulins. However, this notion needs to be verified by establishing sequence homology and by proving a common genetic origin of globulin, albumin 2, and glutelin.

The most important component of globulins is amarantin, which alone constitutes 90% of the total globulins and approximately 19% of the total grain protein (Romero-Zepada and Paredes-Lopez, 1996). Amarantin is a homohexameric molecule of approximately 300–400 kDa comprising subunits of approximately 59 kDa. Each of the subunits consists of an acidic polypeptide of 34–36 kDa and basic polypeptide of 22–24 kDa linked by disulfide bonds. The additional subunit of 54 kDa present in amarantin has been proposed to act as an inducer of polymerization (Martinez *et al.*, 1997).

The amaranth glutelin showed high similarity with 11S globulins (Abugoch *et al.*, 2003) and comprised three major polypeptides groups of 22–25, 35–38, and approximately 55 kDa. It is likely that both glutelins and 11S globulins may belong to the same structural gene family. The differences in composition of alcohol-soluble proteins have been reported in various studies. Gorinstein, Moshe, *et al.* (1991) observed only low-molecular-weight subunits of 10–20 kDa on SDS-PAGE analysis of the alcohol-soluble proteins, whereas Barba de la Rosa, Gueguen, *et al.* (1992) reported the presence of subunits of both low and high molecular mass. Furthermore, great similarity between the electrophoretic pattern of reduced prolamine and glutelins was also observed in the latter study. The lack of consistency in the composition of various protein fractions of amaranth grain, evident from the literature, may be due to the different procedures used in the extraction and analyses. In view of the nutritional importance of amaranth, it is imperative that a systematic study be undertaken to analyze the proteome of amaranth leaves and grains by employing the latest techniques of proteomics. This will enable the identification and characterization of nutritionally important proteins, the genes for which can then be cloned and expressed heterologously in other crops to enhance their nutritive value.

NUTRACEUTICAL PROPERTIES OF AMARANTH PROTEINS

The albumins and globulins are rich in lysine and valine, whereas prolamins have a comparatively higher content of methionine and cysteine. Glutelins, on the contrary, contain higher levels of leucine, threonine, and histidine. Compared to legume grain albumins, which contain several antinutritional factors, the amaranth albumin fraction is considered safe. The amaranth albumin fraction is comparable with egg-white proteins and can be used as an egg substitute in different products. The 11S globulin fraction is rich in peptides of angiotensin-converting enzyme inhibitor, whereas the glutelin fraction contains antihypertensive activity as well as the anticarcinogenic lunasin-like peptide (Silva-Sanchez *et al.*, 2008), thus signifying its nutraceutical properties.

TRANSGENIC APPLICATIONS

Improving the balance of essential amino acids in important crop plants remains one of the major objectives of plant breeders. Transgenic technology presents an attractive alternative for improving the nutritional quality of grain proteins. Heterologous transgenic expression of storage protein genes with higher levels of limiting amino acids has been reported. Transgenic expression of high levels of a particular amino acid may adversely affect the normal physiology of seed development or produce seeds with a biased amino acid composition. Therefore, expressing a gene for a heterologous protein with a balanced amino acid composition is a better alternative. A gene of a 35-kDa albumin protein (AmA1), which is expressed during early to mid-maturation stages of embryogenesis in the amaranth seed, has been cloned (Raina and Datta, 1992). The amino acid composition of this protein meets the World Health Organization's recommended values for a highly nutritional protein because it is rich in various essential amino acids. Potato is the most important noncereal crop in terms of total global food production; therefore, transgenic expression of this gene in the tubers of this crop has been achieved. Heterologous expression of AmA1 under constitutive (CaMV 35S promoter) and tuber-specific promoter (granule-bound starch

synthase) in potato resulted in significant enhancement in total protein content with an increase in essential amino acids (Chakraborty *et al.*, 2000). Furthermore, the growth and production of tubers in transgenic plants were also higher compared to those of control plants.

Maize, which is a staple food in many countries but lacks essential amino acid in the grains, has also been targeted for heterologous expression of amaranth proteins to enhance the nutritional quality of its protein. A complementary DNA of an 11S globulin storage protein, amarantin, which has a high content of essential amino acids, was expressed in maize under CaMV 35S promoter and an endosperm-specific promoter (rice glutelin-1) (Rascón-Cruz, 2004). Heterologous expression of this gene resulted in an increase of 18% in lysine, 28% in sulfur-containing amino acids, and 36% in isoleucine, in addition to a 32% increase in total seed protein. Furthermore, the heterologously expressed protein was digested by simulated gastric and intestinal fluids, thus confirming the biodigestibility of the transgenic protein. Therefore, these studies validate the potential of using different amaranth genes for supplementing and complementing the proteins in both cereal and noncereal staple crops. However, detailed analysis of proteomes of different amaranth species needs to be carried out to identify the candidate gene(s) that can be employed for transgenic improvement. Furthermore, generation of mutants in amaranth is also required to determine the role of specific proteins in growth and development of the plant so that appropriate improvement of the germplasm can be undertaken through conventional breeding strategies.

TECHNOLOGICAL ISSUES

The diversity in composition among different amaranth genotypes necessitates in-depth characterization of biochemical constituents for its specific applications in the food industry. The smaller size granules in amaranth starch, which are similar to the size of fat globules of cow's milk, can be exploited to mimic fat in a number of food products. Some of the genotypes have higher polyphenols with higher antioxidant activity, which could also be utilized in the development of new products. Amaranth grain has the potential to be used in the development of various food products for people suffering from celiac disease, a disorder that makes the body intolerant to gluten proteins.

SUMMARY POINTS

- Amaranth grain is a good source of dietary fiber and has a high glycemic index. It is low in resistant starch, and its starch has uniquely small granules with a low tendency toward retrogradation.
- Grain amaranth has a higher protein content than most of the cereal grains and is an appropriate food for people who are allergic to gluten.
- Amaranth grain oil is quite similar to that of cereals, being high in unsaturated fatty acids and containing mainly linoleic acid. Its oil also contains tocotrienols that are associated with cholesterol-lowering activity in mammalian systems. Amaranthus grain oil contains a significant amount of squalene.
- Amaranth grain proteins are composed mainly of three major fractions—albumins, globulins, and glutelins—with little or no storage prolamin. Amarantin is the most important component of globulins and constitutes 90% of the total globulins and approximately 19% of the total grain protein.
- Heterologous expression of the amarantin gene (AmA1) in potato resulted in significant enhancement in total protein content with an increase in essential amino acids.

- Amaranth is a good source of minerals such as iron, magnesium, phosphorus, copper, and manganese. Because of its unique composition, it is an attractive food complement and supplement.

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CHAPTER 11

Quinoa: Protein and Nonprotein Tryptophan in Comparison with Other Cereal and Legume Flours and Bread

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

HPLC High-performance liquid chromatography
NAD Nicotinamide adenine dinucleotide
NADP Nicotinamide adenine dinucleotide phosphate

INTRODUCTION

Throughout the world, cereals are an essential part of daily nutrition. The wheat used to produce bread, in addition to being the principal source of energy, is also an important nutritional source of protein. However, the protein quality of cereal flours is poor, or may be adequate if ingested in large enough amounts, and thus it must be improved to satisfy the need

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for the limiting essential amino acids. In fact, protein quality, which determines the nutritional value of a food, depends on the essential amino acid composition. The cereals provide a good content of proteins, but these are deficient in some essential amino acids, particularly lysine, threonine, and tryptophan, the latter usually the second limiting amino acid (Friedman, 1996). Therefore, alternate locally available flours must be studied as a substitute or as an integrator for wheat flour in making bread. In several countries, the production of wheat is insufficient. Thus, to reduce the importation of wheat it is also necessary to use indigenous non-wheat flours. Several investigations (Chavan and Kadam, 1993; Friedman, 1996) have been performed to optimize the biological utilization of proteins and to study the possibility of incorporating other cereal flours, such as rice, maize, barley, finger millet, sorghum bicolor, rye, with that of wheat for producing bread, biscuits, and other bakery products. In addition, to improve the nutritional value of bread, in many countries supplementation with legume flours, which contain a higher amount of protein compared to the cereal flours, is used (Chavan and Kadam, 1993). Legume flours are a relatively cheap protein source, and they can be employed as ingredients in the elaboration of a great variety of foods for human consumption because they can, based on their good protein characteristics, improve the amino acid profile of foods such as bread. In fact, the legume proteins are rich in lysine and tryptophan, of which cereals are deficient. Therefore, the supplementation of bread or bakery products with legume proteins improves the amino acid balance of these products, providing all the essential amino acids necessary to meet human nutritional requirements except for the sulfur amino acids methionine and cysteine, which are deficient (Friedman, 1996). However, legume proteins are better compared to those of wheat.

For these reasons, legume proteins may be used as a protein supplement in wheat bread formulations, improving the nutritional value at the same time. However, legume flours can be added to wheat flour usually at 5–25%, depending on the kind of legume flour used for bread making, in order not to alter the good sensory characteristics of the obtained products that were not always found acceptable (Chavan and Kadam, 1993).

Although much research has been carried out on cereal and legume proteins and their amino acid composition, scant information is available on the presence of protein and nonprotein tryptophan (free and protein-bound) in cereals and legumes. This chapter describes the techniques used to analyze protein and nonprotein tryptophan, reports their contents in cereal and legume flours, compares the concentrations of this amino acid in these flours, and highlights the importance of nonprotein tryptophan in calculating the nutritional value of foods.

In addition, quinoa is taken into consideration as an alternative protein source whose nutritional value is similar to that of milk (Koziol, 1992). Quinoa (*Chenopodium quinoa* Willd of the Chenopodiaceae family) is a dicotyledonous indigenous plant of the Andes growing at an altitude higher than 4000 m. It is still widely cultivated in South America, and it is considered an excellent pseudocereal for its nutritional characteristics. Production in the United States has been successful, and agronomists are investigating suitable regions of the United States and Europe in which to grow this crop as a new food resource (Galwey *et al.*, 1990) because of its high protein content. Its amino acid composition is similar to that of milk protein (Koziol, 1992) and close to the ideal protein balance recommended by the Food and Agriculture Organization of the United Nations. Quinoa has also received considerable attention as a potential crop from the National Aeronautics and Space Administration (Schlick and Bubenheim, 1996).

TECHNOLOGICAL ISSUES

Cereal and legume seeds

The cereals considered were wheat (*Triticum aestivum*); rice (*Oryza sativa*); maize (*Zea mays*); barley (*Hordeum vulgare*); oat (*Avena sativa*); rye (*Secale cereale*); spelt (*Triticum spelta*); pearl

millet (*Panicum miliaceum*); two hybrids of sorghum bicolor (sorghum Kalblank and sorghum DK 34—Alabama); sorghum and millet from Orissa, India; and the pseudocereal quinoa (*Chenopodium quinoa* Willd), variety Sajama, harvested in rural areas of San Juan, Bolivia.

The legume seeds studied were soy (*Glycine max*), bean (*Phaseolus vulgaris* L.), broad bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris*), lupine (*Lupinus* sp.), pea (*Pisum sativum* L.), vetch (*Vicia sativa* L.), and peanut (*Arachis hypogaea*).

Dry seed samples of both cereals and legumes were ground to a fine powder in a mortar with a pestle or in a coffee mill, and the flours were passed through a 300-μm sieve. Only the peanuts were defatted before sieving.

Protein content

Protein determination was carried out by assaying nitrogen using the micro-Kjeldahl method. The nitrogen percentage was converted to crude protein using a factor of 6.25.

Tryptophan

ANALYSIS OF PROTEIN TRYPTOPHAN

Because tryptophan is destroyed by acid hydrolysis when amino acid analysis of protein is carried out, a procedure must be done involving alkaline hydrolysis. The analysis of protein tryptophan in 1 g of dry flour was done in triplicate by high-performance liquid chromatography (HPLC) according to the method of Slump *et al.* (1991), based on the alkaline hydrolysis of flours in $Ba(OH)_2$ performed at 130°C for eight hours. The pH was then adjusted to 4.5 with concentrated HCl after cooling at room temperature. A solution of 5-methyltryptophan was added as an internal standard to correct tryptophan losses during hydrolysis. The volume was adjusted with distilled water to 50 ml. The suspension was mixed and filtered. An aliquot of the filtrate was diluted at least threefold with buffer. The mixture was homogenized and filtered; the filtrate was analyzed by HPLC equipped with a fluorescence detector using a Zorbax extended C18 (3 × 250 mm) column. The eluting solvent was 0.1 Na-acetate (42.5%)/0.1 M acetic acid (42.5%)/methanol (15%).

ANALYSIS OF NONPROTEIN TRYPTOPHAN (FREE + PROTEIN-BOUND)

The procedure was carried out as reported by Comai *et al.* (2007a,b). In brief, 1 g of the dry flour of each type of cereal was defatted by suspension in 10 ml of acetone and stirred for 30 min at 37°C.

The organic layer was removed by centrifugation at 12,000 rpm for 10 min at 0°C. The remaining pellet was treated with an additional 10 ml of acetone, shaken for 10 min, and centrifuged. The organic layer was removed, and the dried sediment was extracted with 10 ml of distilled water for 30 min at 37°C under shaking. After centrifugation, the supernatant, containing the free tryptophan and the water-soluble protein fraction, was collected. The residue was extracted again with a further 10 ml of distilled water for 30 min, and after centrifugation the supernatants were combined. An aliquot (5 ml) was ultrafiltered by an Amicon model 12 ultrafiltration cell with an XM-50 Diaflo membrane (Amicon, Oosterhout, Holland) collecting the first 500 μl of ultrafiltrate used to determine the free fraction of tryptophan on a combined HPLC—fluorescence system using 0.1 ml of the ultrafiltrate.

The remaining part of the supernatant was analyzed for the determination of total water-soluble nonprotein tryptophan, using only 0.02 ml of solution. The sediment obtained after extraction with water was resuspended in 5 ml of 0.1 M potassium phosphate buffer (pH 8.9), shaken for 30 min, and then centrifuged. The supernatant was analyzed by HPLC to determine the amount of nonprotein tryptophan eventually bound to water-insoluble proteins.

Cereal and legume flours

The most used sources of proteins for supplementation of bread are flours of cereals other than wheat and legumes. Chavan and Kadam (1993) and Friedman (1996) reported the nutritional enrichment of bakery products by supplementation with non-wheat cereal and legume flours and the nutritional value of proteins from different food sources, respectively.

NON-WHEAT CEREALS

Several non-wheat cereal flours are used for substitution of a portion of wheat flour to improve the protein content in breads. The non-wheat cereals include rice, maize, barley, oat, rye, spelt, millet, sorghum, and the pseudocereal quinoa:

- Rice flour is used in a ratio of 1:1 with wheat flour in the preparation of bread, and it has been found to improve the baking quality of baked products (Chavan and Kadam, 1993). The rice protein is rich in lysine compared to that of wheat and has a better amino acid balance. Therefore, the quality of protein is better than that of wheat (Friedman, 1996).
- Maize is the cereal that provides more than half of the daily calorie and protein intakes. Defatted maize germ flour has been used as a nutrient fortifier for bread (Tsen *et al.*, 1974). The opaque-2 gene in maize plants increases the contents of lysine and tryptophan, improving the quality of its protein (Mertz, 1978).
- Barley is used largely in the brewing of beer. Investigations have been performed to improve the contents of protein and lysine and the nutritional characteristics in new varieties of barley (Anjum *et al.*, 1991).
- Oatmeal is also used in the preparation of bakery products because it improves protein content and has a good amino acid balance.
- Rye flour, when mixed with wheat flour, seems to improve nutritional properties of bread.
- Millet and sorghum flours can be blended with wheat flour to produce bakery products. Wheat flour has been blended at different levels with sorghum flour to produce breads in Senegal and Sudan (Perten, 1983) and Nigeria (Olatunji *et al.*, 1982). However, lysine appears to be the first limiting amino acid.
- Quinoa is a pseudocereal originating in Latin America that grows at high altitudes. It is a good source of proteins, and because of its high quality, it can replace animal proteins in the diet. It is very rich in lysine compared to cereals, and the amino acid composition of protein is well balanced. Quinoa seeds are used boiled like rice, or the flour is used to thicken soup or as porridge or to make noodles.

Protein Content Protein content differs greatly in dry flours of quinoa and of more common cereals (Figure 11.1A). The protein content of wheat flour is similar to those of spelt and quinoa, but it is higher in comparison with the flour of cereals such as rice, maize, barley, oat, rye, pearl millet, *Sorghum bicolor* Kalblank hybrid, and sorghum from Orissa, India. Millet harvested in a tribal village of Orissa, India, and *Sorghum bicolor* DK 34—Alabama hybrid show markedly lower protein content compared to other cereals.

Protein Tryptophan Protein tryptophan differs greatly in the proteins of cereals. Wheat flour has a value similar to those of spelt and quinoa but much higher than those of the other cereals. Maize contains the lowest amount. *Sorghum bicolor* Kalblank is richer in protein tryptophan than sorghum from Orissa and *S. bicolor* DK 34—Alabama. Also, pearl millet contains more protein tryptophan than does millet from Orissa. Moreover, the values are similar to those of the flours of *S. bicolor* Kalblank, oat, barley, and rice, whereas rye flour has a lower value.

Calculating the values of protein tryptophan as milligrams/100 g of protein, millet flour from Orissa has greater amounts of tryptophan compared with maize, sorghum from Orissa, and rye flours (see Figure 11.1B). Rice, spelt, wheat, quinoa, and the two *S. bicolor* hybrid flours have similar values, as do oat, pearl millet, and barley flours.

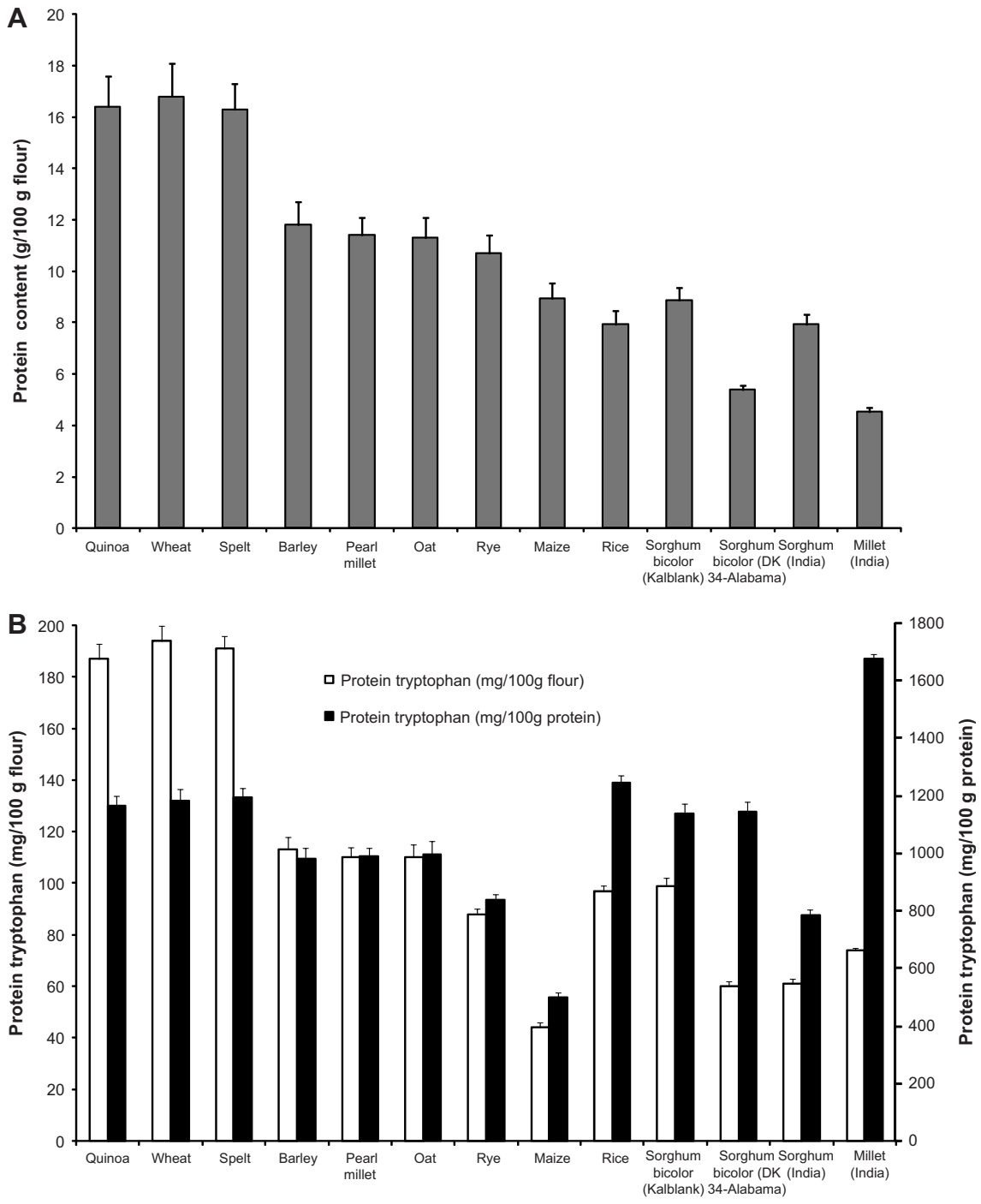

FIGURE 11.1
Protein content (A) and protein tryptophan (B) in flours of quinoa and common cereals. Quinoa has protein content and protein tryptophan values similar to those of wheat and spelt and higher than those of the other cereals. Data are mean ± SEM, n = 3. *Source: Adapted from* Food Chemistry, *100, Comai, S., Bertazzo, A., Bailoni, L., Zancato, M., Costa, C. V. L., and Allegri, G., The content of proteic and nonproteic (free and protein-bound) tryptophan in quinoa and cereal flours, pp. 1350–1355, copyright 2007, with permission from Elsevier.*

Free and Protein-Bound Tryptophan Tryptophan is the least represented amino acid in the vegetable proteins, in which it is present in 1%, whereas the animal proteins contain 1.5%. It is not only required for protein synthesis but also is the precursor of the neurotransmitter serotonin and the hormone melatonin. It is also the major source of the

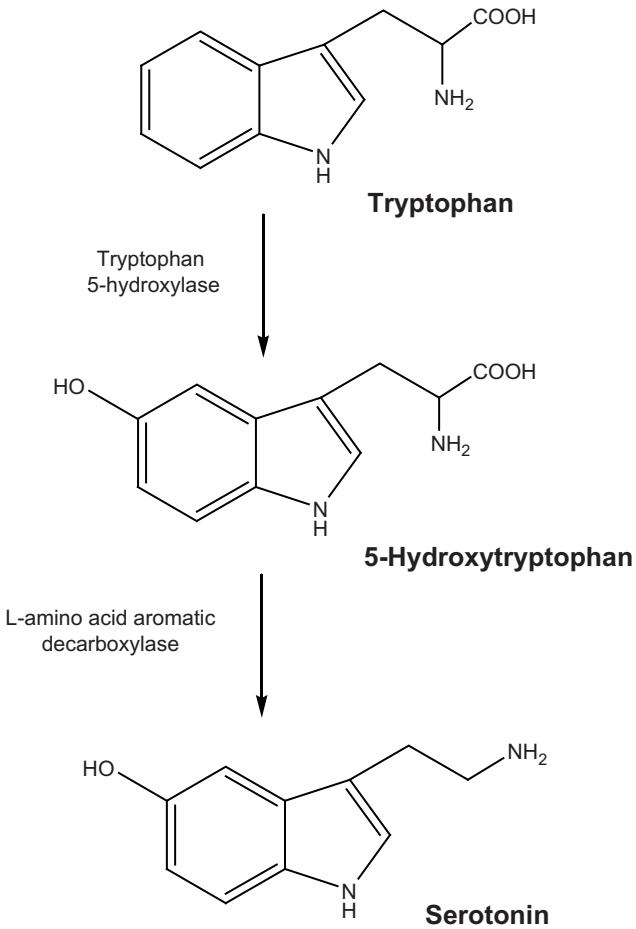

FIGURE 11.2
Tryptophan is the precursor of the neurotransmitter serotonin. Tryptophan is converted to 5-hydroxytryptophan by the enzyme tryptophan 5-hydroxylase (EC 1.14.16.4), the limiting enzyme of the biosynthetic pathway. 5-Hydroxytryptophan is then converted to serotonin by the enzyme L-amino acid aromatic decarboxylase (EC 4.1.1.28).

nicotinamide-containing coenzymes NAD and NADP. In humans, tryptophan is the only amino acid circulating bound to serum albumin in amounts ranging between 80 and 90%, with the remaining 10–20% in the free form. Only the latter is able to enter the brain and be utilized for the synthesis of cerebral serotonin (Figure 11.2) (Knott and Curzon, 1972). Comai *et al.* (2007a,b) found that in cereals and legumes, tryptophan is also present as a constituent of proteins and in the nonprotein form, free and in part bound to proteins. Allegri *et al.* (1993) observed that tryptophan is also present in nonprotein forms in cow and human milk.

Among the cereals, the free form of tryptophan (Figure 11.3) is present in greater amounts in the flours of spelt, barley, and pearl millet, whereas lesser amounts are contained in rice, maize, sorghum and millet from Orissa, sorghum DK34—Alabama, and rye. The flours of sorghum Kalblank, oat, wheat, and quinoa show similar values.

The protein-bound tryptophan in cereal flours is present both in the water-soluble fraction and in the fraction extractable at pH 8.9 (see Figure 11.3). The protein-bound tryptophan, water-soluble fraction, is contained in the highest amount in spelt. Barley, wheat, and pearl millet flours show similar values as do oat, quinoa, rye, and sorghum Kalblank. In rice, maize, sorghum and millet from Orissa, and sorghum DK34—Alabama, the values are lower than in the other cereals.

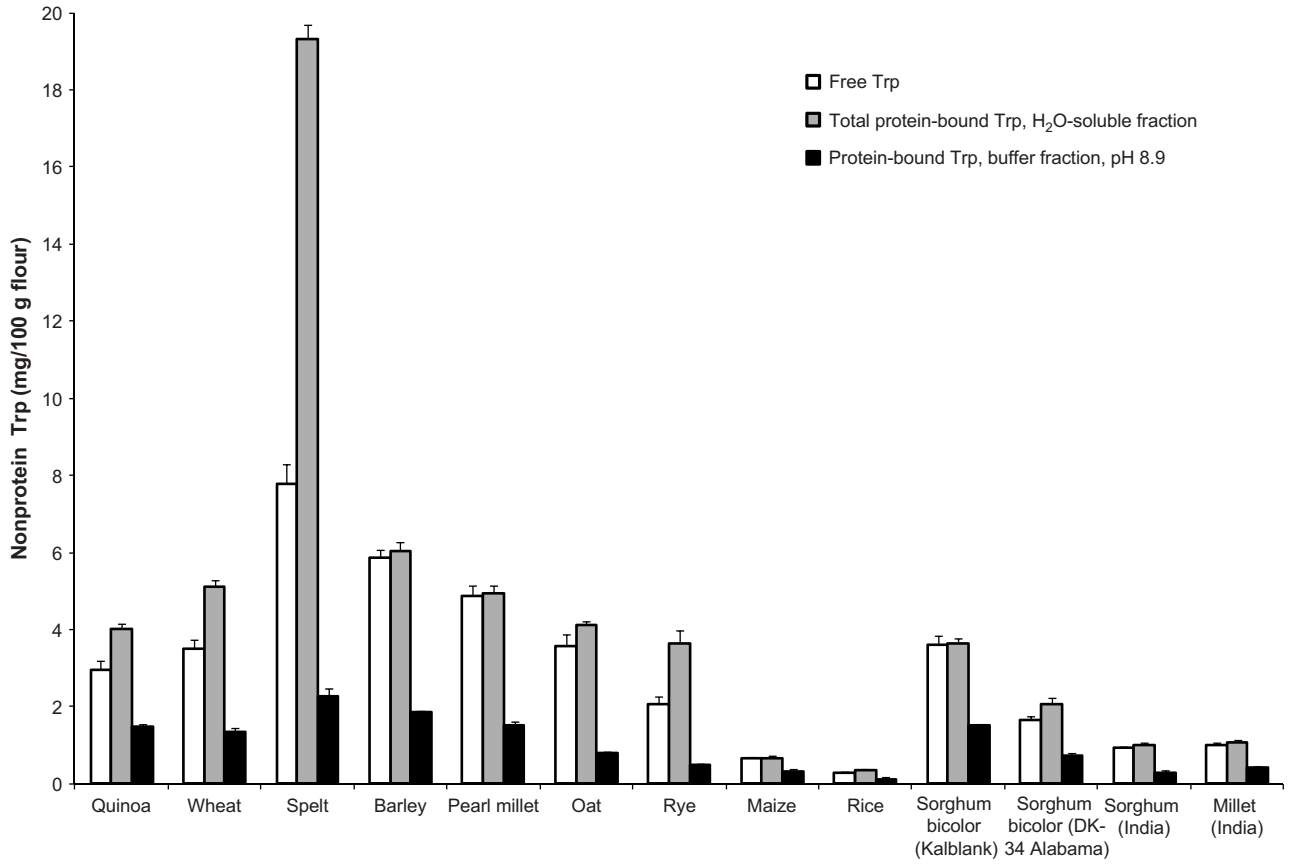

FIGURE 11.3

Levels of nonprotein tryptophan fractions (free, total nonprotein tryptophan (free + protein-bound) water-soluble fraction, and bound to proteins soluble at pH 8.9) in flours of quinoa and common cereals. Spelt has the highest amount of nonprotein tryptophan. Quinoa has values similar to those of oat and rye. Data are mean ± SEM, $n = 3$. *Source: Adapted from* Food Chemistry, *100, Comai, S., Bertazzo, A., Bailoni, L., Zancato, M., Costa, C. V. L., and Allegri, G., The content of proteic and nonproteic (free and protein-bound) tryptophan in quinoa and cereal flours, pp. 1350–1355, copyright 2007, with permission from Elsevier.*

With regard to the protein-bound tryptophan extracted at pH 8.9, sorghum Kalblank, pearl millet, quinoa, and wheat flours contain similar quantities. Spelt and barley have higher contents, and rice, sorghum from Orissa, maize, millet from Orissa, rye, sorghum DK34–Alabama, and oat flours have the lowest.

LEGUME FLOURS

Legumes, such as soybean, bean, broad bean, chickpea, lentil, lupine, pea, vetch, and peanut, together with cereals are the major source of proteins in the human diet. Whereas legumes, which are rich in lysine and tryptophan, are poor in the sulfur amino acids methionine and cysteine, cereals are poor in lysine and tryptophan. Therefore, the proteins of legumes mixed with cereals supply the respective limiting essential amino acids.

Soybean is the most commonly employed legume for enrichment of bread and other bakery products because it supplies proteins and it improves the balance of the amino acid composition.

The influence of soy flour on bakery products has been studied extensively (Chavan and Kadam, 1993). There is an increasing trend to integrate soybean into wheat bread. The nutritional value of soy flour is better than that of wheat and other cereals.

Bean flour has also been tested to enrich the protein content of wheat flour (Sathe *et al.*, 1981).

Broad bean flour has been used as a protein supplement for wheat bread to improve the essential amino acid content. Abdel-Kader (2001) observed that the addition of 20% broad bean flour to Egyptian "Balady" bread increased the protein content 36% and improved the quality of protein.

Chickpea seeds are considered to be a nutritionally important protein source (Tavano *et al.*, 2008). A 15–20% addition of whole chickpea flour to wheat flour produced acceptable breads with improved nutritional composition (Finney *et al.*, 1982). However, Combe *et al.* (1991) observed that in rats, methionine from chickpea flour was not fully utilized, whereas Clemente *et al.* (1998) found that this amino acid was not limiting.

Lentil and pea proteins isolated in an amount of 5–8% have also been added to wheat flour to prepare breads (Hsu *et al.*, 1982).

Lupine flour, in a quantity of 5–20%, can fortify wheat flour when making bread (Gross *et al.*, 1983). Studies by Ballester *et al.* (1984) demonstrated that the supplementation of wheat flour with full-fat lupine flour in an amount of 3–12% in the preparation of bread yielded an acceptable product. The addition of lupine flour improved the content and quality of proteins in composite bread.

Peanuts have been investigated to replace a portion of wheat flour and to enrich the protein content of cookies and biscuits (Chavan and Kadam, 1993). Khalil and Chughtai (1983) reported that the replacement of 20% of wheat flour with defatted peanut flour yielded excellent breads with an increased protein content from 12.5 to 20.5%.

Protein Content Figure 11.4A shows the protein content in common legumes. Lupine and soybean flours have the highest values, followed by peanuts, beans, broad beans, and lentils. Vetch, chickpea, and pea flours have the lowest values.

Protein Tryptophan Protein tryptophan contents appear to vary significantly among legumes (see Figure 11.4B). Soybean flour shows the highest protein tryptophan content and pea flour the lowest. The values of protein tryptophan concentrations in beans are similar to those reported by Mbithi-Mwikya *et al.* (2000), and lupines have values comparable to those found by Mossé *et al.* (1987). Peanut flour has a higher value than chickpea, broad bean, lentil, and vetch flours. Soybean flour has the highest value when the content of protein tryptophan is calculated as milligrams/100 g of protein. Bean and chickpea present higher values than peanut, whereas broad bean, pea, vetch, lentil, and lupine flours show the lowest values.

Free and Protein-Bound Tryptophan Like cereals, legumes also contain tryptophan in free form and bound to proteins (Figure 11.5) (Comai *et al.*, 2007a,b). The highest concentration of free tryptophan is present in chickpea flour. Significantly lower values appear in bean and soybean flours, and the lowest values occur in the other legumes. The contents of free tryptophan are similar between lupine and vetch flours, whereas pea flour is richer than broad bean, lentil, and peanut flours.

Figure 11.5 shows the data for total nonprotein tryptophan, free + protein-bound, water-soluble fraction. The highest content is present in chickpea flour, followed by bean and soybean flours. Lupine and vetch flours contain similar amounts, followed by pea flour. Water-soluble nonprotein tryptophan is contained in similar amounts in broad bean and lentil flours. The lowest value is present in peanut flour. Protein-bound tryptophan is also present in the fraction obtained by extraction at pH 8.9 (see Figure 11.5).

Tryptophan bound to basic proteins is contained in smaller quantities in legume flours with respect to the protein-bound, water-soluble fraction. The highest content is present in bean flour, followed by chickpea flour. Lupine and soybean flours show similar values, but higher than those of vetch, pea, and lentil, whereas broad bean and peanut flours show the lowest values.

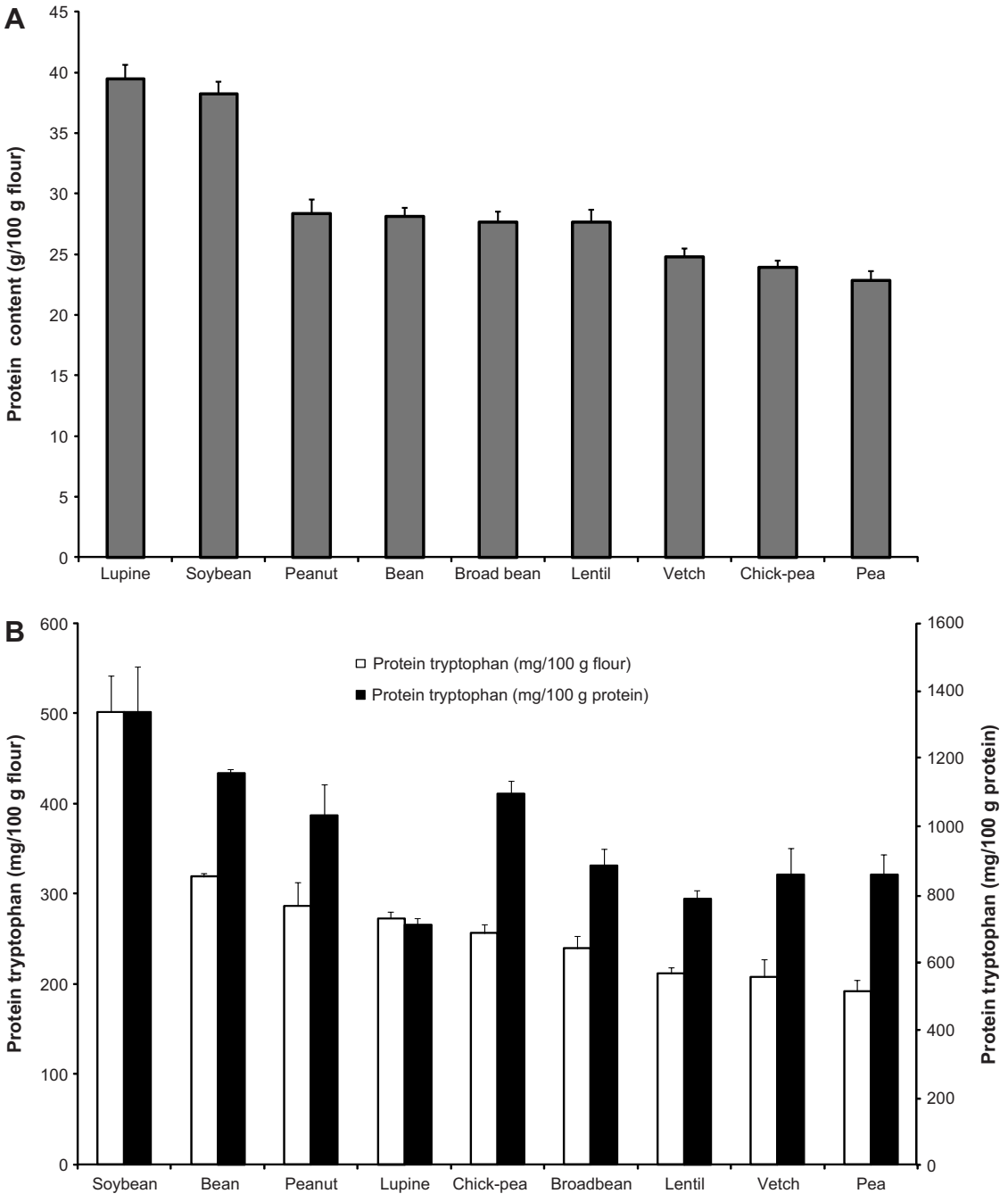

FIGURE 11.4

Protein content (A) and protein tryptophan (B) in flours of common legumes. Soybean and lupine present the highest protein content among the analyzed legumes. Soybean also has the highest amount of protein tryptophan. Data are mean ± SEM, $n = 3$. *Source: Adapted from* Food Chemistry, *103, Comai, S., Bertazzo, A., Bailoni, L., Zancato, M., Costa, C. V. L., and Allegri, G., Protein and non-protein (free and protein-bound) tryptophan in legume seeds, pp. 657–661, copyright 2007, with permission from Elsevier.*

NUTRITIONAL ASPECTS

Several studies have been carried out to improve the nutritive value of bread, supplementing wheat flour with protein-rich non-wheat sources, such as non-wheat cereals and legume flours. Composite flours, obtained by enriching the wheat flour with legume flours at 10–25% or more depending on the type of protein source, have given successful results due to the high

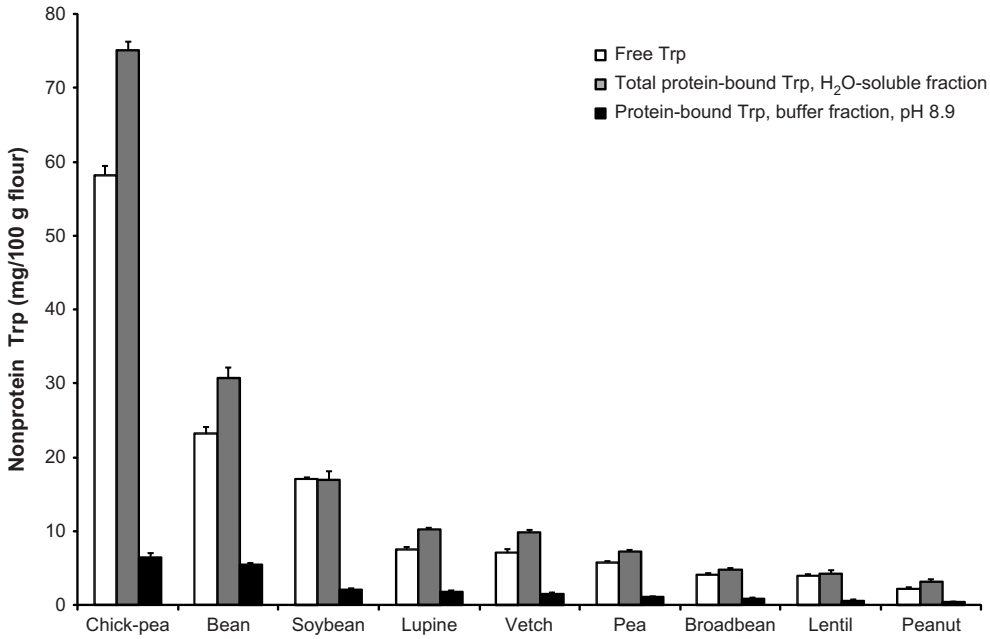

FIGURE 11.5
Levels of nonprotein tryptophan fractions (free, total nonprotein tryptophan (free + protein-bound) water-soluble fraction, and bound to proteins soluble at pH 8.9) in flours of common legumes. Chickpea presents the highest value of nonprotein tryptophan, followed by bean, whereas peanut has the lowest. Data are mean ± SEM, $n = 3$. *Source: Adapted from* Food Chemistry, *103, Comai, S., Bertazzo, A., Bailoni, L., Zancato, M., Costa, C. V. L., and Allegri, G., Protein and non-protein (free and protein-bound) tryptophan in legume seeds, pp. 657–661, copyright 2007, with permission from Elsevier.*

levels of protein in legume flours, which, mixed with wheat flour, have the advantage of improving the nutritional value because of the better composition of amino acids.

Soy flour, which is more economical, is the leading protein source. In fact, several investigations have demonstrated that the fortification of wheat flour with soy flour increases the content and the protein quality of bread and other bakery products. Currently, nutritionists are evaluating the pseudocereal quinoa as an alternative to cereals in the human diet for its nutritional value. Quinoa flour contains protein and protein tryptophan in quantities similar to those of wheat and spelt but markedly higher than those of other cereals. However, the amino acid profile of quinoa is better, being similar to that of milk, than that of common cereals and also of legumes. Therefore, the use of quinoa should be promoted to enrich the nutritional value of bakery products.

The amino acid composition is often used to define the nutritional quality of a protein. Tryptophan is the least represented amino acid in the protein of cereals, which are an essential part of daily nutrition.

In both cereal and legume flours, tryptophan is also present in free and protein-bound forms. Among cereals, spelt flour, which is rich in protein tryptophan, contains both free and protein-bound tryptophan in considerably higher amounts compared to those of all cereals and quinoa. Hence, this cereal should receive more consideration.

Regarding legumes, they are good sources of proteins, and the highest levels were found in lupine and soybean flours; however, protein tryptophan and nonprotein tryptophan (free and protein-bound) are markedly higher in soybean than in lupine flour. Lupines in South America are of great importance in the provision of human food. However, among all legumes tested, chickpea flour shows the highest values in nonprotein tryptophan. Because

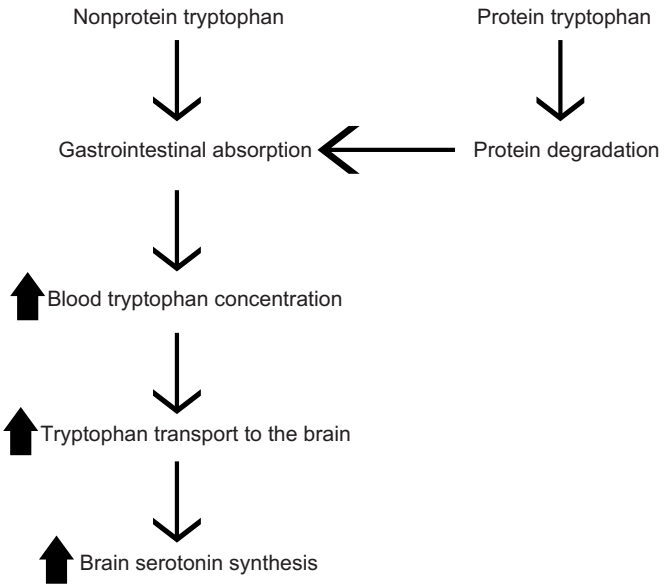

FIGURE 11.6
Tryptophan after gastrointestinal absorption increases brain serotonin synthesis. Nonprotein tryptophan is directly absorbed at the gastrointestinal (GI) level, quickly increasing its brain availability for brain serotonin synthesis. Because protein tryptophan requires protein degradation before its GI absorption, its availability for brain serotonin synthesis is longer.

chickpea flour is also rich in protein tryptophan, it appears to be an important source of vegetable protein, and its use, such as in the fortification of wheat flour, should be encouraged.

Information on the free or protein-bound tryptophan that is nonprotein tryptophan in foods is very scarce. To know its content in foods is of considerable importance because it is one of the essential limiting amino acids of biological value in vegetable proteins. Legumes show a high content of both protein and nonprotein tryptophan. The latter is easily absorbable in humans and can increase the availability of tryptophan to the brain because only the free form can cross the blood–brain barrier, thus influencing the synthesis of the neurotransmitter serotonin (Figure 11.6). In addition, the nutritive value of vegetable proteins must be corrected, considering also the presence of nonprotein tryptophan.

SUMMARY POINTS

- The nutritional value of bread can be improved by making composite flours with non-wheat cereals or supplementing its flour with protein-rich sources, such as legume flours, especially in countries in which the production of wheat is insufficient.
- Quinoa can be an alternative to cereals in the human diet because its nutritional value and high quality proteins are similar to those of milk. Thus, its use should be promoted to enrich the nutritional value of bakery products.
- Tryptophan is present in cereals and legumes not only as protein tryptophan but also as nonprotein tryptophan (Figure 11.7).
- Nonprotein tryptophan should be taken into account when the nutritional value of a food is determined.
- The determination of nonprotein tryptophan in cereals and legumes is very important because this fraction is easily absorbable at the gastrointestinal level, increasing its availability for brain serotonin synthesis.

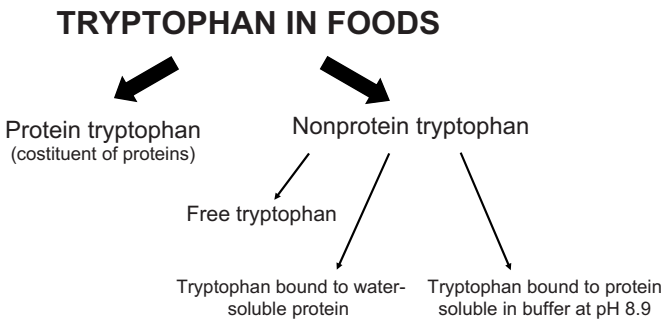

FIGURE 11.7
Tryptophan in foods. Tryptophan is present in foods as protein tryptophan (constituent of proteins) and nonprotein tryptophan (free and bound to water-soluble protein and to protein soluble at pH 8.9).

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CHAPTER 12

Sorghum Flour and Flour Products: Production, Nutritional Quality, and Fortification

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

GAX Glucuronoarabinoxylans
HMW High molecular weight
NSP Non-starch polysaccharides
RDA Recommended Dietary Allowance

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is a tropical cereal. The sorghum is a naked grain—that is, during threshing the glumes are removed from the grain. Thus, there is no husk to remove during milling. The grain is approximately 4 mm in length, and it is more or less

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spherical in shape but somewhat flattened at the germ end (Serna-Saldivar and Rooney, 1995). The 1000 kernel weight ranges from approximately 25 to 35 g. Sorghum grain color varies from almost white to almost black, with shades of red and brown being common (Taylor and Emmambux, 2010). Grain color strongly affects flour color.

Sorghum is uniquely adapted to arid and semi-arid conditions, and it can also survive periods of waterlogging (Doggett, 1988). It is the fifth most important cereal crop worldwide, with more than 63 million tons produced from approximately 47 million ha of land in 2007. The 10 leading sorghum-producing countries in decreasing order are the United States, Nigeria, India, Mexico, Sudan, Argentina, China, Ethiopia, Burkina Faso, and Egypt. In the United States, Mexico, and Argentina, sorghum is mainly used for animal feed. However, in the other countries, particularly in Africa and India, it is mostly used for human food and for brewing beer. Sorghum is a gluten-free cereal (Ciacci *et al.*, 2007), and it contains various phenolic compounds that appear to have health benefits (Dykes and Rooney, 2006), which makes the grain suitable for developing functional foods and nutraceuticals.

GRAIN STRUCTURE WITH RESPECT TO MILLING

The sorghum grain (caryopsis), like all other cereal grains, consists of three distinct parts: the pericarp (outer layer), endosperm (storage tissue), and germ (embryo). Note that the aim of milling is generally not simply to reduce the grain into small particles. Usually, the aim is to also separate the pericarp and germ from the endosperm, with the resulting flour being endosperm of varying purity. The pericarp and germ are removed because most people find the color, texture, and taste imparted by them to food products to adversely affect their acceptability.

Pericarp

The pericarp, which is rich in insoluble dietary fiber, accounts for 4.3−8.7% of sorghum grain (Waniska and Rooney, 2000). It is subdivided into three tissues, namely (from the outer side) the epicarp, mesocarp, and endocarp. The epicarp is covered with a thin layer of wax and contains most of the sorghum grain pigments; hence, it has a major influence on grain color. The mesocarp contains starch granules, which is a feature unique to sorghum and pearl millet (Serna-Saldivar and Rooney, 1995). It has been suggested that the presence of starch granules in the mesocarp may account for the high friability of the sorghum pericarp (Taylor and Dewar, 2001). Friability is a negative attribute of the pericarp for dry milling because it causes fragmentation into fine pieces, thus escaping separation and thereby contaminating the flour.

Some sorghum cultivars have a pigmented subcoat (testa) between the pericarp and the endosperm. The pigmented testa contains condensed tannins (Waniska and Rooney, 2000). Tannins protect these tannin sorghums against insects, birds, and fungal attack. Until recently, sorghum tannins have been viewed as undesirable due to their antinutritive properties. They complex with food macromolecules such as proteins, thereby reducing the digestibility of the macromolecules. However, current research indicates that tannins have health benefits, which are discussed later.

Endosperm

The endosperm is the largest part, constituting 82−87% of the sorghum grain (Waniska and Rooney, 2000), and it contains mainly starch and protein. It is made up of the aleurone layer, the peripheral area, and the corneous (hard) and floury (soft) areas. The latter two account for the largest portion of the endosperm. In sorghum, the aleurone layer is only one cell layer thick. It contains protein bodies, phytin bodies, and oil bodies (spherosomes). The peripheral endosperm region comprises several layers of dense cells containing essentially just protein bodies. The corneous endosperm cells contain a continuous matrix of kafirin protein-containing protein bodies, glutelin matrix protein, and starch granules. In the floury

endosperm, starch granules, matrix protein, and protein bodies are discontinuous with airspaces in the cells. The proportion of starch relative to protein is also higher.

The relative proportion of corneous and floury endosperm is largely genetically controlled. When milled, sorghums with a higher proportion of corneous endosperm yield a more gritty flour due to stronger adhesion between the starch granules and surrounding protein. The tannin sorghums invariably have a high proportion of the softer, floury endosperm.

Germ

The germ is the living part of the sorghum grain, and it consists of two main parts: the embryonic axis and scutellum (Serna-Saldivar and Rooney, 1995). The germ is very rich in lipids, approximately 28% by weight or 76% of total grain lipids. It is also relatively protein rich, approximately 18% by weight or 15% of total grain proteins. The germ proteins are mainly albumins and globulins, which are rich in lysine and other essential amino acids (Taylor and Schüssler, 1986).

GRAIN CHEMICAL COMPONENTS: FUNCTIONAL AND NUTRITIONAL ATTRIBUTES

Starch

Starch constitutes approximately 71% of sorghum grain (Serna-Saldivar and Rooney, 1995). Sorghum starch gelatinization temperature, which ranges from 66 to 81°C, is high compared to that of wheat and possibly slightly higher than that of maize (Taylor and Emmambux, 2010). Starch gelatinization temperature also seems to be quite variable between sorghum cultivars.

Generally, sorghum has lower starch digestibility than maize (Taylor and Emmambux, 2010). On the basis of this, it has been suggested that sorghum may be a particularly suitable food for diabetic and obese people (Dicko *et al.*, 2006). However, there is little, if any, direct evidence to support this contention. It appears that the lower starch digestibility is not an intrinsic property of sorghum starch but, rather, primarily a result of the endosperm protein matrix, cell wall material, and tannins (if present) inhibiting enzymatic hydrolysis of the starch (Taylor and Emmambux, 2010). Protein disulfide bond cross-linking involving the kafirin proteins in the protein matrix around the starch granules seems to be of major importance in reducing starch digestibility (Ezeogu *et al.*, 2008).

Proteins

Sorghum grain protein content is quite variable, ranging from approximately 7 to 16% with an average of approximately 11% (Serna-Saldivar and Rooney, 1995) (Table 12.1). The major sorghum grain proteins are prolamin storage proteins, as in virtually all other cereal grains, and are known as kafirins (Belton *et al.*, 2006). Kafirins are classified into four major species based on differences in molecular weight, solubility, structure, and amino acid composition and sequence. These are the α-, β-, γ- and δ-kafirins (Table 12.2).

Kafirins have low nutritional quality because they are poor in essential amino acids, particularly lysine (Taylor and Schüssler, 1986). They are poorly digestible, especially when cooked in water, as occurs during most food preparation processes (Duodu *et al.*, 2003). However, an important positive health issue with respect to the kafirins is that because they are so different in structure from the wheat gliadin and glutenin storage proteins (see Table 12.2), it has been conclusively shown that sorghum does not elicit morphometric or immunomediated alteration of duodenal explants from patients suffering from celiac disease (Ciacci *et al.*, 2007). Celiac disease, a syndrome characterized by damage to the mucosa of the small intestine, is caused by ingestion of wheat gluten and similar proteins (Catassi and Fasano, 2008). It is

TABLE 12.1 Nutrient Composition per 100 g of Whole Sorghum and Morvite (Fortified, Precooked Sorghum Flour)

Nutrient	Sorghum[a]	Morvite[b]	Morvite (% RDA for persons 10 years or older)[b]
Energy (kJ)	1374	1506	
Protein (g)	11.6	7.1	13
Lipid (g)	3.4	3.8	
Carbohydrate (g)	77.0	77.8	
Dietary fiber (g)	6.3–11.5	2.7	
Na (mg)	6	208	
Ca (mg)	29	120	15
Fe (mg)	4.5	2.1 (electrolytic)	15
Zn (mg)	1.4	2.25	15
I (μg)	No data	23	15
Se (μg)	No data	8.25	15
Vitamin A (μg retinol equiv.)	10–20	200	20
Vitamin B_1 (mg)	0.24	0.35	25
Vitamin B_2 (mg)	0.15	0.4	25
Niacin (mg)	3.0	4.5	25
Vitamin B_6 (mg)	0.48	0.5	25
Folic acid (μg)	84	50	25
Pantothenic acid (mg)	No data	1.5	25
Vitamin C (mg)	0	39	65
Vitamin E (α-tocopherol) (mg)	1.2	1.5	15

[a]*Data from USDA Nutrient Database (www.nal.usda.gov/fnic/foodcomp/search) and Serna-Saldivar and Rooney (1995).*
[b]*Manufacturer's data.*

becoming recognized that celiac disease is a major health problem in Western countries, affecting at least 1 in 150 people. The only treatment is lifelong avoidance of foods containing wheat and similar cereals such as rye, triticale, and barley. Hence, sorghum is a viable and important alternative for making baked products such as bread.

However, a major challenge in using sorghum in bread baking is the very poor viscoelastic properties of kafirin dough compared to that made from wheat gluten (glutenins plus gliadins). When mixed with water, gluten proteins become hydrated and form a three-dimensional network, which is responsible for the unique viscoelastic property of wheat dough (Belton, 1999; Oom *et al.*, 2008). Because kafirins are more hydrophobic than the gluten proteins, related to the high levels of leucine (see Table 12.2), kafirins are difficult to hydrate. Belton *et al.* (2006) suggested that the poor hydration of kafirins may also be linked to their mainly α-helical structure, in contrast to high-molecular-weight (HMW) glutenin subunits of wheat, which have a high level of β-sheet and β-turn structure. As can be seen in Table 12.2, the kafirins are much smaller proteins than the HMW glutenins, which probably also has a bearing on their lack of elasticity. In addition, because kafirins are encapsulated in protein bodies (Duodu *et al.*, 2003), they are probably unavailable for participation in dough fibril formation, unlike gluten proteins, which are present in the continuous matrix after seed desiccation (Shewry, 1999).

Lipids

Sorghum contains approximately 3.4% lipids (see Table 12.1), rather more than wheat but less than maize, the majority of which are neutral triglycerides (triacylglycerols). The triglycerides of sorghum are rich in unsaturated fatty acids. The predominant fatty acids are linoleic (C18:2; 38–49% of the total) and oleic (C18:1; 31–38% of the total) (Serna-Saldivar and Rooney, 1995). Sorghum is also rich in tocopherols (vitamin E; approximately 1.2 mg/100 g) (see Table 12.1).

TABLE 12.2 Classification and Properties of Sorghum Kafirins and Wheat Gliadins and Glutenins

Protein	Group	Subunit Structure	Molecular Mass (kDa)	Total Fraction (%)	Partial Amino Acid Composition (mol %)
Kafirins	α	Monomeric, oligomeric, and polymeric[a]	22–27[a]	66–84[a]	22 Gln, 9 Pro, 0.7 Gly, 15 Ala, 15 Leu, 1 Cys, 0.6 Met[a]
	β	Monomeric and polymeric	16–20	7–13	18 Gln, 19 Pro, 13 Ala, 12 Leu, 5 Cys, 6 Met
	γ	Oligomeric and polymeric	28–29	9–16	14 Gln, 23 Pro, 9 Gly, 9 Leu, 7 Cys, 1 Met
	δ	Unknown	13–15	Unknown	16 Met, 1 Trp[a]
Gliadins	γ	Monomeric[b]	26–36[b]	40–50[b]	32–36 Gln, 16–17 Pro,3 Gly, 3 Ala, 2–3 Cys, 1.5 Met[b]
	α	Monomeric	30–34		35 Gln, 15–18 Pro, 2.3 Gly, 2–3 Ala, 2 Cys, <1 Met
	ω	Monomeric	30–50		40–53 Gln, 20–29 Pro, 0.7–1 Gly, <1 Ala, 0 Cys, 0 Met
	LMW gliadin	Monomeric	16–19		23–27 Gln, 9 Pro, 5 Gly, 7 Ala, 5 Leu, 6–9 Cys, 3–5 Met
Glutenins	HMW glutenin	Polymeric	45–106[b]	30–40[b]	33–36 Gln, 11–13 Pro, 18–20 Gly, 3–4 Ala, 4 Leu, 0.5–1 Cys, <1 Met
	LMW glutenin	Polymeric	32–42		33–35 Gln, 13–16 Pro, 2–3 Gly, 2–3 Ala, 8 Leu, 2–3 Cys, 1.6–2 Met

HMW, high molecular weight; LMW, low molecular weight.

[a]*Data from Belton et al. (2006).*
[b]*Data from Shewry et al. (2009).*

Non-starch polysaccharides

Sorghum contains approximately 6–11% non-starch polysaccharides (NSPs; dietary fiber), probably a slightly lower level than that in wheat. The major NSPs of the sorghum endosperm are water-unextractable (insoluble) glucuronoarabinoxylans (GAX) (Taylor and Emmambux, 2010; Taylor *et al.*, 2006). Because they are water-insoluble, the sorghum GAX are probably not functional in bread making, unlike the wheat arabinoxylans. Concerning their nutritional attributes, they probably have good laxation properties but do not have the cholesterol-lowering effects associated with soluble dietary fiber.

Phytochemicals

Sorghum grain contains several types of potentially health-promoting phytochemicals, including various phenolic compounds, plant sterols, and policosanols. Examples of the health benefits that have been indicated for sorghum phytochemicals include antioxidant, anti-inflammatory, cancer-preventive, anti-arrhythmic activities associated with the phenolics; satiety-promoting activities specifically associated with the tannin-type phenolics (Dykes and Rooney, 2006); and cholesterol-lowering activity associated with the policosanols (Taylor *et al.*, 2006).

PHENOLIC COMPOUNDS

The quantity of phenolic compounds in sorghum grain can be substantial, particularly in red-pigmented, tannin-type sorghums (Table 12.3). The main groups of phenolic compounds in sorghum grain are phenolic acids, flavonoid-type compounds, and tannins (proanthocyanidins) (Dykes and Rooney, 2006) (Figure 12.1). Note that the tannins are only present in tannin (type II and type III) sorghums. Sorghum phenolic acids are mainly derivatives of benzoic acid (see Figure 12.1A) and cinnamic acid (see Figure 12.1B). They are concentrated in the pericarp and occur mostly in bound form (esterified to cell wall polymers). Ferulic acid is the most abundant bound phenolic acid in sorghum. Other phenolic acids abundant in sorghum include syringic, protocatechuic, caffeic, *p*-coumaric, and sinapic.

Flavonoids form the largest group of phenolic compounds in sorghum. They are made up of a benzopyran nucleus with aromatic substituent at carbon 2 of the C ring (see Figure 12.1C). Many sorghum flavonoids have been isolated and identified, with anthocyanins being the major class found in sorghum (Dykes and Rooney, 2006). The anthocyanins contribute most of the color of sorghum. Sorghum anthocyanins are unique because they do not contain the hydroxyl group in the 3-position of the C ring and thus are called 3-deoxyanthocyanins. The two common sorghum 3-deoxyanthocyanidins are the yellow, apigeninidin, and the orange, luteolinidin (see Figure 12.1C). In sorghum, the 3-deoxyanthocyanins are concentrated in the pericarp, and the highest levels are found in sorghums with black pericarp.

Sorghum tannins are probably exclusively of the "condensed" type (proanthocyanidins), which are HMW polyphenols that consist of polymerized polymers of flavonoid subunits, mainly flavan-3-ols and/or flavan-3,4-diols (see Figure 12.1D). The proanthocyanidins in tannin sorghums are mainly the B type because they are linked mostly by C4 → C8 interflavan bonds with (-)-epicatechins as extension units and catechins as terminal units (Dykes and Rooney, 2006). Of the phenolics, the tannins have the highest antioxidant activity when considered on a molar basis.

FLOUR MILLING

The technology of milling sorghum grain into flour is not nearly as well-developed as that of wheat milling (Taylor and Dewar, 2001). Mechanical milling of sorghum grain is normally done using either disk mills or hammer mills. This is often preceded by removal of the bran layers (pericarp and germ), a process referred to as decortication or dehulling. Decortication is

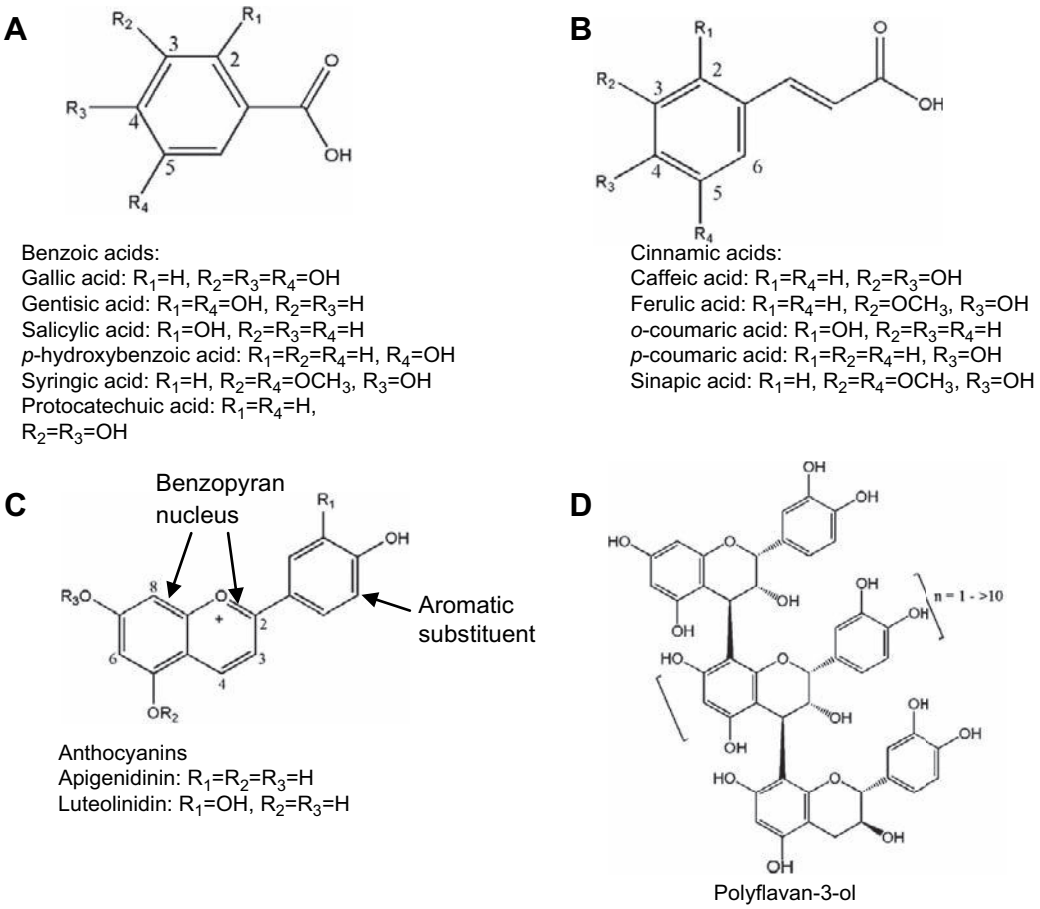

FIGURE 12.1
Basic structures of sorghum phenolic compounds. (A and B) Phenolic acids, (C) flavonoids, and (D) proanthocyanidins (condensed tannins). *Source: Reproduced with permission from Dykes, L., and Rooney, L. W. (2006). Sorghum and millet phenols and antioxidants.* J. Cereal Sci. *44, 236–251.*

generally performed using dehulling equipment employing the dry abrasion principle. These technologies are not particularly efficient, and the quality of the flour can be variable.

Small roller mills, simple versions of the type used for wheat milling, are a recent development in sorghum milling. These small roller mills combine both decortication and reduction in particle size. Research by Kebakile *et al.* (2007) indicates that small roller mills are advantageous over other sorghum milling technologies because they produce flour of higher extraction rate (flour yield) and much higher throughput. An important issue with respect to milling sorghum is that its endosperm consists of two main components—a hard outer part, the corneous (also referred to as vitreous) endosperm, and a softer, inner part, the floury endosperm. The hard corneous endosperm resists reduction to fine particle size (Chandrashekar and Mazhar, 1999). Hence, milling the corneous endosperm to flour size can result in high levels of starch damage, which can adversely affect the bread making quality of the flour.

Removal of the bran strongly affects flour composition. There is an increase in protein content due to removal of the dietary fiber-rich pericarp (Table 12.3). However, the protein quality is adversely affected, with the amount of lysine—the first indispensable amino acid in sorghum—being reduced by approximately 20% (Taylor and Schüssler, 1986) due to removal of at least part of the germ, which is rich in high-quality protein. Germ removal also reduces the lipid content, including the tocopherols, of the flour. Minerals and B vitamins are also

TABLE 12.3 Effect of Milling Extraction Rate on Protein, Oil, and Ash (Total Minerals)

Flour	Extraction Rate (%)	Protein (g/100 g)	Oil (g/100 g)	Ash (g/100 g)
Whole grain	100	11.59	3.60	1.90
Roller milled	83.6	14.12	2.79	1.34
Decorticated and then hammer milled	75.7	13.73	2.61	1.22

Source: Data from Kebakile *et al.* (2007).

TABLE 12.4 Effect of Milling Extraction Rate on Phosphorus, Iron, and Zinc

Extraction Rate (%)	Total Phosphorus (mg/g)	Phytate Phosphorus (mg/g)	Iron (ppm)	Zinc (ppm)
100	4.0	3.1	179	36
90	3.4	2.7	65	30
80	2.4	1.6	83	21
73	1.9	1.2	76	10

Source: Data from Klopfenstein and Hoseney (1995).

TABLE 12.5 Effect of Milling Extraction Rate on Total Phenolics, Condensed Tannins, and Antioxidant Activity in a Red, Tannin Cultivar

Extraction Rate (%)	Total Phenolics (g Catechin Equiv./100 g)	Condensed Tannins (g Catechin Equiv./100 g)	Antioxidant Activity (μMol Trolox Equiv./g)
100	1.25	7.73	373
90	0.87	6.73	322
70	0.42	0	178

Source: Data from Chiremba *et al.* (2009).

substantially reduced as they are concentrated in the germ and aleurone layer. However, mineral bioavailability may be improved (Klopfenstein and Hoseney, 1995) as the level of the antinutrient phytic acid, which binds divalent minerals such as iron, zinc, and calcium, is also reduced because it is similarly located (Table 12.4). Antioxidant activity of the flour is also reduced when the bran is removed (Table 12.5). This is because phenolics primarily responsible for antioxidant activity in sorghum and the non-tannin anthocyanis pigments are concentrated in the pericarp and the condensed tannins (proanthocyanidins), if present, are concentrated in the testa layer, which is directly above the aleurone layer of the endosperm (Dykes and Rooney, 2006).

PRODUCTS MADE FROM SORGHUM FLOUR

Traditional products

Throughout Africa, the major sorghum food product is porridge. This is prepared by cooking sorghum flour with water. Porridges range in solids content from approximately 10% for a thin gruel to 30% for a stiff porridge of mashed potato-like consistency. Depending on regional tastes, the sorghum porridges may be cooked at neutral pH, acidified to pH < 4.0 by lactic acid fermentation or acidification with fruit juice, or alkaline (pH 8.2) due to cooking with wood ash. These treatments affect the nutritional value of sorghum porridge. As stated previously, wet cooking in general substantially reduces the protein digestibility of sorghum foods (Duodu *et al.*, 2003). This adverse effect is alleviated by lactic acid fermentation (Taylor and Taylor, 2002). Other nutritional benefits of lactic acid fermentation can include improved starch digestibility, increased levels of B vitamins, reduced antinutrients such as tannins and phytic acid, and, most important, the rendering of the porridge product microbiologically safe

(Taylor and Dewar, 2000). Alkaline cooking adversely affects sorghum protein quality and availability (Klopfenstein and Hoseney, 1995).

In North Africa and India, sorghum flour is widely used to make flatbreads. In the production of the major African flatbreads, which are kisra produced in Sudan (Figure 12.2A) and injera produced in Ethiopia and Eritrea, a slurry of flour undergoes lactic acid fermentation. In injera making, a part of the cooked flour in then added back. The fermented flour is then diluted into a batter, which is poured onto a circular hot plate. The resulting flatbreads are moist and flexible and have a cellular structure formed by the fermentation gases. Kisra is approximately 3 mm thick, and injera is thicker (approximately 6 mm) probably because of the precooking of part of the flour. In contrast, the major Indian sorghum flatbread, known as roti or chapatti, is a thin, dry, crisp product with a puffed texture due to steam production during baking.

These traditional products from sorghum flour are almost exclusively made in the home, and generally the sorghum used to produce the flour is homegrown. Hence, the options for flour fortification are limited. An exception is instant acidified sorghum porridge powder, called "Morvite," which is commercially manufactured in South Africa (see Figure 12.2B). To make porridge, one simply mixes boiling water or milk with the powder. The porridge powder is made by pre-gelatinizing the starch using technologies such as extrusion cooking or gun puffing. The product is fortified with a range of minerals and vitamins so that a 100-g flour serving generally meets 15–25% of an adult's micronutrient Recommended Dietary Allowance (RDA) (see Table 12.1). Other similar vitamin- and mineral-fortified sorghum powder porridge products are available (see Figure 12.2B). These are variously enriched with soya to provide 40% of an adult's protein RDA and also with fruit, which when made with milk can provide 50 and 25% of a 2- or 3-year-old's protein and energy RDAs, respectively.

FIGURE 12.2
Traditional-type foods from sorghum flour. (A) Sudanese kisra flatbread being removed from a hot plate after baking. (B) Instant sorghum porridge products from South Africa: left and front, Morvite porridge; center, fruit-enriched infant porridge; and right, protein-fortified porridge.

Modern products

Because sorghum is considered a safe alternative to wheat for celiacs and because of the need to find local alternatives to wheat for bread making in tropical countries where wheat cannot be grown, the production of bread, cakes, and cookies from sorghum is being widely investigated (Schober and Bean, 2008; Taylor *et al.*, 2006).

The production of good-quality non-wheat bread with a light, airy texture is a skilled craft. A number of different techniques are used. However, the scientific reasons for most of them remain a matter of conjecture. The general principle of successful non-wheat bread making is that the solute molecules in the dough, mainly starch, have substitute for the gas-holding, viscoelastic properties of the wheat gluten molecules. Specifically, the solute molecules have to interact together and with the water molecules to hold the gas produced during yeast fermentation, allowing the dough to expand during fermentation and to set into a firm cellular structure during baking. In this context, sorghum has no special characteristics compared to other gluten-free cereals such as maize or rice. However, the more bland taste of white, tan-plant sorghum cultivars compared to other cereals and other sorghum types seems to be preferred in bread and cakes.

The non-wheat bread dough is prepared with a much higher proportion of water, 80—110% relative to flour, compared to approximately 65% for wheat dough (Schober and Bean, 2008). Thus, the dough is actually a stiff batter of similar consistency to that used for cake making. The high amount of water presumably allows greater starch granule expansion during baking, perhaps enabling the formation of a more stable gas cell structure. Commonly, raw starch, normally maize or cassava starch, is also included in the recipe at approximately 30% on a flour basis (Schober and Bean, 2008). This is also probably related to a requirement for greater starch granule expansion during baking. As indicated previously, flour from the corneous endosperm of sorghum is subject to high levels of starch damage. Damaged starch granules have high water absorption but at lower temperature than intact granules.

Hydrocolloids such as xanthan gum and hydroxypropyl methyl cellulose are commonly included in commercial gluten-free bread formulations (Schober and Bean, 2008; Taylor *et al.*, 2006). The probable function of the hydrocolloids is to increase the viscosity of the aqueous phase of the dough so as to reduce the rate of gas loss from the dough. Hydrocolloids add considerably to the ingredient cost of gluten-free breads. A much cheaper, but somewhat less effective, alternative is to precook a portion of the flour to produce pregelatinized starch (Taylor *et al.*, 2006), as is done in injera making. This porridge is then added to the flour, water, and other ingredients during dough making.

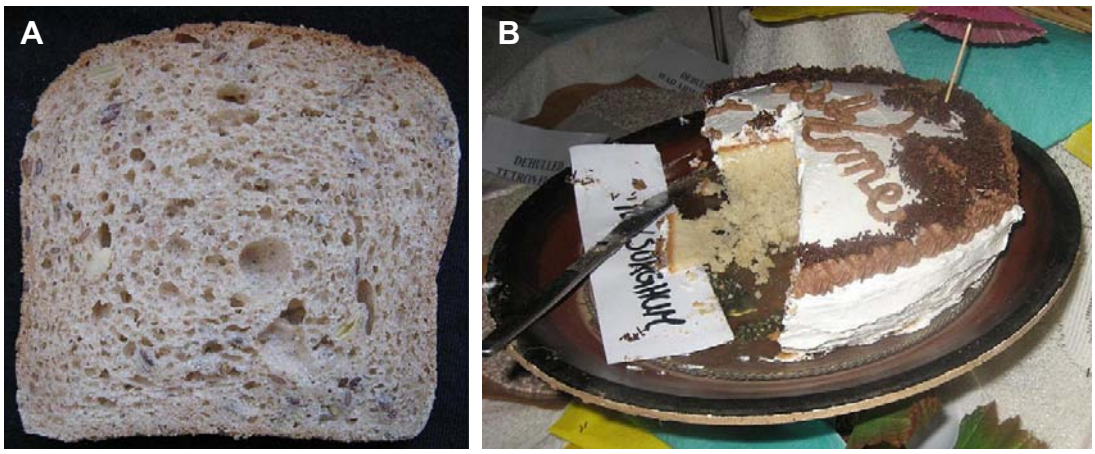

FIGURE 12.3
Modern-type foods made with sorghum flour. (A) Slice of gluten-free bread from Sweden (note the relatively coarse and open crumb structure). (B) 100% sorghum flour cake made by the Food Research Processing Centre, Khartoum, Sudan.

Notwithstanding the use of these techniques, gluten-free breads are invariably denser in texture than wheat breads. They have a coarser crumb structure, with proportionally larger gas cells and thicker cell walls (Figure 12.3A). Gluten-free cakes and muffins more closely resemble their wheat flour counterparts (see Figure 12.3B). This is probably because the role of gluten is less important in cake making. The flour is a much lower proportion of the solutes because there are high levels of sugar and fat, and eggs are generally included (Taylor *et al.*, 2006), which all play a role in gas cell stabilization.

Cookies (biscuits) are easily made from sorghum flour (Chiremba *et al.*, 2009; Taylor *et al.*, 2006) because unlike bread and cakes, they are not leavened. No special ingredients are required. However, sorghum cookies tend to be denser and have a harder texture than their wheat counterparts and can have a somewhat gritty mouthfeel (Chiremba *et al.*, 2009). We have experimentally produced protein-fortified sorghum cookies with the addition of defatted soya flour for use as a supplementary food to combat protein-energy malnutrition. Consumer sensory evaluation by primary schoolchildren of these protein-fortified sorghum cookies showed them to be as acceptable as 100% wheat cookies (unpublished data).

NEW DEVELOPMENTS IN SORGHUM FLOURS

Bread making technology

As explained previously, a problem with making good-quality gluten-free breads from sorghum and other grains is that expensive additives such as hydrocolloids are required. This is especially disadvantageous in countries with developing economies in the tropics, where sorghum is a major crop and should be utilized in bread making. As explained previously, the kafirin proteins are not functional in terms of providing the required viscoelastic characteristics to the dough. However, research (e.g., Oom *et al.*, 2008) has shown that if kafirin or zein, the very similar prolamin protein of maize, are mixed with solvent at elevated temperature (75°C), the resulting dough has viscoelastic properties. This is due to the fact that the temperature of 75°C is higher than the glass transition temperature of the kafirin and zein proteins. This finding offers the possibility of developing a process to produce good-quality breads from sorghum or maize flour without using expensive additives.

Biofortification

As indicated previously, because sorghum is primarily a food in tropical developing countries, the vast majority of sorghum flour food products are made in the home, and they are often produced from own-cultivated grain. Thus, flour fortification is problematical. A more viable option is biofortification. Biofortification can be defined as increasing the concentration of nutrients in crops using conventional plant breeding or recombinant DNA technology (genetic engineering). Polleti *et al.* (2004), who reviewed the progress made in the nutritional fortification of cereals, stated that effective biofortification of cereal staples can be done for the poor in rural areas; has low recurrent costs; is sustainable in the long term; and, in the case of genetic improvement, it requires only an upfront investment. Biosorghum, a Bill and Melinda Gates Foundation Grand Challenges in Global Health project, is using recombinant DNA technology to develop a biofortified sorghum, specifically for smallholder farmers in Africa. Biosorghum will have increased iron and zinc bioavailability through reduction in phytate levels, improved essential amino acid composition and protein digestibility through suppression of specific kafirin proteins, and will contain substantial levels of provitamin A (β-carotene) through expression of β-carotene synthesis.

TECHNOLOGICAL ISSUES

The technology of sorghum milling needs to be improved in terms of milling efficiency, flour functional quality, and retention of micronutrients and phytochemicals. To achieve this, sorghum roller milling technology needs to be further developed.

The technology of sorghum leavened bread production also needs to be further developed so as to improve bread quality and avoid the use of expensive additives. In-depth research is needed to determine how to enable the sorghum kafirin proteins to provide dough viscoelasticity.

Biofortification of sorghum needs to be further developed and implemented in countries in which sorghum is a major staple so as to improve the nutritional status of rural people.

SUMMARY POINTS

- Sorghum is a major staple crop and food in arid, tropical countries in Africa and in India.
- Sorghum has some valuable nutritional characteristics. It is gluten-free and can contain high levels of phytochemicals, particularly antioxidant-rich phenolics.
- Sorghum flour milling technology has not been developed to the same level as wheat milling. Flour extraction rate (flour yield) substantially affects the chemical composition of the flour; with decreasing extraction rate there is a considerable loss of micronutrients and phenolics.
- Sorghum flour is widely used to produce traditional food products, including porridges of many types and flatbreads.
- Micronutrient-fortified instant sorghum porridges are popular foods in South Africa.
- Leavened breads can be produced from sorghum flour, although the quality is not particularly good and expensive additives such as hydrocolloids generally have to be added to compensate for the absence of gluten.
- Reasonable quality cakes and cookies can be readily made with sorghum flour because the absence of gluten is not so important.
- Research has shown that the sorghum proteins can exhibit wheat gluten-like functional dough properties at elevated temperature.
- Biofortified sorghum with improved mineral bioavailability, improved protein quality, and substantial levels of provitamin A is being developed using recombinant DNA technology.

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CHAPTER 13

Buckwheat Flour and Bread

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

HDL High-density lipoprotein
LDL Low-density lipoprotein
NO Nitric oxide

INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Möench) is a dicotyledon that belongs to the Polygonaceae family. The cultivation has many advantages over other grain crops, such as a short growing period (70–90 days) and less tedious care during cultivation. Its grain can be stored for a long time without alternation due to its high content of compounds with antioxidant properties. Originating from East Asia, buckwheat is widely cultivated in North America and is mainly consumed in Central European countries, the United States, Canada, and Asian countries as a constituent of foods such as pancakes, noodles, and buckwheat bread blended with flour

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from other cereals. Recently, buckwheat flour has been introduced in many countries because the flour is nutritionally rich and foods prepared using the flour have beneficial effects on human health.

Tartary buckwheat or bitter buckwheat (*F. tataricum* (L.) Gaertn.), although not as common as buckwheat, is cultivated in southeast China and at higher latitudes in Tibet, Nepal, and India. The rutin content and the antioxidative activity of tartary buckwheat seeds are more than 100 times and 3 or 4 times higher than those of buckwheat, respectively, and the effectiveness on health appears to be nearly equal to that of buckwheat.

Figure 13.1 summarizes the components of buckwheat flour and bread and their functions and reactions in the human body. The richness of buckwheat flour and bread in essential amino acids, essential fatty acids, vitamins B_1 and B_2, and essential minerals (Bonafaccia, Marocchini, *et al.*, 2003; Guo and Yao, 2006) may contribute to the improvement of human health. Rutin, a flavonol glycoside, also contributes to improvements in health because it has anti-inflammatory and hypotensive effects, and it can reduce the fragility of blood vessels. The metabolism of rutin in the stomach has been reported in relation to the formation of nitric oxide (NO) and the production of the oxathiolone derivative of rutin (Takahama *et al.*, 2007, 2009). Proanthocyanidins can also react with nitrite in the stomach, producing NO.

Foods prepared from buckwheat flour may be useful for diabetics because (1) proanthocyanidins can inhibit digestive enzymes including α-amylase (Lee *et al.*, 2007), (2) D-*chiro*-inositol can function as a mediator for anti-hyperinsulinemia (Kawa, Przybylski, *et al.*, 2003; Yao *et al.*, 2008), and (3) starch is converted to indigestible form during the preparation of bread using buckwheat flour (Skrabanja *et al.*, 2001). Apart from its richness in essential amino acids, buckwheat protein can reduce the concentration of cholesterol in the serum, liver, and gallbladder; suppress the formation of gallstones by enhancing the secretion of cholesterol in the feces; and alter cholesterol metabolism (Tomotake *et al.*, 2006). The indigestible starch and undigested protein of buckwheat flour may contribute to the recovery from constipation. In addition, diets containing buckwheat grains or flour appear to have prebiotic properties (Préstamo *et al.*, 2003). These diets enhance the growth of intestinal bacteria beneficial to humans but inhibit the growth of pathogenic bacteria. In this way, buckwheat flour is a useful material for the preparation of healthy foods; however, it contains proteins that induce

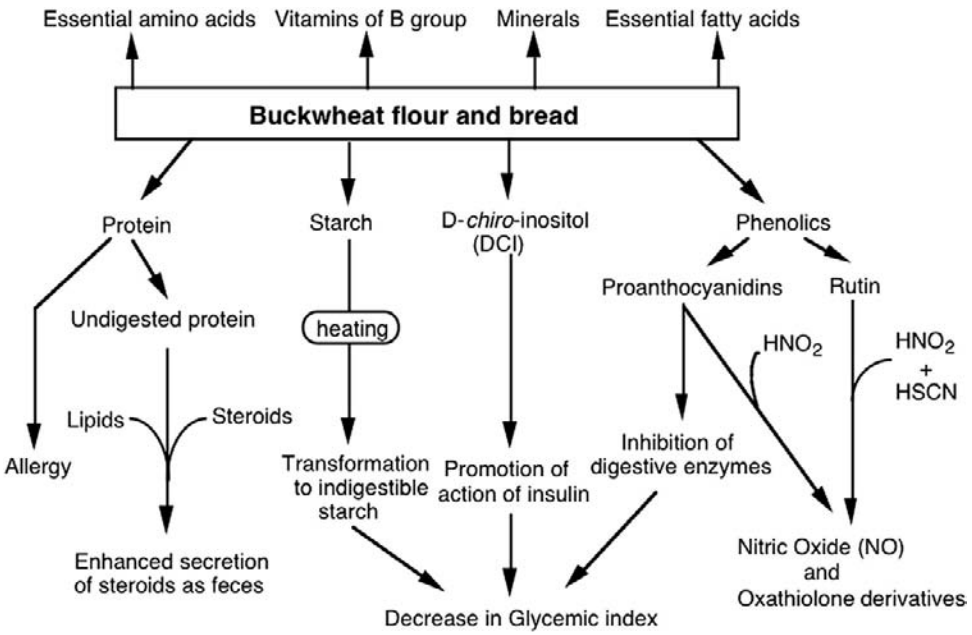

FIGURE 13.1
Components of buckwheat flour and bread and their postulated functions and reactions in the human body.

anaphylaxis in humans (Heffler *et al.*, 2007). This chapter discusses studies on the use of buckwheat and tartary buckwheat for the fortification of health and disease prevention, focusing on the functions of starch, protein, D-*chiro*-inositol, and phenolic compounds.

BIOACTIVE COMPONENTS AND THEIR FUNCTIONS

Advantages of starch

Buckwheat flour contains 70–91% (w/w) starch depending on the flour types, and the starch consists of 25% amylose and 75% amylopectin. A proportion of the starch becomes hydrolysis resistant during boiling or baking of the groats and the flour. The glycemic index – a measure of the effects of carbohydrates on blood glucose concentrations calculated using white bread (bread made from wheat flour from which the bran and often the germ have been removed) as the reference food – of boiled buckwheat groats and bread prepared from the same amount of wheat flour and buckwheat flour is 61 and 66, respectively (Skrabanja *et al.*, 2001). The glycemic index of bread prepared from whole wheat flour is approximately 70. Bread with a glycemic index of 55–70 is classified as bread with a medium glycemic index. The insulin index – a measure used to quantify the typical insulin response to various foods using white bread as the reference food – of boiled buckwheat groats and bread prepared with the same amount of wheat flour and buckwheat flour is 53 and 74, respectively (Skrabanja *et al.*, 2001). Therefore, foods prepared from buckwheat flour and groats with hydrolysis-resistance starch have a potential use for diabetics.

Advantages and disadvantages of protein

Buckwheat flour and tartary buckwheat flour contain 10–20% (w/w) protein. The protein contains albumin, glutelin, prolamin, and globulin, which are rich in essential amino acids (Guo and Yao, 2006). Albumin is relatively rich in histidine, threonine, valine, phenylalanine, isoleucine, leucine, and lysine. Globulin has high levels of methionine and lysine. Prolamin has high levels of histidine, threonine, valine, isoleucine, and leucine. Glutelin is rich in histidine, threonine, valine, isoleucine, and leucine. Disulfide bonds are present in these four proteins (Guo and Yao, 2006). Proteins of the albumin family with disulfide bonds appear to be responsible for the allergic response that is induced by buckwheat products (Satoh *et al.*, 2008).

Advantages of metals, vitamins, and fatty acids

Buckwheat flour and tartary buckwheat flour are rich in Fe (60–100 ppm), Zn (20–30 ppm), and Se (20–50 ppb) (Bonafaccia, Gambelli, *et al.*, 2003). In addition, Cr, Rb, Co, Sb, Ba, Ni, Ag, Hg, and Sn are also found in the flour and bran of buckwheat and tartary buckwheat. In both species, trace elements are concentrated in the bran. The flour is also rich in vitamins B_1, B_2, and B_6. Unsaturated fatty acids [C18:1 (n- 9) (oleic acid), C18:2 (n-6) (linoleic acid), and C18:3 (n-6) (γ-linolenic acid)], which are the components of biomembranes and the precursors of prostaglandins, thromboxane, and leukotriene, are also concentrated in the bran (Bonafaccia, Gambelli, *et al.*, 2003).

Advantages of phenolic compounds

The concentration of rutin is 19–168 ppm in the flour fractions and 131–476 ppm in the bran fractions of buckwheat flour (Kreft *et al.*, 1999). Rutin is also found in the leaves, stems, and flowers at concentrations of approximately 300, 1000, and 46,000 ppm, respectively. Rutin has antioxidant properties, and there are numerous reports characterizing the pharmacological functions of rutin. Minor flavonoids, the *C*-glycosylflavones (orientin, isoorientin, vitexin, and isovitexin), are found in buckwheat flour. In addition, proanthocyanidins, which can function as antioxidants, are also detected (Ölschläger *et al.*, 2008). Anthocyanins are found in the stems of the buckwheat plant. The antioxidative activity of

these phenolic compounds may be observed in the stomach as the reduction of nitrite to NO. Accompanying the reaction of proanthocyanidins with nitrite, the transformation of proanthocyanidins to stable radicals and the nitration and nitrosation of proanthocyanidins have been observed. The ability of proanthocyanidins to inhibit digestive enzymes facilitates their use as anti-diabetic and anti-obesity agents (Lee *et al.*, 2007). The inhibition may be due to the binding of proanthocyanidins to the digestive enzymes disturbing their tertiary structures. It has been reported that buckwheat-enhanced wheat bread, whose taste is moderately acceptable, can be developed as a food with more effective antioxidant properties than wheat bread (Lin *et al.*, 2009). The antioxidant properties of buckwheat flour products are affected by different thermal processing methods, and the decrease in its antioxidant activity is related to the content of phenolic compounds in the processed buckwheat flour (Zhang *et al.*, 2010).

Ascorbate cooperates with flavonoids, and it has been suggested that ascorbate can protect flavonoids, such as rutin and quercetin, from oxidation. This ascorbate-dependent protection may occur when flavonoids are oxidized in lipophilic environments — for example, in the cell membrane, which is inaccessible to ascorbate. Flavonoid radicals or the quinones formed in lipophilic environments may be reduced by ascorbate in a similar manner as the ascorbate-dependent reduction of vitamin E radicals formed in biomembranes.

IMPROVEMENT OF HUMAN HEALTH AND THE PREVENTION OF DISEASES

Improvement of cholesterol and lipid metabolism

Oats, the grain of which is rich in water-soluble fiber, have been known to lower serum lipid and cholesterol concentrations in humans since 1960s. The effects of buckwheat diets on the concentrations of serum lipid and cholesterol were also studied in humans, and the results suggested that buckwheat intake was associated with lower serum concentrations of total cholesterol and low-density lipoprotein (LDL) cholesterol (He *et al.*, 1995). The reduction of the total serum cholesterol concentrations by buckwheat diets has also been observed in rats, and the decrease in the serum cholesterol concentrations is associated with a decrease in high-density lipoprotein (HDL) cholesterol concentration without affecting the LDL cholesterol concentration (Préstamo *et al.*, 2003). Many investigators have confirmed the buckwheat diet-dependent decrease in the serum cholesterol concentration, although there are some discrepancies. The protein present in buckwheat flour is suggested to have a hypocholesterolemic effect (Kayashita *et al.*, 1995; Tomotake *et al.*, 2006), and this effect is associated with the suppression of gallstone formation (Tomotake *et al.*, 2006). This hypocholesterolemic effect has also been reported for tartary buckwheat sprout powder (Kuwabara *et al.*, 2007). The mechanism of the hypocholesterolemic effect of buckwheat protein has been postulated to be due to the increased fecal excretion of steroids, which is induced by binding of the steroids to undigested protein (Tomotake *et al.*, 2006) (Figure 13.2). Hydrophobic interactions may participate in the binding. In addition, the upregulation of hepatic cholesterol 7-α-hydroxylase mRNA by components in buckwheat has also been postulated as a mechanism for the hypocholesterolemic effect of buckwheat protein (Kuwabara *et al.*, 2007). Cholesterol 7-α-hydroxylase is the initial and rate-limiting enzyme of the bile acid synthesis pathway in the liver.

Although it has not been observed in humans, the phospholipid content in HDL decreases following the administration of a buckwheat diet to rats (Préstamo *et al.*, 2003); buckwheat protein also decreases the serum concentrations of triacylglycerols and phospholipids in mice (Tomotake *et al.*, 2006). Such an anti-hyperlipidemic effect of powdered buckwheat leaf and flower has also been reported in rats, and the effect may be mediated by the excretion of lipids in the feces (Lee *et al.*, 2010). The inhibition of lipase by proanthocyanidins may also contribute to the anti-hyperlipidemic effect of buckwheat diets (Lee *et al.*, 2007).

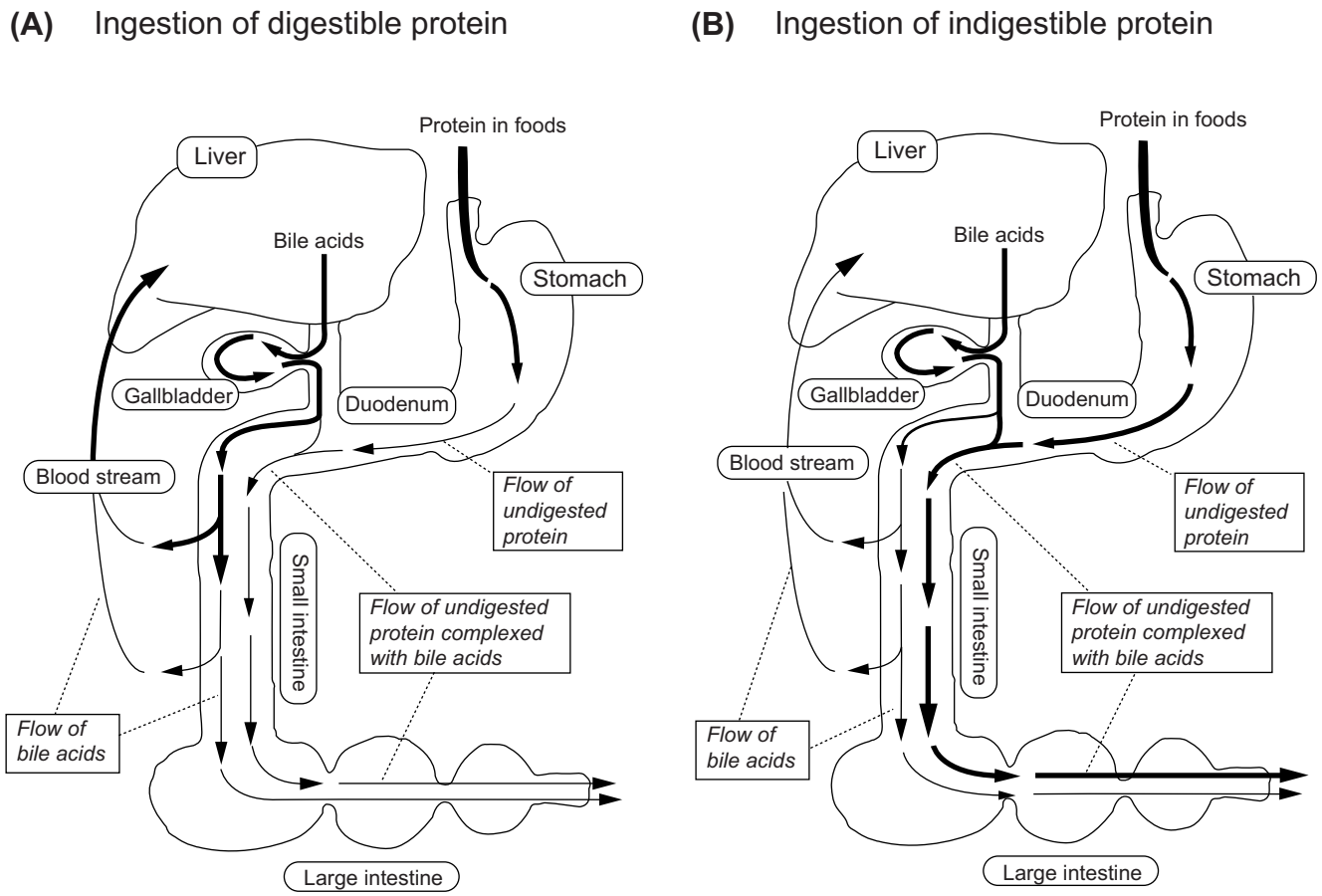

FIGURE 13.2
Decrease in serum steroid concentrations by undigested proteins. The width of each curve expresses the flux of undigested protein, and the joining of the two fluxes at the duodenum indicates the binding of bile acids to undigested protein. Free bile acids are absorbed in the small intestine.

The decrease of serum concentrations of cholesterol and lipid by the ingestion of products prepared from buckwheat flour may be related to the decrease in blood pressure and the prevention of arteriosclerosis observed following the consumption of buckwheat diets.

Improvement of insulin resistance

Buckwheat contains D-*chiro*-inositol (Figure 13.3), which can lower the concentration of blood glucose in streptozotocin-diabetic rats (Kawa, Taylor, *et al.*, 2003) and type 2 diabetic mice (KK-Ay) (Yao *et al.*, 2008). D-*chiro*-Inositol can be incorporated into mammalian cells as the free form and exists in cells as inositol phosphates and inositol phospholipids. Women with polycystic ovary syndrome have insulin resistance and hyperinsulinemia, and these symptoms are considered to be due to the deficiency of D-*chiro*-inositol containing phosphoglycan (see Figure 13.3), which mediates the action of insulin. The administration of D-*chiro*-inositol increases the action of insulin in patients with polycystic ovary syndrome, improving the function of ovaries and decreasing the blood pressure and the concentrations of androgen and triacylglycerol in blood (Cheang *et al.*, 2004; Nestler *et al.*, 1999). These data suggest that products containing buckwheat flour may be used to treat diabetes and polycystic ovary syndrome.

Insulin delivers its signal through a cell membrane receptor, which is formed by two α and two β subunits. The binding of insulin to the extracellular domain of the α subunits leads to the activation of the intracellular tyrosine kinase domain of the β subunits. The activated tyrosine

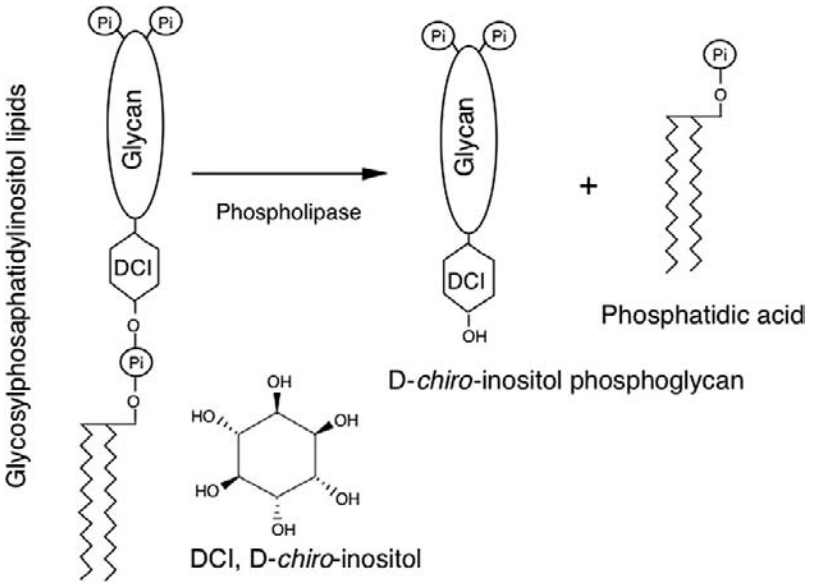

FIGURE 13.3
Glycosylphosphatidylinositol lipid and its hydrolysis by phospholipase.

kinase initiates a multitude of downstream signals leading to the uptake of glucose, the synthesis of glycogen, and so forth. This mechanism of action of insulin is insufficient to explain type 2 diabetes or insulin resistance. In 1986, water-soluble components derived from glycolipids were suggested to mediate the cellular responses to insulin, and one of the components was D-*chiro*-inositol phosphoglycan (see Figure 13.3).

Figure 13.4 summarizes the mechanism of the improvement of insulin resistance by D-*chiro*-inositol. In patients with insulin resistance, there is a low concentration of glycosylphosphatidylinositol lipids at the outer leaflet of the cell membrane, which may be due to the enhanced loss of D-*chiro*-inositol from the urine (Kawa, Przybylski, *et al.*, 2003). In the presence of a low concentration of glycosylphosphatidylinositol lipids in the cell membrane, the hydrolysis rate of the lipids may be slow, even when phospholipases are fully activated by insulin, because the reaction rate between the lipases and the substrate is limited by the concentration of the substrate when the substrate concentration is not much higher than the substrate affinity. The slow hydrolysis results in the slow uptake of D-*chiro*-inositol phosphoglycan, lowering its concentration in the cytosol. The administration of D-*chiro*-inositol may increase the concentration of cytosolic inositol, leading to an increased concentration of glycosylphosphatidylinositol lipids in the cell membrane. Therefore, the activated phospholipases can hydrolyze the phospholipids, rapidly increasing the concentration of D-*chiro*-inositol phosphoglycan in the cytosol. The increase in the concentration of D-*chiro*-inositol phosphoglycan can enhance various activities related to glucose metabolism, such as the transportation of glucose into cells and the synthesis of glycogen. The enhancement of cellular glucose metabolism can result in a decreased concentration of glucose in the bloodstream.

PREBIOTICS

Prebiotics are nondigestible food ingredients that stimulate the growth or activity of bacteria, which are beneficial to health, in the digestive system. Soluble fiber or dietary fiber exhibits some prebiotic effects. The methanol extracts of buckwheat flour, which may contain proanthocyanidin with a high molecular weight, enhance the growth of lactic acid bacteria but inhibit the growth of *Clostridium perfringens* and *Escherichia coli*, suggesting that intestinal bacteria can respond to diets prepared from buckwheat grain. Préstamo *et al.* (2003) reported that a buckwheat diet in rats caused a slight decrease in some pathogenic bacteria and an increase in *Lactobacillus plantarum*, *Bifidobacterium* spp., and *Bifidobacterium lactis*, which have beneficial effects, suggesting that diets prepared from buckwheat have prebiotic effects.

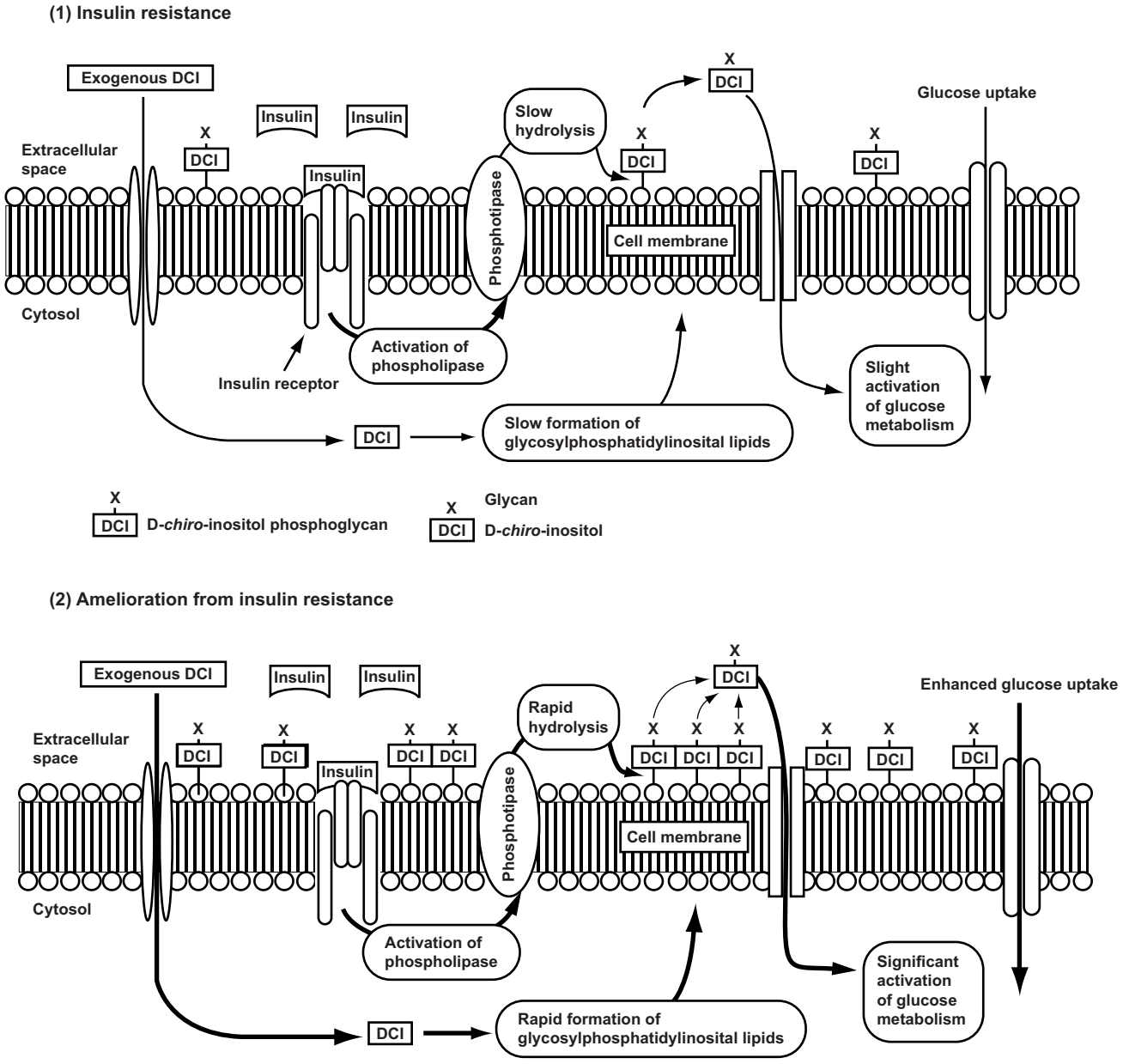

FIGURE 13.4
Hypothesized scheme of the amelioration of insulin resistance by D-chiro-inositol. The width of each curve expresses the flux of each component, rate of reaction, or degree of activation. (Top) insulin resistance; (bottom) ameliorated insulin resistance. Membrane transporters may be related to the incorporation of D-*chiro*-inositol and D-*chiro*-inositolphosphoglycan into the cytosol. *Source*: Adapted from Cheang *et al.* (2004) and Jones and Varela-Nieto (1999).

ADVERSE REACTIONS

Allergy

Allergic reactions provoked after the ingestion of small amounts of buckwheat flour were first reported in 1909, suggesting that the antigenicity of buckwheat is extremely strong. The adult patient described in that study suffered from asthma, rhinitis, urticaria, and angioedema. The hypersensitive symptoms of buckwheat allergy also include gastrointestinal disorders and conjunctiva congestion (Nakamura and Yamagushi, 1974/1975). The routes of exposure are by ingestion and inhalation. Italian pizza containing buckwheat flour can cause anaphylaxis after the administration of a cumulative dose of 2.3 g of the flour (Heffler *et al.*, 2007).

Buckwheat flour induces a type I allergic reaction – that is, an immunoglobulin E (IgE)-mediated immediate-type reaction (Nakamura and Yamagushi, 1974/1975). Some of the allergenic proteins in buckwheat flour have been identified. One is a 24-kDa protein isolated from tartary buckwheat seeds (215 amino acid residues), with strong IgE binding activity in an enzyme-linked immunosorbent assay to sera collected from patients who were allergic to buckwheat (Wang *et al.*, 2004). The gene encoding this protein has been cloned and expressed in a strain of *E. coli* (Wang *et al.*, 2006). BWp16 is a 16-kDa allergenic protein found in buckwheat with 149 amino acid residues. This protein belongs to the 2S albumin family, which contains the water-soluble seed storage proteins of cereals and has disulfide bonds derived from cysteine residues. The disulfide bonds are proposed to play an important role in the allergenicity of the protein, and mutational analysis of recombinant BWp16 demonstrated that mutations to the cysteine residues resulted in weaker IgE binding activity in serum from patients than the wild-type recombinant BWp16 (Satoh *et al.*, 2008). These data may be useful for the development of varieties of buckwheat with low allergenicity.

METABOLISM OF PHENOLIC COMPOUNDS IN THE STOMACH

Reaction of phenolic compounds with nitrous acid producing nitric oxide

Saliva contains nitrate, which is reduced to nitrite by nitrate-reducing bacteria in the oral cavity. The nitrite produced is mixed with food in the oral cavity by mastication. The mixture of nitrite and food components is swallowed, transforming the nitrite in the mixture to nitrous acid (HNO_2) ($pK_a = 3.3$) in the stomach. The transformation is possible because the pH of gastric juice is approximately 2. Because nitrous acid is a reactive component, phenolic compounds present in foods can react with nitrous acid as follow (Takahama *et al.*, 2009):

$$\mathrm{PhOH} + \mathrm{HNO_2} \rightarrow \mathrm{NO} + \mathrm{PhO\ radical} + \mathrm{H_2O} \qquad (13.1)$$

where PhOH is a phenolic compound. It has been reported that nitrous acid can oxidize rutin, quercetin, kaempferol, catechins, caffeic acid, chlorogenic acid, gallic acid, and proanthocyanidin but not 3,4-dihydroxybezoic acid, suggesting that their reducing activities are dependent on their antioxidative activities (Takahama *et al.*, 2002, 2007). The mastication of dough prepared from buckwheat flour and the acidification of the masticated dough results in the oxidation of rutin producing NO (Takahama *et al.*, 2009), suggesting the progress of Eq. (13.1) in the stomach after the ingestion of food. The production of NO in the stomach and the effects of foods and beverages on its production have been reported (Gago *et al.*, 2007). In the absence of dietary phenolic compounds, the ascorbic acid in the gastric juice can reduce salivary nitrite to NO.

The NO produced in the stomach causes an increase in blood circulation and thickening of the mucosal tissues of the stomach. Such functions of NO may be related to the prevention of ulcer development in the stomach. In addition, NO can inhibit the proliferation of bacteria in the stomach. The inhibition of bacterial proliferation might be due to the binding of NO to Fe^{2+} in the heme moiety of heme-containing proteins. During the production of NO by Eq. (13.1), the nitration and nitrosation of phenolic compounds can proceed. Nitrous acid is a nitrosating agent, and the risk for the ingestion of nitrite- and nitrate-containing foods has been discussed in relation to cancer. Current studies suggest that nitrous acid-dependent nitrosation of amines producing nitrosamines seems to be negligible in the stomach because inhibitors of the nitrosation of amines, ascorbic acid and phenolic compounds, are present in the gastric juice.

Formation of an oxathiolone derivative

When rutin is mixed with nitrite and thiocyanate under acidic conditions (pH 2), a stable oxathiolone derivative of rutin (5,7-dihydroxy-2-(7-hydroxy-2-oxobenzo[*d*][1,3]oxathiol-4-yl)-4*H*-chromen-4-one 3-*O*-β-rutinoside) is produced (Figure 13.5) (Takahama

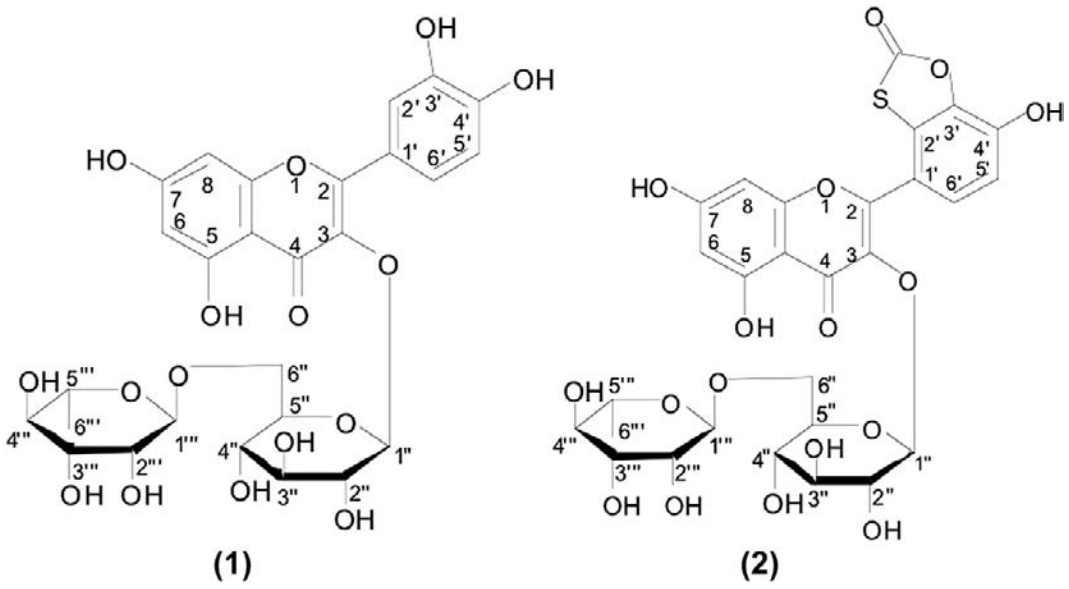

FIGURE 13.5
Chemical structures of (1) rutin and (2) the oxathiolone derivative of rutin.

et al., 2009). This component is also produced by the addition of both nitrite and thiocyanate to an acidic suspension of buckwheat flour and by the acidification of masticated buckwheat dough, suggesting that its formation is possible in the stomach after the ingestion of foods containing buckwheat flour or rutin.

The oxathiolone derivative of rutin can be produced as shown in Figure 13.6. Rutin may be transformed to the quinone form via a rutin radical to react with thiocyanate producing 2-thiocyanaterutin, which can be transformed to the oxathiolone derivative by hydrolysis. Thiocyanic acid might contribute to the formation of 2-thiocyanaterutin. The contribution of thiocyanic acid ($pK_a = 0.8$) could be deduced from the observation that the oxathiolone derivative of caffeic acid was detected at pH 2.0 but not pH 3.3, although caffeic acid was oxidized by nitrite at both pH values (unpublished observation).

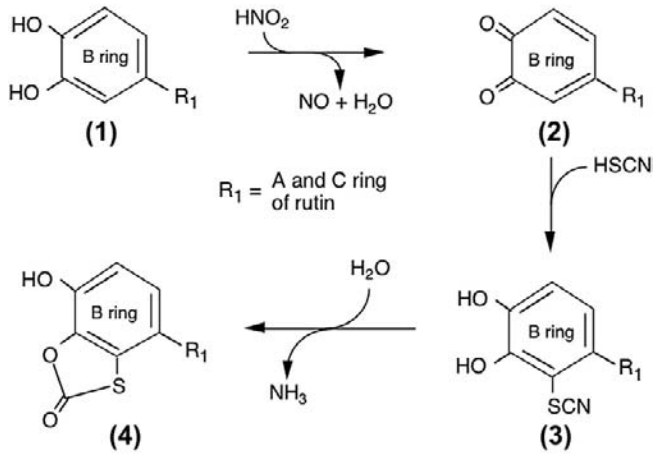

FIGURE 13.6
A postulated mechanism for the formation of the oxathiolone derivative of rutin in the stomach.

Chlorogenic acid, which is a common phenolic compound contained in foods and beverages, is also oxidized to its quinone form by nitrite under the acidic conditions. This quinone can be transformed to its oxathiolone derivative in the presence of thiocyanic acid as shown in Figure 13.6 (Takahama *et al.*, 2007). A component with an oxathiolone moiety (6-hydroxy-1,3-benzoxathiol-2-one) is used for the treatment of acne, and its derivatives can inhibit the activity of carbonic anhydrase and inhibitory κB kinase-β. Oxathiolone derivatives of chalcones can inhibit the growth of *Micrococcus luteus* and *Staphylococcus aureus*. Our results showed that the oxathiolone derivative of rutin inhibited the growth of *M. luteus*, but not that of *E. coli*, under aerobic conditions. Further studies are required to elucidate the function of oxathiolone derivatives of phenolic compounds, which may be produced in the stomach.

SUMMARY POINTS

- Heating of buckwheat flour results in the formation of indigestible starch that can contribute to the improvement of glycemic and insulin indexes. Inhibition of digestive enzymes by proanthocyanidins may also contribute to the improvement of glycemic and insulin indexes.
- D-*chiro*-Inositol can contribute to the improvement of glycemic and insulin indexes by ameliorating insulin resistance.
- Undigested buckwheat flour protein in the gut can contribute to the decrease in serum concentrations of cholesterol and lipid.
- Diets prepared from buckwheat flour can function as prebiotics.
- Allergens of buckwheat products have been identified, and the genes encoding the proteins have been cloned and expressed. This may lead to the development of allergen-free buckwheat.
- Rutin and proanthocyanidins in buckwheat flour can enhance the generation of NO in the stomach. Rutin can be transformed in the stomach to its oxathiolone derivative, the function of which remains to be elucidated.
- Diets prepared from buckwheat flour may be useful for human health.

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CHAPTER 14

Non-Starch Polysaccharides in Maize and Oat

Ferulated Arabinoxylans and β-Glucans

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

FA Ferulic acid
RDA Recommended Daily Allowance

INTRODUCTION

Cereals represent a major component of the human diet worldwide. The principal cereal crops grown throughout the world are maize, wheat, barley, rice, oats, rye, and sorghum. Cereals supply carbohydrate and protein as well as a variety of micronutrients. In addition, cereal-based foods can also supply significant amounts of dietary fiber. Cereal processing takes two basic forms, mechanical (e.g., milling) and thermal (e.g., baking), which increase the bioavailability of the nutrients in the grain and customer satisfaction (Eastman *et al.*, 2001).

Maize bran is an important by-product of the maize flour industry. Maize bran contains heteroxylans (approximately 50%), cellulose (approximately 20%), and phenolic acids

Flour and Breads and their Fortification in Health and Disease Prevention. DOI: 10.1016/B978-0-12-380886-8.10014-5

(approximately 4%, mainly ferulic and diferulic acid) (Saulnier *et al.*, 1995). Ferulic acid (FA) is the predominant phenolic compound in maize bran, and it is mainly found bound to cell wall polysaccharides (Fry, 1986). Phenolics can act as free radical terminators, chelators of metal catalysts, or singlet oxygen quenchers (Shahidi and Wanasundra, 1992). Consumption of free radicals and oxidation products may be a risk factor for cancer and cardiovascular disease, and dietary phenolics may have health benefits (Huang *et al.*, 1992). According to Hashimoto and Grossmann (2003), the main non-starch polysaccharides in maize bran are arabinoxylans (approximately 30%). Maize bran arabinoxylans have positive effects on cecal fermentation, production of short-chain fatty acids, and reduction of serum cholesterol, among others (Hopkins *et al.*, 2003; Lopez *et al.*, 1999).

Research on oat (*Avena sativa*) has increased in recent years because it has been reported to reduce serum cholesterol levels and attenuate postprandial blood glucose and insulin responses, which has been related to the presence of β-glucans (Cui, 2001). β-Glucans are the main component of non-starch polysaccharides of oat. β-Glucan is a cell wall polysaccharide found in the endosperm and in the subaleurone layer of oat. Oat contains 3.2−6.8% β-glucan, which varies with cultivar and environmental effects (Colleoni-Sirghie *et al.*, 2003).

This chapter discusses maize bran and oat as a source of ferulated arabinoxylans and β-glucan, respectively.

MAIZE BRAN

The tortilla industry is important in Mexico because half of the total volume of consumed food is maize. Maize bran is a by-product of the tortilla commercial process. Before tortilla preparation, nixtamalization of maize kernel is needed. Nixtamalization consists of cooking maize grains in a lime solution and soaking for 2−8 h. The remaining material is then ground to obtain nixtamal (dough or masa), which is used to prepare a variety of products, with tortilla being the most popular, whereas the supernatant (called nejayote) and the maize bran are discarded. The nixtamalization process degrades and solubilizes maize cell wall components, thus facilitating bran removal (Arámbula-Villa *et al.*, 2001). Alkali-soluble noncellulosic cell wall polysaccharides present in maize bran (mainly arabinoxylans) show interesting nutritional benefits. A maize nixtamalization facility processing 500 kg of maize every day generates approximately 100 kg of maize bran on a daily basis. Thus, alternatives for maize bran utilization are needed. According to Holguin-Acuña (2007), maize bran from the tortilla industry contains 4% fat, 33% fiber, 10% protein, and 2.5% ash (Table 14.1). This composition is similar to that reported by Kleinhans *et al.* (2005) in maize bran fractions from ethanol production. The arabinoxylans content in maize bran has been reported to vary from 30 to 38% (Gourson *et al.*, 1999; Holguin-Acuña, 2007). According to Saulnier *et al.* (1995) and Holguin-Acuña (2007), the FA content in maize bran is 3.4−3.7%.

TABLE 14.1 Chemical Composition of Maize Bran[a]

Fat	4.0 ± 0.50
Fiber	33.03 ± 0.60
Protein	10.0 ± 0.85
Arabinoxylans	38.6 ± 1.52
Ash	2.47 ± 0.01
Ferulic acid	3.70 ± 0.001
Starch	8.20 ± 0.00

Source: Adapted from Holguin-Acuña (2007).
[a]*Results are expressed in g/100 g dry matter. All results obtained from triplicates.*

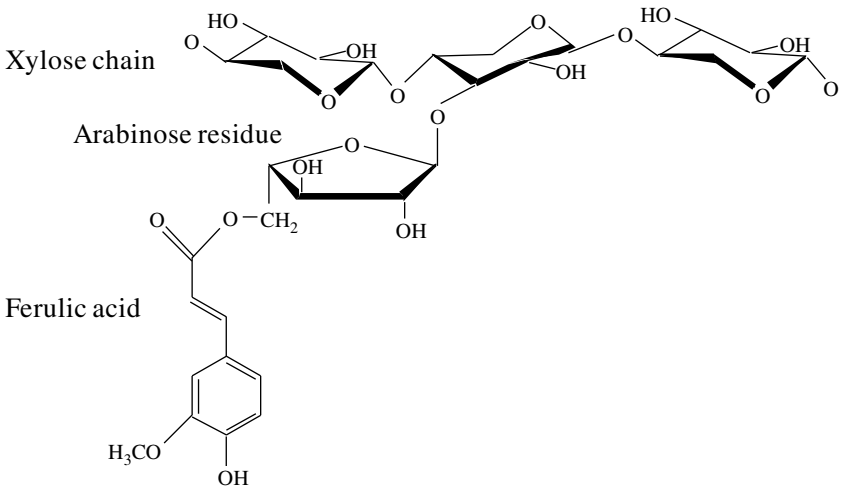

FIGURE 14.1
Representative chemical structure of ferulated arabinoxylan from maize.

FERULATED ARABINOXYLANS

Arabinoxylans are composed of a linear backbone of β-(1→4)-linked D-xylopyranosyl units to which α-L-arabinofuranosyl substituents are attached through the *O*-3 and/or *O*-2,3 positions of the xylose residues (Izydorczyk and Biliaderis, 1995). Some of the arabinose residues are ester linked on (*O*)-5 to FA (Smith and Hartley, 1983) (Figure 14.1). The molecule of arabinoxylans from rice, sorghum, and maize bran is more complex than that from wheat, rye, and barley because in addition to arabinose side branches, there are small amounts of xylose, galactose, and uronic acids (Izydorczyk and Biliaderis, 2007). Adams *et al.* (2003) reported that a small fraction of the arabinoxylan might be branched by β-(1→4)-linked xylose residues randomly located along the chain.

Niño-Medina *et al.* (2009) reported a ferulated arabinoxylan extracted from the wastewater of maize nixtamalization called "nejayote." Maize bran is an abundant by-product of the commercial maize dry milling process in Mexico. The heteroxylans portion of maize bran can be extracted with alkaline (Whistler, 1993) or acid solutions (Saulnier *et al.*, 1995) to produce water-soluble maize bran gum. Carvajal-Millan *et al.* (2007) reported an arabinoxylan-enriched water-soluble maize bran gum extracted under alkaline mild conditions from maize bran. Gels were obtained by using arabinoxylans recovered from nejayote and maize bran by laccase/O_2 covalent cross-linking of FA.

OAT

Oat (*A. sativa*) is extensively planted as a forage crop in northern Mexico, where drought often results in smaller oat crops that fail to meet the requirements of the market. In fact, oat cultivars have been developed for drought conditions. As an important animal feed resource, Mexican oat cultivars have been studied on the basis of forage yield and nutritional value. Ramos-Chavira *et al.* (2009) reported the following chemical composition of *A. sativa* Mexican grains harvested under drought conditions: fat, 9.6%; fiber, 2.9%; protein, 13.2%; ash, 2.3%; and starch, 63% (Table 14.2). This composition is similar to those reported for oat genotypes grown in different environments (Peterson *et al.*, 2005). The β-glucan content reported in this Mexican grain was 3.9%, which is in the range of that reported for other *A. sativa* varieties (3.2−6.8%) (Colleoni-Sirghie *et al.*, 2003). On the other hand, Ramos-Chavira *et al.* (2009) reported an FA content in oat (0.02%) higher than that reported by Wojdyło and Oszmański (2007) in other *A. sativa* cultivars from Poland (0.011−0.008%).

TABLE 14.2 Chemical Composition of Oat Flour[a]

Fat	6.89 ± 0.60
Fiber	4.67 ± 0.10
Protein	14.56 ± 0.70
β-Glucan	4.14 ± 0.3
Ash	2.67 ± 0.10
Ferulic acid	0.07 ± 0.01
Starch	67.00 ± 3.00

Source: Adapted from Ramos-Chavira (2008).
[a]*Results are expressed in g/100 g dry matter. All results obtained from triplicates.*

β-Glucan

The main component of soluble dietary fiber of oats is (1→3), (1→4)-β-D-glucan, also referred to as β-glucan (Cui, 2000; Izydorczyk and Biliaderis, 2007). β-Glucan is a linear polysaccharide made up entirely of sequences of (1→4)-linked D-glucopyranosyl units separated by single (1→3)-β-linked units (Izydorczyk and Biliaderis, 2007) (Figure 14.2). β-Glucan is a cell wall polysaccharide found in the endosperm and in the subaleurone layer of oats and barley (Colleoni-Sirghie *et al.*, 2003). Oat (*A. sativa*) contains 3.2−6.8% β-glucan; the percentage varies with cultivar and environmental effects (Colleoni-Sirghie *et al.*, 2003).

HEALTH BENEFITS

The increasing incidence of chronic diseases and obesity and the demonstrated link between the intake of dietary fiber and various health benefits have increased consumer interest in foods enriched with dietary fiber (Önning, 2007). Mahalko *et al.* (1984) reported that consumption of 52 g maize bran daily decreased very low-density lipoprotein cholesterol, triglycerides, and glycosylated hemoglobin. The nutritional value of arabinoxylans as a fiber component has not been investigated to the same extent as other polysaccharides. However, some studies have revealed positive effects of water-soluble maize arabinoxylans on cecal fermentation, production of short-chain fatty acids, reduction of serum cholesterol, and improved adsorption of calcium and magnesium (Hopkins *et al.*, 2003; Lopez *et al.*, 1999). Arabinoxylans are not digested in the small intestine but provide fermentable carbon sources for bacteria that inhabit the large bowel (Hopkins *et al.*, 2003). Hopkins *et al.* investigated the degradation of cross-linked and non-cross-linked arabinoxylans by the intestinal microbiota in children and found that FA cross-linking reduced the rate of arabinoxylan fermentation. Similar results were found in adults.

Research on oat (*A. sativa*) has increased in recent years because clinical studies have shown oat β-glucan to reduce serum cholesterol levels and attenuate postprandial blood glucose and insulin responses in a viscosity-related manner (Cui, 2001). On the other hand, because of the high viscosity of their solutions, β-glucans have a potential application in food texturizing

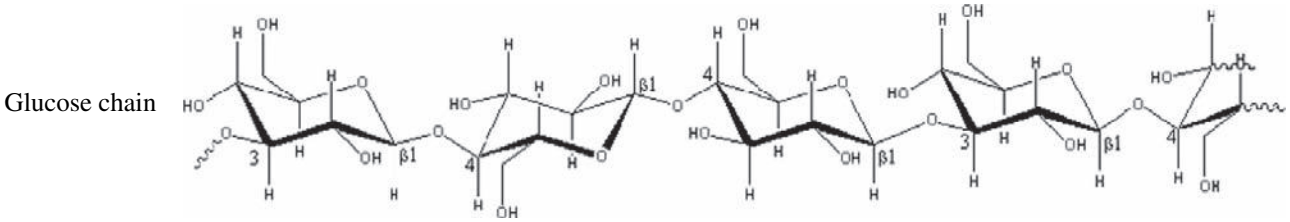

FIGURE 14.2
Representative chemical structure of β-glucans from oat.

TABLE 14.3 Chemical Composition of Maize Bran/Oat Flour Extruded Breakfast Cereal[a]

Maize Bran/Oat Flour (%, w/w)	Protein (%, w/w)	Fat (%, w/w)	Fiber (%, w/w)	Ash (%, w/w)	Starch (%, w/w)
0	14.4[a]	6.4[a]	0.8[f]	2.7[a]	76[b]
10	13.3[b]	5.7[b]	1.7[e]	2.7[a]	79[b]
20	12.6[b]	4.9[c]	3.1[d]	2.6[a]	77[a]
30	12.6[b]	4.1[d]	4.3[c]	2.4[a]	73[a]
40	11.9[b]	3.5[e]	5.6[b]	2.4[a]	72[a]
50	10.4[c]	3.3[e]	6.7[a]	2.4[a]	77[a]

Source: Adapted from Holguin-Acuña (2007).
[a]*Results are expressed in g/100 g dry matter. Mean values in the same column with different letters are significantly different* ($p < 0.05$).

TABLE 14.4 Non-Starch Polysaccharides, Ferulic Acid, and Antioxidant Capacity in Maize Bran/Oat Flour Extruded Breakfast Cereal[a]

Maize Bran/Oat Flour (%, w/w)	Arabinoxylans (g/100 g Dry Matter)	β-Glucans (g/100 g Dry Matter)	Ferulic Acid (mg/g Dry Matter)	Antioxidant Capacity (Fe^{2+} mmol)
0	2[d]	2.2[c]	0.03[d]	1.3[d]
10	3[d]	1.7[c]	0.3[d]	1.8[c]
20	5[c]	1.3[b]	1.5[c]	1.9[c]
30	7[b]	1.1[b]	2.1[b]	2.5[b]
40	21[a]	0.9[a]	3.2[a]	2.8[b]
50	22[a]	0.6[a]	3.6[a]	3.5[a]

Source: Adapted from Holguin-Acuña (2007).
[a]*Mean values in the same column with different letters are significantly different* ($p < 0.05$).

(Brennan and Cleary, 2005). In addition, some β-glucans can form physical gels, which have been proposed as fat mimetics and as encapsulation agents (Izydorczyk and Biliaderis, 2007).

Holguin-Acuña *et al.* (2008) prepared an extruded breakfast cereal by using different percentages of maize bran/oat flour (0, 10, 20, 30, 40, and 50%, w/w). The composition of these formulations is presented in Table 14.3. These authors found that a 100-g serving of breakfast cereal containing 30% maize bran, which was the most accepted by consumers, provides 8.1 g of complex polysaccharides (1.1 g of β-glucans and 7.0 g of arabinoxylans) (Table 14.4). The Recommended Daily Allowance (RDA) of dietary fiber is 20−35 g/day, according to the "Dietary Reference Intakes for Energy, Carbohydrate." It has been indicated that products that contain 0.75 g β-glucans or 1.78 g *Psyllium* per serving are permitted to carry a health claim stating that the product "will reduce the risk of coronary heart disease." Similarly, a 100-g serving of breakfast cereal containing 30% maize bran provides 0.2 g of FA, which is 20% of the RDA for adults. Baublis *et al.* (2000) produced a wheat-based breakfast cereal with an FA content of 0.74 mg/g cereal, which is less than half of the FA content in the sample containing 30% of maize bran (2 mg/g cereal). According to Holguin-Acuña *et al.* (2008), the antioxidant capacity value of the cereal breakfast containing 30% of maize bran is similar to that reported by Benzie and Strain (1996) for ascorbic acid, α-tocopherol, or uric acid (approximately 2.0 mmol Fe^{2+}). This antioxidant activity is attributed to FA from maize bran. The medicinal action of FA is mainly due to its antioxidant capacity, free radical scavenging, and chelation of redox active metal ions (Baublis *et al.*, 2000).

TECHNOLOGICAL ISSUES

Maize bran and oat flour may be alternatives for non-starch polysaccharide- and antioxidant-enriched cereal products, but technological and nutritional evaluation of these food products

is necessary. The maize/oat products to be obtained would have health benefits such as lowering of blood cholesterol and sugar as well as antioxidant properties.

SUMMARY POINTS

- Maize and oat are sources of non-starch polysaccharides and antioxidants, which are beneficial to health.
- The presence of ferulated arabinoxylans in maize bran allows an alternative use of this by-product of the food industry and would offer new advantages for future industrial applications of this product.
- Low-value *A. sativa* cultivar has a β-glucan content similar to those reported for normal oat grains, which could represent a commercial advantage compared to other oat cultivars commonly used in the food industry.
- Maize and oat as sources of non-starch polysaccharides and antioxidants continue to be investigated, and new information about their health benefits is being reported.
- More research is needed to elucidate several questions, especially those concerning the effect of food process on health benefits of these compounds.

Acknowledgments

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CHAPTER 15

Gluten-Free Bread: Sensory, Physicochemical, and Nutritional Aspects

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

HPMC Hydroxypropyl methylcellulose
NaFeEDTA Sodium iron (III) ethylenediaminetetraacetate

INTRODUCTION

Celiac disease (gluten-sensitive enteropathy or gluten intolerance) is a genetically based autoimmune enteropathy caused by a permanent sensitivity to gluten (Rubio-Tapia and Murray, 2010). In susceptible individuals, the ingestion of gluten induces an immunologically toxic reaction that results in damage to the mucosal surface of the small intestine—specifically small bowel mucosal villous atrophy with crypt hyperplasia (Catassi and Fasano, 2008).

Celiac disease affects approximately 1% of most populations but remains largely unrecognized (Rubio-Tapia and Murray, 2010), despite advances in diagnosis. Upon diagnosis, the celiac disease patient is directed to a gluten-free diet for life. The gluten-free diet excludes the intake of storage proteins found in wheat, rye, barley, and hybrids of these grains, such as kamut and triticale. This diet prevents morbidity and reduces the incidence of the associated gastrointestinal malignancy, but it is difficult to adhere to (Kupper, 2005), particularly because it

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excludes many products that contain gluten. It is therefore to the benefit of celiac disease patients to develop new gluten-free products suitable to their needs that will increase their dietary choices and improve quality of life in general.

AN EMERGING NEED FOR NEW GLUTEN-FREE PRODUCTS

The unequivocal need for the development of new gluten-free products is emerging for several reasons. First, the daily dietary requirements for essential nutrients of celiac disease patients are not fully covered by existing products. Products of the gluten-free group, compared with their gluten-containing counterparts, are lower in protein, vitamins, minerals, and dietary fiber (Anton and Artfield, 2007; Thompson, 2000). Moreover, gluten intolerance is frequently associated with low absorption of nutrients. Consequently, celiac patients face several nutrition-related problems, such as weight loss, iron deficiency anemia, osteoporosis, fatigue syndrome, and diabetes. In addition to nutrient content, gluten-free products based on starch are less tasty than the gluten-containing counterparts. In the case of bread, there is also a high staling tendency due to the absence of gluten (Gallagher *et al.*, 2003).

Second, the target group of gluten-free products is currently expanding to include, in addition to celiac patients, people looking for nonallergenic ingredients and generally people who are more careful about their diet not just for health reasons. This constitutes a new market that needs a variety of products. According to research, 15–25% of parents in the United States seek gluten-free products for their children as part of a balanced diet.

Third, gluten-free products can function as prototypes for the development of other products targeted to specific groups with specific nutritional needs (e.g., diabetics).

Gluten-free products constitute a growing sector in the food business. Between January 2008 and June 2009, Mintel's Global New Products Database found gluten-free to be the 10th most popular claim for new product launches throughout Europe.

INGREDIENTS FOR THE FORMULATION OF GLUTEN-FREE BREADS: PHYSIOCHEMICAL AND SENSORY ASPECTS

Maize and rice are the main ingredients used for preparing gluten-free bakery products. Maize and rice products are similar in taste, thus offering the consumer limited choice. Rice flour is one of the most suitable cereal flours for preparing gluten-free products because it is natural, hypoallergenic, and has a bland taste. It provides a high amount of digested carbohydrates but a low amount of proteins (prolamins), thus indicating the need for other components to reinforce the batter matrix and the nutritional content of the final product.

There has been increasing interest in new gluten-free breads, whose formulations mainly involve the incorporation of starches of different origin, other non-gluten proteins such as dairy proteins, gums, and their combinations (Mariotti *et al.*, 2009). These ingredients can mimic the viscoelastic properties of gluten and result in improved structure, mouthfeel, acceptability, and shelf life of these products (Gallagher *et al.*, 2004). The successful production of gluten-free products is a challenge, considering that gluten plays a major role in food structure and is involved in the formation of the three-dimensional network, which influences the textural and sensorial properties of the final product (Crowley *et al.*, 2000). Furthermore, the development of new gluten-free products is planned in order to reflect changing consumer lifestyles and needs.

Pseudocereals such as quinoa and amaranth can be used to enhance nutritional content, particularly the protein content of the final product. Moreover, proteins from different sources such as soybean, pea, egg albumen, and whey can be added to increase the nutritional value of gluten-free products. By adding proteins, improvement in the quality of gluten-free bread due to the formation of a continuous protein phase is also reported (Moore *et al.*, 2004). It follows that the selection of the proteins used in a gluten-free formulation is a critical issue.

An example of the use of pseudocereals in gluten-free breads is the use of amaranth flour (Figure 15.1). Amaranth flour has high protein content, ranging from 14.5 to 17.8%. The protein from amaranth flour is of high quality due to the high amounts of lysine- and sulfur-containing amino acids (Gorinstein *et al.*, 2002). In gluten-free products, amaranth flour can be an alternative component for successful development of these complex mixtures. Tosi *et al.* (1996) described the use of amaranth flour in gluten-free biscuits, whereas Schoenlechner *et al.* (2006) described a substitution of gluten-free flour by amaranth flour up to 100% for the development of such products. Amaranth has been successfully used in gluten-free pasta production (Chillo *et al.*, 2007). Finally, gluten-free bread with enhanced nutritional value can be produced using amaranth flour. In small amounts, amaranth flour can also improve dough workability and volume of bread crumb. Furthermore, antifungal activity of amaranth has been shown (Rizzello *et al.*, 2009).

In a study on the sensory properties of gluten-free breads containing amaranth flour (Schoenlechner *et al.*, 2010), the addition of fat and albumen resulted in samples with higher scores on mouthfeel, texture, and volume in comparison with samples without the addition of fat or albumen. Samples were given to a trained panel (8 people at the University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria) and to an untrained panel (52 people at the Agricultural University of Athens, Greece) to evaluate the sensory characteristics of the samples. According to both sensory evaluation protocols, the combined effect of fat and albumen resulted in more desirable properties of the final products rather than the effect of each ingredient separately. A 2^3 factorial screening experimental design was applied to investigate the influence of several variables on the quality of gluten-free bread that contained amaranth. The variables were the amount of water (60, 70, and 80%), albumen (0, 2.5, and 5%), and fat (0, 2, and 4%). The effect of water and albumen content on response attributes such as crumb firmness, crumb porosity, loaf circumference, and crumb relative elasticity are shown in response surface plots (Figure 15.2). It was shown that water is a critical variable that significantly influences the physical properties of the produced gluten-free breads. Increased water addition significantly decreased crumb firmness (Figure 15.2A) and slightly increased relative elasticity (Figure 15.2B), which was accompanied by a significant increase in bread circumference (Figure 15.2D). Moreover, it sharply increased the average pore size (Figure 15.2C). An increase in average pore size was accompanied by a decrease in pore number. Variations in albumen concentration resulted in minor changes in the previously mentioned response variables; average pore size and bread circumference were slightly decreased. To a greater extent, albumen significantly influenced only crumb viscoelastic character; relative elasticity was increased by the increased addition of albumen.

An alternative protein source for the development of gluten-free breads is carob seed. Carob seed germ, in particular, has not been valorized as a functional protein source and has been

FIGURE 15.1
Gluten-free breads containing amaranth. Freshly baked (left) and frozen bread stored for 10 days (right). Gluten-free breads have larger air cells than those of wheat bread, but this structure is maintained during storage; thus, freezing can prolong their shelf life.

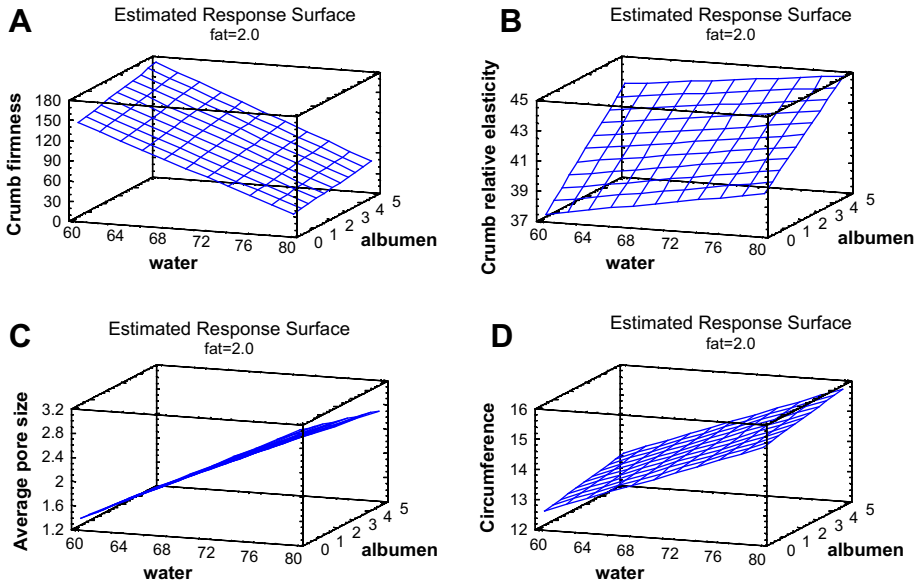

FIGURE 15.2
The effect of water and albumen content in amaranth-based gluten-free breads on the response attributes crumb firmness (A), crumb relative elasticity (B), crumb porosity (C), and loaf circumference (D), shown in response surface plots. The physical characteristics of gluten-free breads were mainly influenced by the initial dough water content. Specifically, higher water addition decreased crumb firmness and increased sharply the average air cells size. *Source*: *Reprinted with permission from Schoenlechner, R., Mandala, I., Kiskini, A., Kostaropoulos, A., and Berghofer, E. (2010). Effect of water, albumen and fat on the quality of gluten-free bread containing amaranth.* Int. J. Food Sci. Technol. *45, 661–669.*

neglected for many years by the food industry. However, the high protein content of the germ, almost 50%, and its high content of lysine and arginine make carob germ fairly attractive for special dietary needs (i.e., clinical nutrition and gluten-free products) (Dakia *et al.*, 2007). Caroubin, the protein of carob germ, also has similar viscoelastic properties as gluten. Thus, its potential as a food ingredient is high, and it could be considered a low-cost competitor to other food proteins such as dairy or soy proteins (Bengoechea *et al.*, 2008).

Various sources of fiber are in use in gluten-free breads or being investigated for the development of new gluten-free breads. For example, psyllium fiber was added to gluten-free breads (Mariotti *et al.*, 2009), resulting in an increased fiber content of the bread (190–450% higher than that of the control breads) and a softer crumb during a 4-day storage period. Furthermore, it enhanced the physical properties of the produced dough due to the filmlike structure that it is able to form.

In one study, gluten-free bread enriched with resistant starch (20% substitution of the corn starch used) raised the total dietary fiber content up to 89% compared to the control sample (Korus *et al.*, 2009). Furthermore, gluten-free breads contained more water than the control and restricted bread staling. Compared to common sources of insoluble fibers, resistant starch may have advantageous features that are both functional and nutritional. It is a natural white source of dietary fiber, has a bland flavor, and gives a better appearance and texture and masks flavor less than sources of insoluble fibers (Sajilata *et al.*, 2006). Concerning its nutritional characteristics, an interesting attribute of resistance starch is its pattern of fermentation in the colon, principally the profile of short-chain fatty acids (Baixauli *et al.*, 2008).

Few studies have investigated the effect of fibers that may have a prebiotic effect when added to gluten-free products. Korus *et al.* (2009) added a range of different prebiotics to gluten-free breads. Among them, inulin resulted in breads with high sensory scores and also reduced staling. Inulin (8% inclusion level) increased fiber content from 1.4% (control) to 7.5% (control + inulin) and crust color was enhanced (Gallagher *et al.*, 2003). As inulin increases the dietary fiber content of gluten-free bread, the nutritional value of the resulting product is increased (Gallanger *et al.*, 2004).

Gums are important ingredients in gluten-free bread formulations that may improve the texture and the appearance of the final products. The addition of gums provides high dough consistency, improves gas-retaining capacity, and results in products with a longer shelf life.

Different gums and different combinations may be applied in gluten-free product development. Among them, hydroxypropyl methylcellulose (HPMC), locust bean gum, guar gum, carragenan, and xanthan gum provide the best results in the final products (Gallagher *et al.*, 2004; Lazaridou *et al.*, 2007). Gum combinations such as xanthan—locust bean gum are effective in improving dough structure (Demirkesen *et al.*, 2010).

Complex formulations that appear promising in terms of technological improvement and nutritional quality can be developed. Clearly, new products differ in their degree of innovation and, consequently, the effort invested in their development. Gluten-free products can be classified into the following categories: (1) reformulations (e.g., high-fiber gluten-free versions of traditional antecedents), (2) new forms of existing products (e.g., frozen and par-baked), (3) repackaging of existing products, and (4) innovative products (e.g., use of novel cereals) that are technologically challenging and require good marketing (Kelly *et al.*, 2008). Furthermore, ingredients for a new food product can be based on different criteria, such as function in the product, cost, or availability. In the case of gluten-free products, labeling concerns are a key issue; for example, a product that is labeled as causing an allergic reaction in consumers with celiac disease is to be avoided. The staling process is important in gluten-free products because they contain a large amount of starch and its retrogradation influences the quality of the final product. Hence, a reduced staling rate in gluten-free products is quite desirable. Water migration and transformation in the starch fraction are important factors that can control staling.

FROZEN GLUTEN-FREE BREADS

Frozen storage can be used for the production of gluten-free products with extended shelf life. Par-baking bread production has great market potential in gluten-free cereal processing because a fresh product can be produced with a simple bake-off stage (Kelly *et al.*, 2008). Most of the gluten-free bread and rolls available on the market are par-baked, but there are no publications in this area (Keller *et al.*, 2008). Figure 15.1 shows gluten-free bread frozen stored. As expected, the structure of such products is different from that of a wheat bread. Pore size and number differ; usually, the produced sample has larger pores and a lower volume than wheat bread. Frozen storage can be successfully used for the production of gluten-free breads. The sensory characteristics of wheat breads containing gluten and gluten-free breads preserved by using either microwave treatment or frozen storage are presented in Figure 15.3 (Liassi and Mandala, 2007). According to the sensory characteristics presented, wheat breads seemed to be firmer, with lower deformability, and less crispy than gluten-free breads. On the other hand, gluten-free breads were more adhesive and had greater chewiness. Frozen stored breads (wheat breads and gluten-free breads) had the best appearance, aroma, taste, and textural characteristics. In addition, sensory attributes in ambient conditions and after microwave treatment had only slight differences.

IRON-FORTIFIED GLUTEN-FREE BAKERY PRODUCTS

The immunologically toxic reaction that is induced by gluten in susceptible individuals results in damage to the mucosal surface of the small intestine (Rubio-Tapia and Murray, 2010). This interferes with the absorption of nutrients, including iron. Thus, the importance of celiac disease as a possible cause of iron deficiency anemia is increasingly being recognized. It is estimated that celiac disease may account for 3—5% of the prevalence of iron deficiency anemia (Grisolano *et al.*, 2004). Moreover, the gluten-free products are often low in micronutrients, increasing the risk of deficiencies (Thompson, 2000). Fortified or enriched gluten-free products are rare, but it has been suggested that the development of such products would improve the quality of the diet (Kupper, 2005). Fortification is an effective approach to increase dietary iron intake, provided that certain conditions apply (Hurrell *et al.*, 2004).

Successful fortification requires an iron compound that is adequately absorbed and does not affect the sensory properties of the products. Iron has been known to affect the sensory

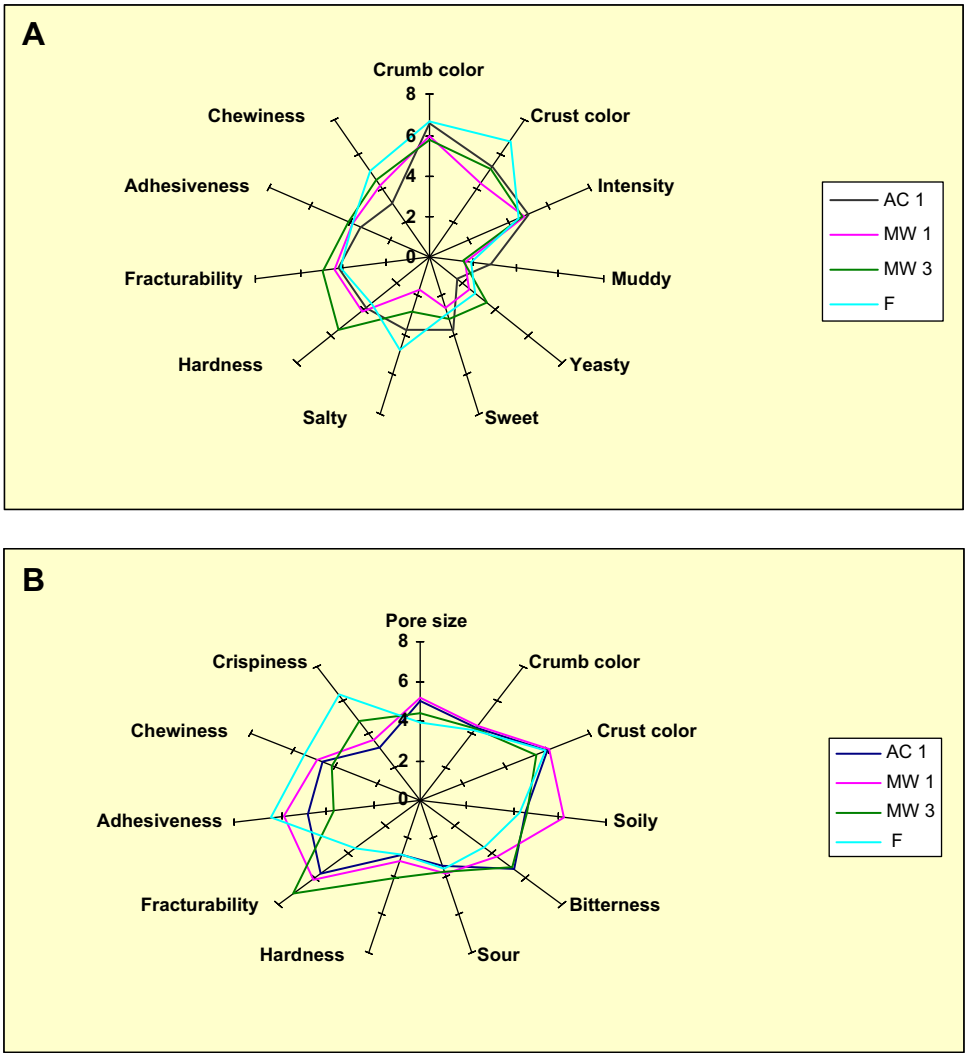

FIGURE 15.3
Sensory characteristics regarding the appearance, aroma, taste, and texture of (A) control gluten-containing breads and (B) gluten-free breads stored in ambient conditions (AC), after microwave treatment (first and third day of storage; MW1 and MW3, respectively), and after frozen storage (F). The sensory characteristics of the breads presented (wheat or gluten-free) were negatively influenced by microwave treatment or by storage in ambient conditions. On the contrary, frozen stored breads had good appearance, aroma, taste, and texture.

properties of a fortified food, particularly color and taste (Hurrell *et al.*, 2004). Ferrous sulfate is the most popular source of iron for the fortification of various foods; however, other iron forms may exhibit higher bioavailability than ferrous sulfate and may present alternative choices (Lynch and Stoltzfus, 2003). Other iron compounds, such as iron gluconate or sodium iron (III) ethylenediaminetetraacetate (NaFeEDTA) (Hurrell *et al.*, 2004), have been studied as well.

The fortification of gluten-free breads, however, presents further challenges. In general, the taste of gluten-free bread is inferior to that of white wheat bread; therefore, the moderate scores obtained were typical for gluten-free bread and were considered acceptable for such products. In one study (Kiskini *et al.*, 2007), we produced gluten-free bread, fortified with iron, using selected iron compounds (iron pyrophosphate, iron pyrophosphate with emulsifiers, NaFeEDTA, electrolytic iron, ferrous gluconate, ferrous lactate and ferrous sulfate). Furthermore, we tested the sensory characteristics of the iron-fortified product (mouthfeel texture, crumb color, aroma, and taste) and compared iron dialyzability of various iron compounds. We found that the most acceptable products were those fortified with ferrous pyrophosphate with emulsifiers and ferrous pyrophosphate. Ferrous dialyzable iron (ferrous iron with a molecular weight less than 8000, an index for prediction of iron bioavailability) was measured under simulated gastrointestinal conditions. Ferrous dialyzable iron in gluten-free bread fortified with iron pyrophosphate with emulsifiers, NaFeEDTA, ferrous bis-glycinate,

ferrous gluconate, or ferrous sulfate was higher than that in gluten-free bread fortified with electrolytic iron, ferrous lactate, or iron pyrophosphate. When comparing iron-fortified wheat or gluten-free breads (Kiskini *et al.*, 2010), we observed that the effect of iron on the quality characteristics of the breads investigated depended on iron type but not on iron solubility. Color, crust firmness, specific volume, cell number and uniformity, as well as aroma, were the attributes that were mainly affected in iron-enriched wheat bread (Figure 15.4). According to principal component (PC) analysis, the total variation in all data was explained up to 96.18% by PC1 (50.06%) and PC2 (46.12%). Component 1 was defined by the L/b value and the crust firmness. Component 2 was defined by the specific volume, the firmness, the viscoelasticity, and the moisture content of the crumb (see Figure 15.4).

In gluten-free breads, differences between unfortified and fortified samples included color, crust firmness, cell number, moisture odor, metallic taste, and stickiness. In some cases, the sensory scores were better for fortified samples. In general, we observed that differences in the sensory characteristics of breads due to iron fortification were less pronounced in gluten-free compared to wheat breads (Kiskini *et al.*, 2010).

A frozen iron-fortified gluten-free bread was developed to meet the market request for frozen breads. In these frozen formulations, the iron compound remained in the aqueous environment of the dough for a longer storage time than it would have in a fresh white wheat bread product, thus interacting with the remaining ingredients and creating a challenge for maintaining its sensory characteristics. In this experiment (Kiskini *et al.*, 2007), the fortified products in the form of dough remained at −18°C for 10 days before they were baked and tested. Differences in reactivity of the iron compounds in the physicochemical environment of the dough or of the baked bread may explain the observed differences in scores assigned to the different formulations. However, it was difficult to explain why some iron-fortified samples were assigned better scores than gluten-free breads. In the case of iron pyrophosphate with emulsifiers, the scores were significantly higher than those of gluten-free breads in most attributes tested. This suggests

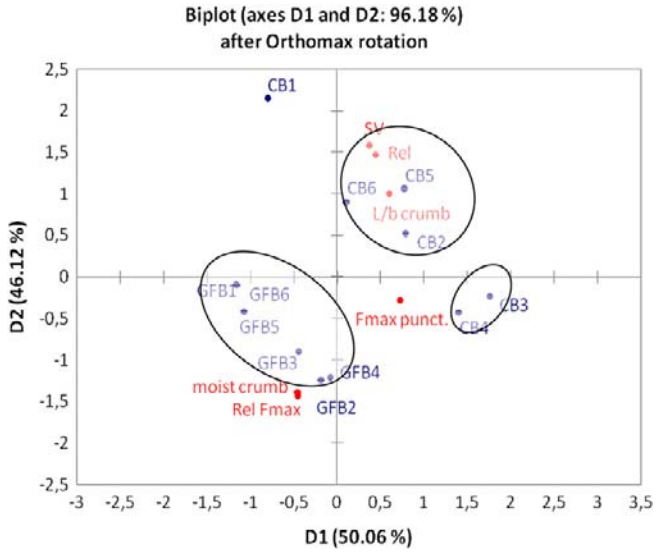

FIGURE 15.4
Physical attributes and sample categorization in a biplot of two principal components. Numbers in bread samples correspond to iron-type fortificants: CB1/GFB1, unfortified; CB2/GFB2, ferric pyrophosphate; CB3/GFB3, ferric pyrophosphate with emulsifiers; CB4/GFB4, sodium iron EDTA; CB5/GFB5, ferrous sulfate; CB6/GFB6, elemental iron. Differences in the physical characteristics of breads due to iron fortification were less pronounced in gluten-free compared to wheat breads. The unfortified wheat bread was significantly different from all other samples. The ferric pyrophosphate-fortified bread (CB2/GFB2) declined the most from the standard unfortified sample. *Source*: *Reprinted with permission from Kiskini, A., Kapsokefalou, M., Yanniotis, S., and Mandala, I. (2010). Effect of different iron compounds on wheat and gluten-free breads.* J. Sci. Food Agric. *90, 1136–1145.*

that iron fortification by this compound improves some sensory characteristics of gluten-free breads. A plausible explanation may be that the emulsifier used in this formula led to a good moisture distribution in the crumb and a desirable aerated structure. It was observed that the crumb of this product had medium-sized air pores of good uniformity. Furthermore, its grain characteristics (pore size and distribution) had the greatest similarities to those of the unfortified sample. These observations suggest that further investigation of the physicochemical characteristics of the iron-fortified gluten-free breads is needed because it may reveal important effects of iron compounds on the properties of the baked products. Furthermore, it may explain differences noted by the trained panelists. Nevertheless, these results are promising for the development of iron-fortified gluten-free baked products. Identification of the appropriate iron compound that will not cause adverse quality changes is still a challenge.

TECHNOLOGICAL ISSUES

Innovative products that are technologically challenging require the development of complex formulations that provide consumers superior quality products. Ingredients for the development of a new gluten-free bread type should be carefully chosen, and their selection should be based on their high added value. Furthermore, they should meet the criteria of allergy concerns, and any suspicious allergic substance is strictly inappropriate for celiacs and should be avoided.

New products differ in their degree of innovation and consequently the effort invested in their development, but high-quality products with good market prospects should be based on a great degree of innovation. Thus, innovative gluten-free products should be carefully designed and further developed in order to fulfill consumers' needs.

SUMMARY POINTS

- Celiac disease is caused by a permanent sensitivity to gluten.
- The celiac disease patient is directed, for life, to a gluten-free diet.
- The gluten-free diet excludes the intake of storage proteins found in wheat, rye, barley, and hybrids of these grains, such as kamut and triticale.
- New gluten-free products will increase the dietary choices of celiac patients or other nutritionally conscious consumers and improve their quality of life.
- Gluten-free ingredients employed in the formulation of gluten-free breads include maize; rice; amaranth; quinoa; starches of different origin; non-gluten proteins such as dairy proteins, gums, hydrocolloids, and their combinations; inulin; psullium; and gums (HPMC, locust bean gum, guar gum, carragenan, and xanthan gum).
- Fortified gluten-free breads, particularly those fortified with iron, meet the increased nutrient needs of celiac patients.
- Achieving desirable physicochemical and sensory characteristics for the gluten-free breads remains a challenge that drives continuous research in the field.

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CHAPTER 16

Dietary Fiber from Brewer's Spent Grain as a Functional Ingredient in Bread Making Technology

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

BSG Brewer's spent grain
BU Barbander units
CL Celluclast
LE Lipapan Extra
ME Maxlife 85
PCE Pentopan Mono and Celluclast
PE Pentopan Mono

Flour and Breads and their Fortification in Health and Disease Prevention. DOI: 10.1016/B978-0-12-380886-8.10016-9

DIETARY FIBER

Dietary fiber has received much attention by nutritionists in resent years as an important ingredient in enhancing human health. A variety of definitions of dietary fiber exist, mainly based on that provided by Trowell *et al.* (1976), who defined dietary fiber as "the plant polysaccharides and lignin which are resistant to the enzymes of man." The following are definitions of dietary fiber:

> Dietary fiber is the remnants of the edible part of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human large intestine. It includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fiber exhibits one or more of either laxation (fecal bulking and softening; increased frequency; and/or regularity), blood cholesterol attenuation, and/or blood glucose attenuation. (American Association of Cereal Chemist, 2001)
>
> Dietary fiber consists of one or more of: edible carbohydrate polymers naturally occurring in the food as consumed; carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic, or chemical means; synthetic carbohydrate polymers. (Food Standard Agency, 2007)
>
> Dietary fiber is defined as food material, particularly plant material, that is not hydrolyzed by enzymes secreted by the human digestive tract but that may be digested by microflora in the gut. Plant components that fall within this definition include non-starch polysaccharides (NSP) such as celluloses, some hemi-celluloses, gums and pectins, as well as lignin, resistant dextrins, and resistant starches. (Institute of Food Science and Technology, 2007)

However, dietary fibers are highly complex substances that can be classified as insoluble fibers, or those that do not dissolve in water, and soluble fibers, or those that do dissolve in water. The amount of each type of fiber varies in different plant foods.

HEALTH BENEFITS OF DIETARY FIBER

Consuming a high level of dietary fiber food has a number of important physiological effects in humans and has been associated with the prevention of several diseases. According to results published in the literature, adults in the United States consume less than half (Slavin, 2005) of recommended levels of 25–30 g dietary fiber per day (Cummings and Stephen, 1980), whereas adults in the United Kingdom (51% of men and 69% of women) fell short of the minimum recommended intake of 18 g per day (Food Standard Agency, 2007).

In a review paper by Slavin (2005), a strong positive correlation of dietary fiber intake with prevention of obesity was reported, and there was an inverse relationship with body weight, body fat, and body mass index, probably because the addition of dietary fiber generally decreases food intake. It was suggested that dietary fiber can control weight through promoting satiation, decreasing absorption of macronutrients, and altering secretion of gut hormones.

Dietary fiber intake reduces the risk of chronic heart disease and diabetes (Jenkins *et al.*, 2004), which was associated with the consumption of insoluble cereal fiber, whereas a reduction of total and low-density lipoprotein cholesterol levels (Brown *et al.*, 1999) was associated with viscous fibers. Similar metabolic effects have been seen with increasing meal frequency or using low glycemic index foods. Schulze *et al.* (2004) examined the association between glycemic index, glycemic load (amount of carbohydrates multiplied by the average glycemic index), and dietary fiber with the risk of type 2 diabetes in young women. It was found that higher glycemic index was significantly associated with an increased risk of diabetes, higher carbohydrate intake, and higher glycemic load. Diets with a high glycemic index and low in cereal fiber increase the risk of type 2 diabetes, particularly in women with a sedentary lifestyle and a family history of diabetes.

Dietary fiber is one of the primary substrates for growth of the microflora in the large bowel, which increases stool bulk and improves laxation (Cummings and Englyst, 1995). Thus, the bulk associated with undigested residue contributes directly to stool bulk as undigested material or indirectly through the growth of microflora, which are a part of the stool weight.

Furthermore, a high intake of dietary fiber may reduce the risk of duodenal ulcer in men aged 40 years or old (Aldoori *et al.*,1997) and colorectal cancer (Larsson *et al.*, 2005), particularly with the consumption of hard, whole grain rye bread of more than 4.5 servings per day.

DIETARY FIBER IN BREAD MAKING TECHNOLOGY

Traditionally, bread is considered a nutritious food rich with carbohydrates, protein, dietary fiber, and vitamins and essential components of the daily diet. To provide more variety in functional breads, different sources of dietary fiber have often been used in recipes, such as wheat bran, barley, oat, rye, and rice brans (Katina *et al.*, 2006; Rakha *et al.*, 2010; Sudha *et al.*, 2007; Wang *et al.*, 2002). Fiber-supplemented breads show a pronounced decrease in quality parameters, and there is a significant effect on mixing and viscoelastic properties and fermentation behavior during bread preparation. Dietary fiber addition increases water absorption (Sudha *et al.*, 2007), decreases loaf volume (Katina *et al.*, 2006), and affects farinograph parameters and shelf life (Katina *et al.*, 2006). Arabinoxylans are the major high-molecular polymers of cell walls and components contributing to the dietary fiber value in breads. Biliaderis *et al.* (1995) studied the functional role of various amounts of arabinoxylans in bread making technology and found that arabinoxylans affect textural properties of breads depending on the amounts added, the molecular size of these polymers, and the bread making quality of base flours. All these changes seem to be a result of "dilution of the gluten network, which in turn impairs gas retention rather than gas production" (Autio and Laurikainen, 1997) and "disruption of the starch–gluten matrix and restriction of gas cells to expand in a particular dimension" (Gan *et al.*, 1995).

Good quality, high dietary fiber breads with an acceptable texture and taste are essential; therefore, it is necessary to make adjustments in various process parameters. The other solutions are presoaking or fermenting bran before it is added to the dough and/or forming sourdough or using enzymes. Apart from textural improvement, sourdough fermentation has a well-establish role in improving nutritional properties and flavor. Bran fermentation in water allows enhanced water absorption and textural modification of bran particles, resulting in improved structure of the gluten network and softer breads due to altered water migration between starch, protein, and bran particles during storage (Katina *et al.*, 2006). Furthermore, the combination of bran sourdough and enzyme mixture is more pronounced in improving loaf volume, the structure of the gluten network, and the shelf life of baked breads and in reducing starch crystallization during storage.

EXPLOITATION OF BREWER'S SPENT GRAIN AS A SOURCE OF DIETARY FIBER

There is a trend to find new sources of dietary fiber as functional ingredients. A number of researchers have studied the value of cereal by-products and their utilization.

Brewing industries in the European Union produce approximately 3.4 million tons of brewer's spent grain (BSG) annually, of which UK brewers contribute more than 0.5 million tons. BSG represents up to 85% of the total residues from the brewing process, which amounts to approximately 20 kg/hl beer. BSG is available at very low cost and is traditionally used for landfill and cattle feed. However, new technologies for the use of this valuable by-product as an ingredient in food products are of great interest due to the minimization of environmental impact and risk to human health, benefits for businesses from cheap or no-cost material, and improved value products for consumers.

Production of brewer's spent grain

The schematic presentation of the brewing process resulting in the production of BSG is given in Figure 16.1. Beer making comprises two separate processes: malting and brewing. Malting is the process in which barley is prepared and soaked in water long enough for germination to begin. Mashing is the process of heating grains mixed with water at controlled temperatures for designated periods of time to activate enzyme activity that converts starches to fermentable sugars (mainly maltose and maltotriose) and nonfermentable sugars (dextrins) and degrades proteins, polypeptides, and amino acids (Mussato *et al.*, 2006). This enzymatic conversion is known as wort. The insoluble grain husks are allowed to settle to form a bed in the mash tun, whereas filtered wort is used to produce beer. The residual separation of the barley extract is known as BSG.

Characteristics of brewer's spent grain

BSG is considered lignocellulosic material rich in hemicellulose (39%), proteins (24%), cellulose (14%), lipids (6%), and lignin (4%) (Mussato *et al.*, 2006). Pentose content (the sum of xylose and arabinose) of BSG has been found to vary between 21.0 and 27.3% dry weight for oven-dried samples (Santos *et al.*, 2003). It represents an untapped resource for obtaining industrially important hydrocolloids, such as arabinoxylan and protein (Mandalari *et al.*, 2005).

Because of the potentially good nutritional value of BSG, it may possibly be used as a novel source of dietary fiber for developing new products with full regulatory approval because the brewery process uses materials suitable for human food consumption.

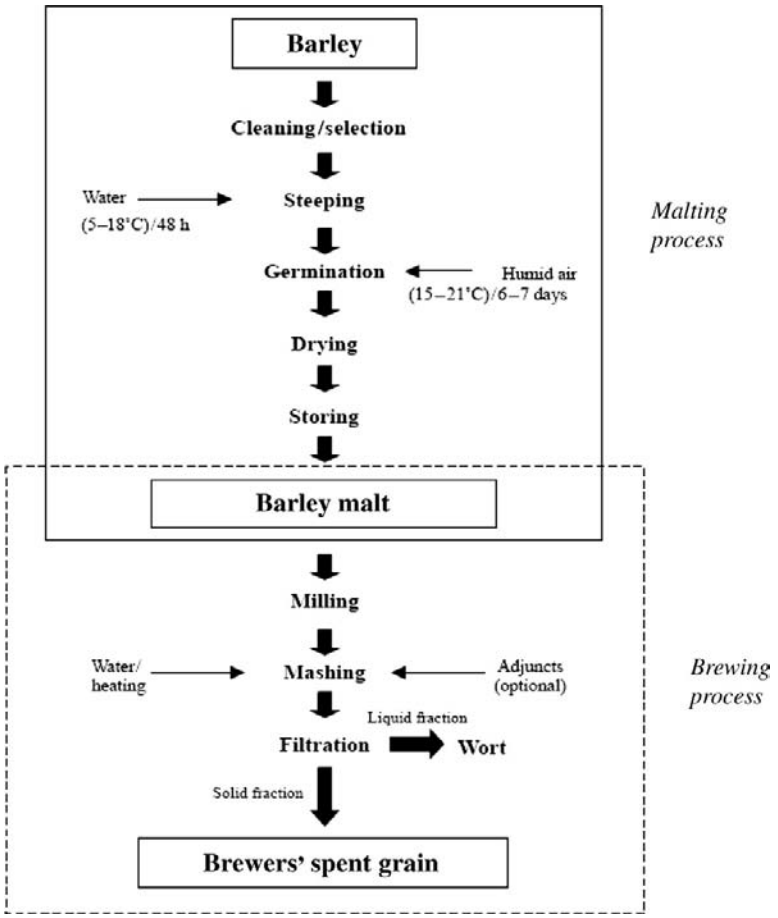

FIGURE 16.1
Schematic representation of the process to obtain BSG from natural barley. *Source: Adapted with permission from Mussatto, I. S., Dragone, G., and Roberto, C. I. (2006). Brewer's spent grain: Generation, characteristics and potential applications.* Journal of Cereal Science, *43, 1—14.*

FIGURE 16.2
Example of BSG obtained from Joseph Holts Brewery, Manchester, UK.

Preparation of dry brewer's spent grain

The most common preservation method used in the industry for drying BSG is oven drying. Santos *et al.* (2003) studied the variability in composition of moisture, protein, fat, ash, and total phenolics of eight lots of BSG (Mahou SA, Madrid, Spain) using oven- and freeze-drying techniques and compared the results with those of frozen samples. The results revealed that different techniques showed small variation in composition of dried BSG. Oven drying resulted in a small decrease in protein and fat content in comparison with that of the frozen sample and was similar to that of freeze-drying.

In a study by Stojceska and Ainsworth (2008), a commercial sample of BSG (Joseph Holts Brewery, Manchester, UK) (Figure 16.2) with a composition of 75% moisture, 1% ash, 4.8% protein, 16% fiber, 2.1% fat, and 1.1% carbohydrate content was dried using a reel oven (Teknigas, Sussex, UK) at 150°C for 4 h. The source of BSG was based on brewing of barley and hops and was the remains after extraction of the wort, before fermentation. The composition of dried BSG was 20.30% protein, 53.39% fiber, 8.32% fat, and 10.76% carbohydrate content. Table 16.1 presents different chemical compositions of BSG obtained from different world-wide breweries. The lowest total dietary fiber of 5.3% in BSG was reported by Prentice *et al.* (1978), whereas the highest levels of 71.2 and 76% were reported by Öztürk *et al.* (2002) and Kissell and Prentice (1979), respectively. Crude protein levels varied from 13% (Öztürk *et al.*, 2002) to 50.7% (Kissell and Prentice, 1979). However, the composition of BSG probably

TABLE 16.1 Chemical Compositions of BSG Used in the Literature

Reference	Composition of BSG
Kanauchi and Agata (1997)	59% total dietary fiber, 24% proteins, 2.4% ash, 1.6% lipids
Kissell and Prentice (1979)	26–76% total dietary fiber, 18.7–50.7% proteins, 2.75–4.73% ash
Ranhotra *et al.* (2006)	29.1–35.8% dietary fiber, 26.9–34.9% proteins
Santos *et al.* (2003)	26.45% proteins, 5.8% fat, 18.12% phenolics
Stojceska and Ainsworth (2008)	53.39% total dietary fiber, 20.30% proteins, 8.32% fat, 10.76% carbohydrates content
Zerai *et al.* (2008)	32% proteins
Prentice and D'Appolonia (1977)	5.4–51% total dietary fiber
Öztürk *et al.* (2002)	60.3–71.2% total dietary fiber, 13–36% proteins, 2.54–3.69% ash

depends on barley variety, time of harvest, the characteristics of hops, and brewery technology (Santos *et al.*, 2003).

Application of brewer's spent grain in bread making technology

BSG has been used as a cheap source of dietary fiber supplement in conventional dough (Finley and Hanamoto, 1980; Prentice and D'Appolonia, 1977; Stojceska and Ainsworth, 2008) and sourdough breads (Stojceska and Ainsworth, 2010).

THE EFFECT OF BREWER'S SPENT GRAIN ON NUTRITION PROPERTIES

The addition of BSG into bread dough formulations at the levels of 0–30% significantly ($p < 0.001$) increased the total dietary fiber level from 2.3 to 11.5% and fat level from 3.4 to 4.4% (Table 16.2) (Stojceska and Ainsworth, 2008). Protein content varied between 10.7 and 11% and was not related to the addition of BSG.

THE EFFECT OF BREWER'S SPENT GRAIN ON FARINOGRAPH PARAMETERS AND WATER ABSORPTION

Table 16.2 presents farinograph parameters and water absorption of BSG supplemented breads. Water absorption increased with fiber addition, varying between 58 and 61% at a fixed dough consistency of 700 BU. Dough development time (3.5–18 min) and dough stability (6.5–18 min) increased, whereas degree of softening (5–25 BU) decreased, as the level of fiber increased.

THE EFFECT OF BREWER'S SPENT GRAIN ON LOAF VOLUME, TEXTURE, AND SHELF LIFE

The loaf volume, texture, and shelf life (Table 16.3) were also affected by the addition of BSG, probably as a result of an increased level of arabinoxylans, the main polymers in BSG. Similar results for BSG breads were reported by Finley and Hanamoto (1980) and Prentice and D'Appolonia (1977). The specific loaf volume of BSG breads containing different amounts of fiber varied between 2.06 and 3.22 ml/g, with a significant correlation between fiber content and resulting loaf volume of $r = -0.8$ ($p < 0.0001$). The greatest loaf volume reduction was detected at 30% BSG addition. The shelf life of the breads containing different amounts (0–30%) of BSG and different enzymes was tested at 1, 2, 5, and 8 days. Fiber addition significantly ($p < 0.001$) increased crumb firmness in samples containing 20 and 30% BSG, whereas no significant difference was found with 10% addition. This significant difference was observed at each day of storage with the 20 and 30% BSG samples. Biliaderis *et al.* (1995) reported that the molecular weight of arabinoxylans significantly increased the firmness of crumb but the amount of arabinoxylans significantly decreased in wheat flour breads over 7 days of storage. This could be explained by the fact that cereal brans consist of different

TABLE 16.2 Nutritional Analyses, Water Absorption, and Farinograph Characteristics of BSG Breads[a]

BSG (%)	0	10	20	30
Fiber (%)	2.3[a]	6.3[b]	9.7[c]	11.5[d]
Protein (%)	11.0[a]	10.7[c]	11.4[d]	10.9[a]
Fat (%)	3.4[a]	3.4[a]	3.8[b]	4.4[c]
Water absorption (%)	58.0[a]	61.0[b]	60.5[b,c]	60.0[c]
Dough development time (min)	3.5[a]	7.0[b]	13.0[c]	18.0[d]
Stability (min)	6.5[a]	9.5[b]	10.0[b]	18.0[c]
Degree of softening (BU)	25.0[a]	15.0[b]	20.0[c]	5.0[d]

Source: Adapted from Stojceska and Ainsworth (2008).
[a]*Different letters in the same row indicate statistically significant values (p = 0.05).*

TABLE 16.3 Loaf Volume and Texture Information of BSG Breads

Enzymes	BSG (%)	Specific Loaf Volume (ml/g)	Hardness (N)			
			Day 1	Day 2	Day 3	Day 4
No enzymes	0	3.2	15.1	15.9	21.2	45.6
	10	2.7	20.7	18.6	26.1	44.3
	20	2.2	33.6	30.9	39.3	77.0
	30	2.1	38.5	32.6	37.6	80.2
ME	0	3.4	17.1	21.3	24.4	55.1
	10	2.5	26.5	18.1	26.2	44.3
	20	2.3	34.3	33.2	31.4	70.0
	30	2.4	30.5	30.3	33.3	59.1
LE	0	3.5	11.4	11.9	19.0	34.6
	10	3.9	5.3	5.5	8.4	26.1
	20	2.8	18.9	16.9	21.8	35.9
	30	2.5	19.6	16.7	20.7	44.1
PE	0	4.0	12.3	11.9	20.5	39.1
	10	3.7	13.8	13.1	21.8	38.2
	20	2.7	28.9	28.5	37.8	67.7
	30	2.7	22.1	22.7	32.1	63.5
PE + CL	0	3.9	10.6	11.6	19.2	43.6
	10	3.7	13.9	12.4	19.2	45.9
	20	3.1	12.4	13.3	20.8	47.4
	30	2.4	30.9	22.9	33.7	53.2

CL, Celluclast; LE, Lipapan Extra; ME, Maxlife 85; PE, Pentopan Mono.
Source: Adapted from Stojceska and Ainsworth (2008).

tissues and thus the actual fine structures of isolated arabinoxylans are very diverse (Mandalari *et al.*, 2005).

IMPROVING THE QUALITY OF BREWER'S SPENT GRAIN BREADS BY USING ENZYMES

In a study by Stojceska and Ainsworth (2008), the texture of BSG breads was improved by the addition of the enzymes Maxlife 85 (ME) (Danisco Ingredients, Denmark), Lipopan Extra (LE), Pentopan Mono (PE), and Celluclast (CL) (Novozymes, Denmark).

The addition of ME into the bread formulations with specific loaf volume ranged between 2.4 and 3.4 ml/g, resulting in a significant ($p < 0.001$) decrease as the amount of fiber increased. There was no significant difference compared to the control samples containing different amounts of fiber. Specific loaf volume of samples containing the enzymes LE (2.4–3.9 ml/g), PE (2.7–4 ml/g), and Pentopan Mono and Celluclast (PCE) (2.4–3.9 ml/g) behaved in a similar way, with significantly ($p < 0.0001$) lower specific loaf volume at 20 and 30% of BSG. No significant difference in specific loaf volume was found among these samples at different fiber levels except at 10% BSG, where LE was significantly ($p < 0.001$) higher than PE and PCE samples. Comparing these samples (LE, PE, and PCE) with their equivalent of control samples and ME samples, all of them showed significantly ($p < 0.0001$) higher specific loaf volume.

ME breads showed a significant ($p < 0.001$) increase in hardness as fiber increased, whereas no significant difference was found compared with their equivalent control sample. The same trend was observed during each day of storage. LE showed no significant difference in crumb firmness at 0 and 10% BSG, whereas a significant ($p < 0.0001$) difference was observed at 20 and 30% BSG. Compared with the equivalent control samples, LE gave significantly ($p < 0.0001$) lower crumb firmness at all levels of BSG (0–30%) for each day of storage. PE

showed a significant ($p < 0.0001$) increase in crumb firmness as the amount of BSG increased up to 20%, but it decreased at 30% BSG. Compared with the equivalent control samples, the only significant ($p < 0.001$) decrease in crumb firmness was detected at 30% BSG. Again, this was apparent during each day of storage. PCE showed a significant difference in hardness ($p < 0.0001$) at all levels of dietary fiber. Compared with its equivalent control samples, the only difference was found at 20 and 30% BSG. Bread containing LE, PE, and PCE showed a clear tendency toward a softer crumb and a reduced rate of staling compared with the control samples and samples containing ME. PE showed significantly ($p < 0.0001$) increased hardness during storage compared with LE and PE at 20% BSG. In this study, the best results in terms of crumb firmness were obtained with LE, with a significant ($p < 0.001$) delay in staling compared with the equivalent control samples (Stojceska and Ainsworth, 2008).

IMPROVING THE QUALITY OF BREWER'S SPENT GRAIN BREADS WITH A COMBINATION OF SOURDOUGH AND ENZYMES

The work of Stojceska and Ainsworth (2008) was extended by forming sourdough and a combination of enzymes (Stojceska and Ainsworth, 2010). The specific loaf volume of sourdough breads was not different from that of conventional breads (Stojceska and Ainsworth, 2008). The combination of sourdough and different enzymes improved the texture and shelf life of BSG breads, resulting in lower crumb hardness and delay in staling. Compared to conventional BSG breads (Stojceska and Ainsworth, 2008), sourdough extended shelf life, which was more pronounced with a combination of enzymes. The best results were obtained with a combination of LE and PE with ME and PE with CL, which is probably the result of redistribution of water from pentosan to the gluten phase, reduced starch retrogradation rate, and degradation of cell wall components leading to altered water distribution between starch and protein (Katina *et al.*, 2006).

Potential health benefits from brewer's spent grain

A number of studies suggest that BSG may have a role in the prevention of certain diseases. Odes *et al.* (1986) studied a role for BSG fiber in the treatment of constipated patients. Nineteen ambulatory patients with chronic, laxative-dependent constipation were treated with 20–25 g BSG fiber daily for 4 weeks. The following symptoms improved with treatment: bowel movement frequency in 15 patients (79%), flatulence in 12 (63%), abdominal pain in 10 (53%), stool consistency in 8 (42%), and laxative dependence in 14 (74%). Fifteen patients (79%) showed improvement in some or all of these factors, whereas 4 patients were largely unresponsive to fiber.

Zhang *et al.* (1991) reported a beneficial effect of BSG on the physiological function of the colon. Two different diets were studied: a high-fiber diet with 62 g BSG supplementation in breads, muffins, and breakfast flakes and a low-fiber diet without BSG supplementation. Two experimental groups of five subjects each were studied for 1 week. It was found that the cholesterol and net cholesterol excretion per day on the high-fiber diet were significantly higher than those on the low-fiber diet. BSG fiber intake increased the dry weight of the ileostomy contents and decreased the concentration of sterols in ileostomy effluent.

Hassona (1993) measured total lipids and cholesterol in rats fed breads containing milled BSG at levels of 10–25% with the fiber content of 4.9, 6.4, and 7.5% for 28 days. The results indicated impaired growth weight (7.1–10.0%) compared with that of the control. Total lipids and total cholesterol were reduced by 5.7–8.0 and 6.0–8.3%, respectively.

Aman *et al.* (1994) studied the excretion of total dietary fiber in 10 human subjects with ileostomies who consumed a low-fiber diet (15 g total dietary fiber/day) or the same diet supplemented with 62 g/day of BSG in a crossover design study. Food and excreta were collected and analyzed on Days 2, 3, and 7 of each dietary period. Analysis of specific dietary

fiber components showed that the increased excretion was mainly due to fucose, mannose, galactose, and uronic acid residues. High-fiber diet consumption showed significantly greater excretion of the same non-starch polysaccharide residues as those for consumption of the low-fiber diet but with a simultaneous decrease in excretion of arabinose, xylose, and glucose residues (12%, $p < 0.01$), which were the major fiber components in the diet.

Kanauchi and Agata (1997) developed a new product by milling and sieving BSG; the mix of glutamine-rich protein and dietary fibers of cellulose, hemicelluloses, and lignin protein was called germinated barley foodstuff. It was found that this product increased fecal dry weight and the number of feces and jejunal mucosal protein content in rats, indicating that it might improve defecation for people with constipation.

BSG was used as a protein source for fish diet (Zerai *et al.*, 2008), replacing 25, 50, 75, and 100% of fish meal protein during a 10-week period. The results showed that brewer's waste can effectively substitute up to 50% of the fish meal protein of a typical commercial feed with no adverse effect on growth of tilapia, whereas 75% fish meal replacement showed significantly lower weight gain.

Scientists are in agreement that BSG is an important source of dietary fiber with a beneficial effect on health. However, further research is needed to establish the precise function of BSG fiber on human health.

CONCLUSION

Dried BSG has great potential to be used as a functional ingredient that may provide beneficial effects on human health. By incorporating BSG up to 30% in bread making technology, the level of dietary fiber will increase up to fivefold. Loaf volume, texture, and shelf life of BSG can be improved by forming sourdough and using appropriate enzymes, such as ME, LE, PE, and CL. LE in breads gave the most open network, increased wall thickness, and decreased cell density, which resulted in a higher loaf volume and extended shelf life. PE and PCE were more effective at increasing loaf volume and extending shelf life of breads containing higher amounts of fiber. However, the best results were obtained by a combination of sourdough with LE, PE and ME, and PE with CL.

SUMMARY POINTS

- Consuming a high level of dietary fiber food has been associated with the prevention of several diseases.
- BSG is a by-product from brewery and a cheap source of total dietary fiber that could be used as a functional ingredient in different food products.
- The addition of BSG into bread dough formulation at 30% increases the level of total dietary fiber up to fivefold.
- Sensory characteristics and shelf life of BSG breads can be improved by adding enzymes and forming a sourdough.

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CHAPTER 17

Composite Flours and Breads: Potential of Local Crops in Developing Countries

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

PF Plantain flour
PFSB Plantain flour-supplemented bread
SF Soybean flour
SFSB Soy flour-supplemented breads
WWB Whole wheat bread

INTRODUCTION

Composite flours are mixtures of different vegetable flours—rich in starch, protein, and/or other nutrients—with or without wheat flour. Several institutions, including the Food and

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Agriculture Organization (FAO), have been involved in research designed to find ways of partially substituting wheat flour with those from other sources or replacing wheat altogether. The technology of composite flours represents an interesting option for the management of costs associated with importation of wheat flour in developing countries where wheat is not cultivated for climatic reasons (Olaoye *et al.*, 2006). With the constant increase in the consumption of bread and other baked products in many developing countries, coupled with ever-growing urban populations, the composite flour/bread technology could be very useful.

It has been reported that replacing wheat with 20% non-wheat flour for the manufacture of bakery products would result in an estimated savings of $320 million annually (FAO, 1982). At 30% substitution, the savings would be $480 million annually.

The ingredients used in composite flours depend on the availability of raw materials in the country concerned. The goal is to minimize cost of production compared to the cost of imported wheat because this would enable affordability of the final product by consumers. Africa is endowed with an enormous biodiversity of plant resources, which could be exploited for this purpose. Unfortunately, many of these abundant resources remain largely underutilized. Indigenous crops are low-input produce; as such, they are important for agricultural diversification and can provide a unique opportunity to combat food and nutritional insecurity. Examples of underutilized plants in developing countries, such as Nigeria, include plantain, breadfruit, tigernut, and soybean. Some varieties have been studied and appreciated for their nutritional properties and medicinal values (Akubor, 2003; Rincon and Padilla, 2004; Stover and Simmonds, 1987).

With the current emphasis on healthy bread with a low glycemic index, high protein, and increased dietary fiber, the use of composite flours in baked goods is to be favored. Therefore, given the inherent nutritional and therapeutic advantages of these crops, they could find useful application in the baking industry. Many studies have reported on the possibility of replacing (although partially) wheat flour with those obtained from local crops for the purpose of making bread and other baked products (Ade-Omowaye *et al.*, 2008; Akubor, 2003; Bamidele *et al.*, 1990; Mepba *et al.*, 2007; Olaoye and Onilude, 2008; Olaoye *et al.*, 2006, 2007); however, consumer acceptability of these products is still under study.

PLANTAINS

Brief overview

Plantain is a form of banana, which belongs to the family Musaceae, genus *Musa*. Banana and plantains, which are derived from the wild species *M. acuminata* and *M. balbisiana*, are staple food crops for millions of people in developing countries. In terms of gross value of production, bananas and plantains are the developing world's fourth most important crop after rice, wheat, and maize. They have their greatest importance as a staple food crop in areas of East Africa, where annual consumption is more than 200 kg/capita.

Bananas are believed to have originated from the tropics (Asia and Africa), and more than 700 varieties are known and approximately 100 cultivated. There are three common species of *Musa*. *Musa paradisiaca* is a type of plantain usually cooked before consumption. Another variety is *Musa sapientum* (true banana), which is mostly eaten raw at maturity and may be processed into other forms. *Musa paradisiaca* and *M. sapientum* are very high in starch content (Stover and Simmonds, 1987).

Plantains are one of the important food crops in many developing nations and are grown in more than 100 countries in the tropics and subtropics. They constitute a major source of carbohydrates for millions of people in Africa, the Caribbean, Latin America, Asia, and the

Pacific. A worldwide increase in the production of plantains, up to several tons, has been noted (FAO, 2003).

In Central and West Africa, plantains serve as important staple foods, which along with bananas provide more than 60 million people with 25% of their calories. More than 2 million metric tons of plantains are produced in Nigeria annually, but up to 60% post-harvest loss had been reported; this is attributed to lack of storage facilities and inappropriate technologies for processing (Mepba *et al.*, 2007). In terms of management and cost of production, *Musa* is considered one of the cheapest food crops to produce in comparison to other staples crops.

Health and nutritional benefits of plantains

Plantains are a good source of carbohydrates and dietary fiber. The green fruit contains higher hemicellulose content (approximately 6%) than most fruits and vegetables. In addition to dietary fiber, green bananas contain a high amount of essential minerals such as potassium and various vitamins, such as A, B_1, B_2, and C (Chandler, 1995). The quantities of nutrients in *M. paradisiaca* and *M. sapientum* are very similar, except that the former contains more starch. They are both significantly high in potassium, 400 mg/100 g pulp, and have a trace amount of sodium (approximately 1 mg) and iron. *Musa paradisiaca* is richer in B vitamins, including thiamine, niacin, and riboflavin, as well as vitamin A, compared to *M. sapientum* (Chandler, 1995). Plantains are rich in vitamin C, providing approximately 20 mg for every 100 g of flesh (Chandler, 1995).

Because of the low lipid and high energy values, bananas are recommended for obese and geriatric patients. Bananas are useful for people with peptic ulcers, for treatment of infant diarrhea, and for celiac disease and colitis. The potential of dried unripe plantain or banana pulp powder in the treatment of ulcers has been noted (Dunjic *et al.*, 1993). Plantains contain vitamin A and thus can act as an aid to digestion. The juice from the male bud provides an apparent remedy for stomach problems in many people. The ripe fruit has also been noted for use in the treatment of asthma and bronchitis.

SOYBEANS

Brief overview

Botanically, soybean belongs to the order Rosaceae, family Leguminosae or Papillonaceae or Fabaceae, subfamily Papilionoidae, the genus *Glycine*, and the cultivar *Glycine max*. It is widely believed that the origins of soybean are in China, probably in the north and central regions, 4000−5000 years ago. Soybean cultivation was believed to have reached Africa in the late 1800s, although little is known of the countries to which it was first introduced. According to FAO data, soybean was grown in Africa on an average of 1.16 million ha of land with an average production of 1.26 million tons in 2005 (FAO, 2005). African countries with the largest area of production were Nigeria (601,000 ha), South Africa (150,000 ha), Uganda (144,000 ha), Malawi (68,000 ha), and Zimbabwe (61,000 ha). Other countries with sizeable areas were Rwanda (42,160 ha), the Democratic Republic of the Congo (30,000 ha), and Zambia (15,000 ha). Soybean is also grown on a small scale in more than 10 other African countries.

The amount of protein produced by soybeans per unit area of land is higher than that of any other crop. They also fix atmospheric nitrogen, which reduces the need for farmers to purchase fertilizer. This is a major benefit in African farming systems, where soils have become exhausted by the need to produce more food for increasing populations, and where fertilizers are scarce or expensive. In Nigerian markets, for instance, soybeans cost approximately one-fifth as much as other forms of protein sources, such as dairy and fish, and are easier to store and transport. Unfortunately, the crop is not well exploited in sub-Saharan Africa, where it is mostly seen as a cash crop.

Health and nutritional benefits of soybeans

Soybean is an excellent source of high-quality protein, low in saturated fat, and free of cholesterol. In addition, soybean contains a high dietary fiber, the second largest component, and it has been reported to reduce the risk of colon cancer and other diseases (Anderson *et al.*, 1999; Mateos-Aparicio *et al.*, 2008). Soybean, with its 40% protein, is richer than any other food crop or even livestock. It is also made up of 20% oil, and it contains all the eight essential amino acids, making it the healthiest legume crop. Soybean contributes significantly to overall human nutrition in terms of both calorie and protein intake. The crop appears to be well placed to meet the fast-growing demand for vegetable oil and animal feed in developing countries. Soybean is very rich in nutritive components (Table 17.1).

On October 26, 1999, the U.S. Food and Drug Administration approved a health claim based on the role of soybean protein in reducing the risk of coronary heart disease (Mateos-Aparicio *et al.*, 2008). This claim establishes that soybean protein included in a diet low in saturated fat and cholesterol may reduce the risk of coronary heart disease. It has been suggested that frequent soybean protein consumption lowers cholesterol levels (Mateos-Aparicio *et al.*, 2008). It has also been claimed that soybean dietary fiber plays a role in the reduction of cholesterol levels in some hyperlipidemic individuals and has a major protective effect on cardiovascular disease (Anderson *et al.*, 1999; Mateos-Aparicio *et al.*, 2008). Therefore, the possible use of soybean in baked foods in developing countries could provide economic and health advantages because the consumption of soybean protein and dietary fiber seems to reduce the risk of cardiovascular disease. Furthermore, soybean isoflavones have been associated with a potential role in the prevention and treatment of some diseases (Potter, 1995).

TABLE 17.1 Nutritional Value of Soybeans

Part	Quantity (per 172 g of Edible Part)
Water (%)	63
Calories (kcal)	298
Protein (g)	29
Total fat (g)	15
Fatty acids (g)	
Saturated	2.2
Monounsaturated	3.4
Polyunsaturated	8.7
Cholesterol (mg)	0
Carbohydrate (g)	17
Total dietary fiber (g)	10.3
Calcium (mg)	175
Iron (mg)	8.8
Potassium (mg)	886
Sodium (mg)	2
Vitamin A	
IU	15
RE	2
Thiamin (mg)	0.27
Riboflavin (mg)	0.49
Niacin (mg)	0.7
Ascorbic acid (mg)	3

IU, international unit; RE, retinol equivalent.
Source: U.S. Department of Agriculture (2002).

BREADFRUIT

Brief overview

Breadfruit (*Artocarpus altilis*) was domesticated in the Pacific and has nourished islanders for more than 3000 years. In addition to the Pacific and the Caribbean, the breadfruit tree also grows in some coastal regions of Africa, especially Mozambique and Guinea. Breadfruit is grown in backyards, orchards, marginal lands, and home gardens by local communities in some developing countries such as Nigeria. It is regarded as "tree yam" because the processed fruit is similar in taste to yam, a popular root and tuber crop in West Africa. With intensive utilization, it has the potential to contribute to food security in developing countries. Breadfruit is round, oblong or oval in shape, with green skin composed of polygonal sectors. Mature fruit is approximately 300 mm in length and 200 mm in diameter. Weight varies but usually is between 1000 and 3000 g. One tree can produce between 300 and 500 fruits per year. Normally, the fruit is peeled, and the heart and stem are removed and, in most cases, discarded. This results in an overall yield of edible pulp of approximately 68% of the total fruit weight, although this varies with stage of maturity (Bates *et al.*, 1991). The mature fruit is preferred for human consumption. A mature fruit can be differentiated by appearance; mature fruit has dark green, large polygonal segments compared to smaller, densely packed segments for immature fruits. A very mature fruit softens and can be detected manually, whereas immature fruit exudes a large amount of latex when peeled and has an undesirable taste. Breadfruit is important in the diets of people of the tropics. It is relatively cheap and is readily available in season (normally twice a year).

Health and nutritional benefits of breadfruit

Breadfruit is a staple food sought after because it is an inexpensive source of high energy. Compared to banana, cassava, plantain, taro, and sweet potato, it is a relatively good source of calcium (Monro *et al.*, 1986). Potassium and phosphorus have been reported in relatively good quantities, although amounts may vary between cultivars. Compared to other tropical starchy foods, it is an acceptable source of vitamin C (20 mg/100 mg of pulp) and has good levels of iron, niacin, and riboflavin at all stages of maturity. Although not high in protein, the amino acid profile of its protein is favorable. Breadfruit also contains significantly high amounts of fiber. According to the American Heart Association, fiber decreases bad cholesterol and triglycerides, which increase the risk of heart attack. An increased intake of fiber lowers low-density lipoprotein (bad) cholesterol levels while elevating high-density lipoprotein (good) cholesterol levels in the body. Breadfruit protects the body against heart disease and heart attack (Fassbender, 2008). Breadfruit benefits the body because it contains favorable amounts of omega-3 and omega-6 fatty acids.

The main problem with breadfruit utilization is its high perishability, resulting in high postharvest losses. In some extreme cases, up to 50% losses have been reported. Consequently, only fruits for immediate needs are harvested, thus reducing the opportunities for development of a large-scale international trade in breadfruit (Bates *et al.*, 1991). Its possible incorporation in baked goods such as bread could widen its scope of utilization with consequent reduction in postharvest losses in developing countries.

STUDIES ON THE USE OF COMPOSITE FLOURS OF WHEAT, SOYBEANS, PLANTAINS, AND TIGERNUT IN BREADS

Efforts have been made to promote the use of composite flours, in which flour from locally grown crops and high-protein seeds replace a portion of wheat flour for use in bread, thereby reducing costs and producing nutritionally enhanced bread (Giami *et al.*, 2004; Mepba *et al.*, 2007). Although there currently is a substantial effort to improve composite bread technology, such breads still require at least 70% wheat flour to be able to rise (Olaoye *et al.*, 2006).

Soybean, because of its excellent protein content, has been added to wheat flour in varying proportions to produce acceptable bread (Olaoye *et al.*, 2006). Nutrient enhancement has been reported in bread produced from wheat flour substituted with soybean. In a study by Olaoye *et al.* (2006) on the production of bread from partially substituted wheat flour with soybean flour (SF) at various levels, the authors observed increases in crude protein, crude fiber, ether extract, and ash contents of the soy flour-supplemented breads (SFSBs) with a progressive increase in the proportion of soy flour. According to the authors, the 15% SFSB had the highest values of 8.39, 0.14, 2.46, and 1.17% of the respective components, whereas the lowest values were recorded for the whole wheat bread (WWB) (Table 17.2). The protein content was observed to increase with a progressive increase in the proportion of SF, indicating that supplementation of wheat with SF may have contributed to improvement in the nutritional quality of the bread samples. This was attributed to the high level of protein content in soybean seeds (Olaoye *et al.*, 2006). This is an indication that SFSB may be of nutritional importance in most developing countries, in which proteinaceous foods are too expensive for those who earn low incomes.

The ash and fat contents of the SFSBs were also noted to assume a similar trend as the protein content, with the highest content recorded for the bread samples supplemented with 15% SF. On the contrary, the lowest values were obtained for the bread samples containing wheat flour alone. The sensory evaluation (Table 17.3) on the bread samples indicated comparable results between WWB and SFSB because no significant differences ($p < 0.05$) were observed in the sensory attributes of aroma, internal texture, taste, and general acceptability in 5% SFSB. However, at higher levels of SF supplementation, significant differences were noted and consumer preferences decreased. Olaoye *et al.* (2006) hypothesized that soy flour substitution at 15% or less for bread making could contribute to composite flour technology, with resultant nutritional and cost benefits.

Varying degrees of supplementation of wheat with plantain flour (PF) in bread making were also reported by Bamidele *et al.* (1990). They observed that with increasing levels of supplementation with plantain, the water absorption capacity and dough development time of the composite flour decreased (Table 17.4). However, the mixing tolerance time increased and the mixing quality decreased. It was also observed that the baking quality decreased with increasing level of supplementation when unblanched PF was used, possibly due to the undesirable enzyme activity. In terms of sensory attributes, it was reported that quality decreased with corresponding increase in the proportion of PF.

TABLE 17.2 Proximate Composition and Microbial Count of Soybean Flour-Supplemented and Whole Wheat Bread Samples[a]

Parameter/Bread Sample (%)	A[b]	B[c]	C[d]	D[e]
Crude protein	7.01	7.26	8.03	8.39
Crude fiber	0.03	0.06	0.08	0.14
Ether extract	0.76	1.29	2.32	2.46
Ash	0.64	0.89	1.01	1.17
Moisture	30.98	34.10	35.50	35.59
Carbohydrate	60.58	56.40	53.06	52.25
APC (log CFU/g)	3.20	4.50	4.10	4.30

APC, aerobic plate count; CFU, colony-forming units.
Source: Olaoye *et al.* (2006).
[a]*Values are means of three replicated samples.*
[b]*Bread produced from 100% wheat flour.*
[c]*Bread produced from composite flours of 95% wheat and 5% soybeans.*
[d]*Bread produced from composite flours of 90% wheat and 10% soybeans.*
[e]*Bread produced from composite flours of 85% wheat and 15% soybeans.*

TABLE 17.3 Mean Sensory Scores of the Soybean Flour-Supplemented and Whole Wheat Bread Samples[a]

Attribute/Bread Sample	A[b]	B[c]	C[d]	D[e]
Crust	7.5[a]	5.7[b,c]	6.4[a,c]	4.9[b]
Aroma	6.6[a]	5.7[a]	5.7[a]	5.3[a]
Shape	7.3[a]	5.7[b,c]	6.2[a,b]	4.8[c]
Internal texture	6.6[a]	5.8[a]	5.5[a]	4.6[b]
Taste	6.6[a]	5.4[a]	5.4[a]	5.9[a]
Appearance	7.6[a]	5.3[b]	5.3[b]	5.3[b]
General acceptability	6.9[a]	6.1[a]	5.4[b]	5.1[b]

APC, aerobic plate count.
Source: Olaoye *et al.* (2006).
[a]*Values are means of three replicated samples. Mean scores in rows with different superscript letters are significantly different ($p < 0.05$).*
[b]*Bread produced from 100% wheat flour.*
[c]*Bread produced from composite flours of 95% wheat and 5% soybeans.*
[d]*Bread produced from composite flours of 90% wheat and 10% soybeans.*
[e]*Bread produced from composite flours of 85% wheat and 15% soybeans.*

While reporting findings on the use of PF supplementation in wheat flour for bread making, Olaoye *et al.* (2006) noted similarity in the nutritional contents of WWB and plantain flour-supplemented bread (PFSB) samples. Higher ash content was recorded in the latter compared to the former (Table 17.5). Significant differences ($p < 0.05$) were not observed in the sensory attributes of the two types of breads with respect to shape, appearance, and general acceptability, irrespective of the level of PF substitution. The authors recommended that up to 15% PF substitution in wheat could be adopted in bread making processes without adversely affecting quality.

In a related study conducted by Mepba *et al.* (2007), similar observations were reported in WWB and PFSB. The authors noted that the latter samples were organoleptically acceptable with up to 20% PF supplementation, whereas no significant differences ($p < 0.05$) were observed among the two different bread samples. However, the level of acceptability was observed to decrease with increase in PF supplementation.

In a study conducted by Olaoye and Onilude (2008) in which the baking potential of breadfruit was evaluated, it was observed that the quality and acceptability of bread produced from 15% breadfruit flour substitution were similar to those of bread samples produced from 100% wheat flour. The authors observed that the ash and fiber contents were enhanced in the

TABLE 17.4 Rheological Properties of Composite Flours from Wheat and Plantain

Parameter	WF	WF (90%) + BPF (10%)	WF (85%) + BPF (15%)	WF (90%) + UPF (10%)	WF (85%) + UPF (15%)
% Absorption	56.3	55.4	56.0	54.1	56.2
Arrival time (min)	1.25	1.50	1.25	1.25	1.25
Departure time (min)	5.00	3.50	3.50	4.50	4.50
Stability time (min)	3.75	2.00	2.00	3.25	2.75
Peak time (min)	2.00	2.00	2.00	2.00	1.75
Mixing tolerance index (BU)	60	70	80	70	70
Dough development time (min)	5.2	3.5	3.2	4.2	4.0
Resistance (BU)	580	450	390	820	950
Extensibility	140	140	120	110	85
Resistance/extensibility	4.1	3.2	3.2	7.4	11.2

BPF, blanched plantain flour; BU, Brabender unit; UPF, unblanched plantain flour; WF, wheat flour.
Source: Bamidele *et al.* (1990).

TABLE 17.5 Proximate Composition and Microbial Count of Plantain Flour-Supplemented and Whole Wheat Bread Samples[a]

Parameter/Bread Sample (%)	A[b]	B[c]	C[d]	D[e]
Crude protein	7.01	6.97	6.91	6.88
Crude fiber	0.03	0.03	0.02	0.02
Ether extract	0.76	1.04	1.03	1.03
Ash	0.64	0.71	0.84	0.95
Moisture	30.98	31.50	32.71	33.84
Carbohydrate	60.28	59.75	58.49	57.28
APC (log CFU/g)	3.20	4.30	4.10	4.10

APC, aerobic plate count; CFU, colony-forming units.
Source: Olaoye *et al.* (2006).
[a]*Values are means of three replicated samples.*
[b]*Bread produced from 100% wheat flour.*
[c]*Bread produced from composite flours of 95% wheat and 5% plantain.*
[d]*Bread produced from composite flours of 90% wheat and 10% plantain.*
[e]*Bread produced from composite flours of 85% wheat and 15% plantain.*

composite bread samples. They advocated for their practical application on a commercial scale, a practice that could be of tremendous importance in developing countries in which breadfruit is cultivated.

Furthermore, Ade-Omowaye *et al.* (2008) reported the feasibility of incorporating tigernut (*Cyperus esculentus*) flour into bread making. They noted that the proximate composition of the composite flour samples showed a reduction of approximately 14–38% in protein content, but a significant enhancement in the fiber content (167–967%), depending on the level of tigernut substitution, was recorded. The fat and ash contents, as well as the pH of the flour samples, increased with an increase in the proportion of the tigernut flour. Dough with good viscoelastic properties and acceptable bread with qualities similar to those of 100% wheat bread were produced from 10% tigernut flour addition. The significant increase in the fiber content (167–967%) could be nutritionally advantageous because evidence abounds that a high-fiber diet protects from a number of major diseases in developing countries such as Nigeria, where white bread is one of most common staples among all classes of people. The bulk density of the samples ranged from 0.57 to 0.64, which showed an increasing trend with an increase in tigernut flour addition (Table 17.6). There was an increase in pH values with

TABLE 17.6 Physicochemical Properties of the Wheat/Tigernut Flour Samples[a]

Sample	Titratable Acidity (%)	pH	WAC (%)	Bulk Density (g/ml)
A[b]	0.057[e]	5.80[a]	85.17[a]	0.57[a]
B[c]	0.054[e]	5.95[b]	85.36[a,b]	0.59[a,b]
C[d]	0.045[d]	6.02[b,c]	85.5.0[b]	0.60[b,c]
D[e]	0.041[c]	6.06[b,c,d]	85.61[b,c]	0.61[b,c]
E[f]	0.036[b]	6.10[c,d]	85.85[c,d]	0.62[c,d]
F[g]	0.024[a]	6.16[d]	86.01[d]	0.64[d]

WAC, water absorption capacity.
Source: Ade-Omowaye *et al.* (2008).
[a]*Means followed by different superscripts within a column are significantly different* ($p \leq 0.05$).
[b]*100% wheat flour.*
[c]*90% wheat flour and 10% tigernut flour.*
[d]*80% wheat flour and 20% tigernut flour.*
[e]*70% wheat flour and 30% tigernut flour.*
[f]*60% wheat flour and 40% tigernut flour.*
[g]*50% wheat flour and 50% tigernut flour.*

a corresponding decrease in titratable acidity values of the flour samples as the content of tigernut flour increased. The titratable acidity values ranged from 0.024 to 0.057%, whereas the pH values were between 5.80 and 6.16.

The traditional food habits of people in developing countries seem to pose a great barrier to the acceptability of bread produced from composite flour. However, with concerted effort in educating the people on the nutritional benefit and probably derivable economic advantages that may be associated with the adoption of bread from composite flour, it is envisaged that the composite flour/bread technology could yield impressive results.

TECHNOLOGICAL ISSUES

This review and worldwide research efforts suggest that composite flour/bread technology could play an integral role in cost savings, promoting utilization of local crops, encouraging local farmers, and enhancing agricultural systems in developing countries. Limited reports on the associated nutritional and health benefits of composite breads in developing countries indicate that more research is required for the purpose of validation and probable commercialization. Moreover, the technology could increase cost-effectiveness and promote food security among consumers in developing countries.

ADVERSE REACTIONS

There is a lack of information on possible adverse reactions to composite bread, especially in developing countries. However, adverse reactions may occur in some people, depending on their immune system. Although consumers need to be cognizant of this, research efforts are required to identify possible allergic reactions or toxicity that could be linked with composite breads/flours.

SUMMARY POINTS

- Many crops in developing countries, such as plantains, soybeans, tigernuts, and breadfruits, possess inherent nutritional values and therapeutic properties that could be exploited for enhancement of human nutrition and well-being.
- Such nutritional values could be transformed into human use by wheat-composite flour technology for bread production.
- Although baking potentials of the crops at varying levels of substitution have been documented, further studies are required for process optimization.
- The presence of at least 70% wheat flour is still required for good dough formation in wheat-composite flour for bread production.
- Irrespective of the low level of flour from crops that could be successfully adopted in composite flour/bread technology, it will accrue in great savings of the scarce resources of most developing countries, especially in Africa, where wheat cultivation does not thrive for climatic reasons.

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CHAPTER 18

Legume Composite Flours and Baked Goods: Nutritional, Functional, Sensory, and Phytochemical Qualities

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CHAPTER OUTLINE

INTRODUCTION

Composite flours are flours prepared by mixing or blending cereal, root, tuber, or legume flours at a predetermined ratio. These are then used to prepare various food products, including fermented flat breads, biscuits, and tortillas. Legumes are plants that produce seeds in pods and belong to the family Leguminosae.

An important motivation for the production of composite foods is to improve nutritional quality. Cereal–legume composite foods serve as a good example of this. Legumes are protein-rich relative to cereals and are also generally better sources of essential amino acids,

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particularly lysine. On the other hand, cereals, although lysine-deficient, are relatively better sources of sulfur-containing amino acids such as methionine. The benefit of producing cereal—legume composite foods may be considered as twofold: (1) There is an overall increase in the protein content of the composite food compared to when only the cereal forms the base, and (2) there is a better amino acid balance due to the contribution of lysine by legumes and the contribution of methionine by cereals.

Compositing affects not only nutritional quality but also functional, sensory, and phytochemical qualities of final food products. Various factors play a role, including preprocessing steps followed in the preparation of the flours, the ratio of cereal to legume flours used, as well as the procedures used during the preparation of the end product.

This chapter provides a short introduction to the general production of legume flours used in composites. It then discusses the potential effects of compositing flours (with particular reference to legumes) and subsequent baking on quality parameters such as nutritional, phytochemical, rheological, physical, and sensory properties.

PREPARATION OF FLOURS

Prior to compositing, flours need to be prepared. Usually, the preparation of flours follows a number of simple steps, including heating, dehulling/decortication, defatting (in some cases), and dry or wet milling. It is important to note that each of these simple unit operations can impact either positively or negatively on the nutritional, functional, sensory, and phytochemical qualities of the flours. There is an important relationship between the type of process used and the product characteristics. Figure 18.1 shows a generic diagram of the preparation of flours. Table 18.1 summarizes the potential positive and/or negative effects of selected processing or preparation steps on the nutritional, functional, sensory, and phytochemical qualities of legume flours prior to the compositing step. Processors need to carefully consider both the potential advantages and the disadvantages of using specific procedures in the preparation of flours.

Dry heating

Dry heating prior to dehulling and milling is done to inactivate antinutritional factors such as trypsin inhibitors present in legumes. Dry heating (180°C for 20 min) of marama (*Tylosema esculentum*) beans—an underutilized, drought-tolerant legume in southern Africa—effectively

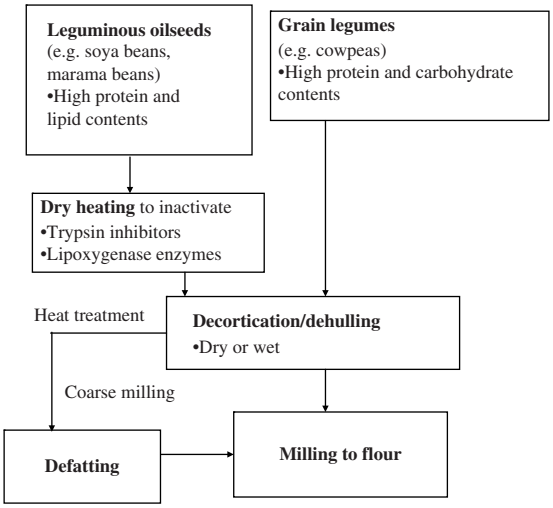

FIGURE 18.1
Outline of processing steps used during the preparation of legume flours.

TABLE 18.1 Effect of Selected Processing or Preparation Steps on the Nutritional, Functional, Sensory, and Phytochemical Qualities of Flours and Their End Products

Processing or Preparation Step	Nutritional Quality		Functional Quality		Sensory Quality		Phytochemical Quality	
	Positive Impact	Negative Impact	Positive Impact	Negative Impact	Positive Impact	Negative Impact	Positive Impact	Negative Impact
Heating of legumes prior to dehulling and milling	Inactivation of antinutritional factors[a,b] Improved *in vitro* protein digestibility[a] Increased protein and ash[c]	Slight decrease in lysine content[a,d] Decrease in thiamine content[e]	In some cases, improved water absorption capacity of flours[a]	Decreased protein solubility[a,f] Decreased foaming capacity,[a] emulsion capacity,[a,c] oil absorption capacity,[a] and water absorption capacity[a]	If controlled properly, pleasant roasted, nutty flavor[d] Heat inactivates lipoxygenase enzyme, thus preventing formation of off-flavors[g,h]	Browning[a,c,d,e] Bitter taste and aftertaste in products prepared from defatted flours[d]	Dry heating results in higher total phenolic content and antioxidant activity in flours from dry heated beans[d]	N/A
Dehulling/ decortication	Decrease in antinutritional components such as condensed tannins Improvement in protein digestibility[f]	Decrease in mineral content	Improves water absorption capacity	N/A	Seed coat removal improves color of flour and removes bitter and astringent components	Reduction of apparent viscosity of cowpea seed paste[e]	N/A	Dehulling/ decortication reduced total phenolic content and antioxidant activity

N/A, not applicable.
[a]*Maruatona (2008).*
[b]*Quin* et al. *(1996).*
[c]*Jideani* et al. *(2009).*
[d]*Kayitesi (2009).*
[e]*McWatters* et al. *(1993).*
[f]*Phillips* et al. *(1988).*
[g]*Stephens* et al. *(1997).*
[h]*Buranasompob* et al. *(2006).*

reduced the trypsin inhibitors present in marama beans (Maruatona, 2008). However, whereas the *in vitro* protein digestibility was improved, the lysine content was somewhat reduced (Maruatona *et al.*, 2009).

The use of dry heating processes in the preparation of legume flours can also affect their functional and sensory properties. Heating of marama beans prior to decortication reduced protein solubility and emulsifying capacity of resulting marama bean flours but significantly improved water absorption capacity (Maruatona *et al.*, 2009). Jideani *et al.* (2009) also established that roasting of *T. esculentum* significantly increased the oil absorption capacity of the resulting flour.

Decortication

Dehulling or decortication of legumes such as cowpeas is the process by which the seed coat is removed. Seed coats contain fibrous components, proteins, phenolic compounds, and various antinutritional factors (e.g. trypsin inhibitors) that could interfere with the nutritional and functional properties of flours. The presence of fibrous components could, for example, interfere with digestibility of weaning foods prepared from cowpea flours or with functional properties of cowpea pastes (Phillips, 1982) used to produce deep-fat-fried or steamed products.

Ease of removal of seed coats of cowpeas depends on how loosely or tightly the seed coat adheres to the cotyledon. Cowpea types with loosely adhering seed coats are easily decorticated through the simple procedures of cracking and aspiration, whereas types with tightly adhering seed coats require wetting. For the latter, soaking of legumes is done to help facilitate the removal of seed coats and subsequent milling. For example, dehulling efficiency was improved by soaking cowpea seeds for 1 min followed by drying at 100°C (Phillips *et al.*, 2003). The effect of dry and wet decortication processes of diverse cowpea varieties on the pasting properties of flour has been investigated. Henshaw *et al.* (1996) found that a dry decortication process (with presoaking of seeds) gave pastes with higher hot paste viscosity than when no presoaking was done. Presoaking was also shown to affect particle size distribution of flour. In a related study, Phillips *et al.* (1988) investigated the maximum temperature at which cowpeas could be dried without altering essential functional, nutritional, and sensory properties. Protein solubility was reduced at temperatures of 90°C or higher. Increases in drying temperatures also resulted in reduced thiamine content and increased browning of the flour.

Defatting

Defatting is required in the case of leguminous oilseeds such as marama beans when protein-rich, stable flours are required. Although full fat flours are deemed to be more energy dense than fully or partially defatted flours, they have lower protein content and are prone to hydrolytic and oxidative rancidity. It has been postulated that dry heating of marama beans prior to dehulling disrupts lipid bodies, thereby allowing oil to be readily expelled from the lipid bodies during the defatting step. This results in higher protein content flour than that produced from unheated whole marama beans (Maruatona *et al.*, 2009). However, in the case of dry cowpea seeds, because of its low fat content (1−2%), production of flour is simpler than that required for flours from leguminous oilseeds (McWatters, 1990). In this case, a defatting step is not required.

Milling

Milling of dry cereal or legume seeds is a size-reduction unit operation used to produce flours or meals. Particle size distribution of flours influences functionality of cowpea paste and final end product quality (Singh *et al.*, 2005). Finely milled flour has a lower gelatinization temperature than that of more coarsely milled flour (Kerr *et al.*, 2001). Milling to too fine a particle size may result in textural quality problems in end products (Kerr *et al.*, 2001).

Conversely, flour with coarse particles has been found to result in viscous pastes (Singh *et al.*, 2004). Wet milling of soaked, decorticated cowpeas has been found to result in highly functional pastes (Kethireddipalli *et al.*, 2002).

NUTRITIONAL, FUNCTIONAL, AND SENSORY QUALITIES OF LEGUME-BASED COMPOSITE FLOURS AND BAKED GOODS

Table 18.2 provides a summary of studies on the use of composite flours in baked foods. The details of these studies are discussed here.

Nutritional quality of composite baked goods

Improved protein content of cereal–legume composite baked foods has been reported by many authors. Investigating the biscuit making potential of millet–pigeon pea flour blends, Eneche (1999) reported that protein content of the composite biscuits increased with increasing proportion of pigeon pea flour (the legume component) in the blend: 7.2% (0% pigeon pea flour), 12.1% (25% pigeon pea flour), 14.4% (35% pigeon pea flour), and 15.2% (50% pigeon pea flour). It was estimated that the protein content of the composite biscuits was higher than that of other commercially available biscuits such as cream crackers, digestives, and plain biscuits. Improved protein content has also been reported for many such composite baked goods, including cakes and cookies of wheat–linseed (flaxseed) composite flours (Bashir *et al.*, 2006); wheat–mothbean composite biscuits (Awan *et al.*, 1995); tortillas made from composites of wheat with various legumes, namely red bean, black bean, and pinto bean (Anton *et al.*, 2008); maize–bean composite tortillas (Mora-Avilés *et al.*, 2007); and wheat–mustard flour composite biscuits (Tyagi *et al.*, 2007). Awan *et al.* (1995) further reported increased net protein utilization, biological value, protein efficiency ratio, and feed efficiency of wheat–mothbean composite biscuits with an increase in mothbean supplementation. Mora-Avilés *et al.* (2007) reported improved levels of tryptophan, lysine, calcium, iron, and zinc in maize–bean composite tortillas compared to tortillas made exclusively with maize.

Composite baked goods are not restricted to the use of legumes as a component of the composite flour. Various food materials in addition to legumes have been used, including potato, African breadfruit, tigernut, and plantain. Although production of composite foods using such nonleguminous components may not necessarily lead to improved protein content, it appears that levels of fiber, which are important for nutrition and health, may be improved. Increased levels of fiber have been reported for wheat–breadfruit composite bread (Olaoye and Onilude, 2008), wheat–tigernut composite flour (Ade-Omowaye *et al.*, 2008), wheat–plantain composite bread (Mepba *et al.*, 2007), and wheat–cactus pear composite flour and bread (Moreno-Álvarez *et al.*, 2009).

Phytochemical quality and antinutritional factors of composite baked goods

Phytochemicals are non-nutritive plant chemicals that exert protective or disease-preventing effects. They have been associated with protection from and/or treatment of chronic diseases such as heart disease, cancer, hypertension, diabetes, and other medical conditions. The range of phytochemicals found in grains is very broad and includes various phenolic compounds, phytosterols, alkaloids, and saponins. Phenolic compounds, for instance, through their antioxidant activity, are hypothesized to have the ability to reduce the risk of developing certain cancers by potentially protecting body cells against oxidative damage caused by reactive oxygen species. On the other hand, many of these phytochemicals together with other seed components are regarded as antinutritional factors. Therefore, with regard to these phytochemicals or antinutritional factors, the objective of producing composite baked goods may either be to retain them in order to exert potential health benefits or to use the processing to eliminate or inactivate them so as to reduce their antinutritional effects.

TABLE 18.2 Summary of Research Studies on the Use of Composite Flours in Baked Foods

Type of Product	Composite Flours Used	Properties Investigated	Reference
Leavened flat bread	Wheat–potato	Proximate analyses of composite flours Sensory analyses of breads	Anjum *et al.* (2008)
Egyptian Balady bread	Wheat–cracked broad bean	Proximate analyses on individual flours Rheological properties of flour blends Sensory evaluation of breads	Abdel-Kader (2000)
Bread	Fermented/germinated cowpea–wheat	Composite flours analyzed for ash, protein, gluten content, α-amylase activity; color; farinograph and extensograph characteristics Breads analyzed for loaf volume and weight, texture, crumb structure and color	Hallén *et al.* (2004)
Bread	Wheat–full fat lupin/soya/triticale	Flours analyzed for physical dough properties Breads analyzed for quality characteristics	Doxastakis *et al.* (2002)
Bread	Wheat–breadfruit	Proximate analyses, sensory evaluation, and aerobic plate count of the bread samples	Olaoye and Onilude (2008)
Bread	Wheat–tigernut	Physicochemical properties of the dough Sensory and physical properties of the breads	Ade-Omowaye *et al.* (2008)
Bread	Wheat–cactus pear stem	Composite flours and breads analyzed for proximate composition Rheological properties of the composite doughs Sensory properties of the composite breads determined	Moreno-Álvarez *et al.* (2009)
Bread	Wheat–barley	Total phenolic content and antioxidant activity of free and bound fractions of the flours and breads	Holtekjølen *et al.* (2008)
Bread	Wheat–cassava	α-Amylase activity and rheological properties of the composite flours Physical characteristics and sensory properties of the breads determined	Khalil *et al.* (2000)
Bread and biscuit	Wheat–plantain	Proximate composition of the composite breads Rheological properties of dough from the composite flours used for bread making Baking characteristics of the bread Physical characteristics Sensory properties of the breads and biscuits	Mepba *et al.* (2007)
Biscuit	Millet–pigeon pea	Proximate analyses of individual flours and biscuits Sensory analyses of biscuit samples	Eneche (1999)
Biscuit	Breadfruit (malted and unmalted)–wheat	Biscuit quality parameters (biscuit flow/spread and biscuit break strength) Sensory evaluation	Nwabueze and Atuonwu (2007)
Biscuit	Wheat–mothbean	Biscuits analyzed for proximate composition, physical characteristics, sensory quality, and biological parameters using weanling albino rats	Awan *et al.* (1995)
Biscuit	Wheat–mustard	Proximate composition and sensory properties of the biscuits and textural characteristics of the doughs and biscuits determined	Tyagi *et al.* (2007)
Cakes and cookies	Wheat–linseed	Proximate composition, physical properties, and sensory attributes of cookies and cakes	Bashir *et al.* (2006)
Cake	Wheat–chickpea	Physical properties of the batters and cakes	Gómez *et al.* (2008)

Continued

TABLE 18.2 Summary of Research Studies on the Use of Composite Flours in Baked Foods—continued

Type of Product	Composite Flours Used	Properties Investigated	Reference
Tortillas	Wheat–bean	Rheological properties of the doughs Physical properties Protein content, total phenolics and antioxidant activity of the tortillas, and phytic acid content and trypsin inhibitor activity of the flours and tortillas	Anton *et al.* (2008)

However, it appears that with regard to composite baked goods, not much research has been conducted to determine the effect of the compositing and processing on levels of these phytochemicals. Anton *et al.* (2008) reported increases in total phenolics and antioxidant activity of wheat–bean composite tortillas with increased substitution with bean flour. Tortillas from composite flours containing colored beans (small red bean, black bean, and pinto bean) had higher total phenolics and antioxidant activity than tortillas from composite flours containing cream white navy beans. The authors proposed that this may be related to the higher concentration of phenolic compounds (e.g., flavonol glycosides, anthocyanins, and condensed tannins) in the seed coats of colored bean varieties compared to those of cream white or less colored varieties.

Regarding antinutritional factors, Anton *et al.* (2008) reported an increase in levels of phytic acid and trypsin inhibitor activity in wheat–bean composite flours as the rate of substitution with bean flour increased. However, processing flours into tortillas significantly reduced phytic acid and trypsin inhibitor levels.

Holtekjølen *et al.* (2008) took advantage of the relatively higher content of phenolics in barley to prepare wheat–barley composite flours and breads with improved phenolic content and antioxidant activity. Incorporation with barley increased antioxidant properties of the flours and breads compared to the control bread, and this was dependent on the barley variety and flour extraction rate. During baking, there was a decrease in free phenolics and an increase in bound phenolics, whereas antioxidant activities remained relatively stable.

Rheological properties of composite doughs

For the preparation of baked goods, rheological properties of the dough are of importance. Wheat, the main ingredient in most baked foods, contains viscoelastic gluten, which confers specific rheological properties to the dough, and this in turn influences the final quality of the baked product. Therefore, it would be expected that during preparation of composite flours and doughs, partial replacement of wheat with another component that may be devoid of gluten would influence rheological properties of the resultant dough. Various rheological parameters are of interest in this regard, including water absorption, dough development time, dough strength, and mixing tolerance.

Generally, the major effect on dough rheological properties arises from dilution of the gluten content on partially replacing the wheat component. Composite doughs of wheat with legumes tend to have higher water absorption and lower strength and stability. In a study on the production of Egyptian Balady bread using composites of wheat and broad bean flour, water absorption, arrival time, and dough development time of the flour blends increased with increasing proportion of broad bean flour in the blend (Abdel-Kader, 2000). Extensographic energy of the dough blends decreased with increasing proportion of broad bean flour (Abdel-Kader, 2000), presumably due to the reduction of gluten content of the composite doughs. Hallén *et al.* (2004) reported that during production of bread from wheat–cowpea

composite flours, gluten content of composite flours decreased with increasing proportion of cowpea due to a dilution effect. Water absorption of the composite flours increased with increasing protein content (or increasing proportion of cowpea) due to the predominantly water-soluble nature of cowpea proteins. There was dough weakening with increasing proportions of cowpea in the composite flour due to the decrease in gluten content. The authors also hypothesized that the supplemental proteins from the cowpea component disrupt the protein–starch complex in wheat flour, leading to dough weakening. Composite flours containing germinated cowpea had lower falling number (higher α-amylase activity) than composites containing fermented and normal cowpea flour (Hallén *et al.*, 2004).

Similar observations of increased water absorption and decreased dough stability on inclusion of the nonwheat component have been reported for other composite doughs, including wheat with lupin, soya, or triticale (Doxastakis *et al.*, 2002); wheat–tigernut composite (Ade-Omowaye *et al.*, 2008); wheat–plantain composite (Mepba *et al.*, 2007); wheat–cactus pear composite (Moreno-Álvarez *et al.*, 2009); and wheat–cowpea composite (Anton *et al.*, 2008).

As would be expected, the rheological properties of the resultant composite dough are dependent on the relative hydrophobicity or hydrophilicity of food components such as proteins endogenous to the composite flours. Whereas incorporation of legumes in composite flours tends to bring about an increase in water absorption and decrease in dough strength and stability, the opposite seems to be the case when a root tuber such as cassava is used. In a study on wheat–cassava composite flours, Khalil *et al.* (2000) reported that there was a progressive reduction in water absorption of flours with an increase in substitution with cassava flour, possibly due to reduced protein content of the composite flours. Mixing time, stability, and strength of the composite doughs also increased as the proportion of cassava flour increased. The addition of malt at 1% decreased mixing time and improved dough stability.

Physical characteristics and sensory quality of composite baked goods

The physical characteristics of composite baked goods influence their sensory quality and acceptability by consumers. Of interest are characteristics such as crust and crumb color, loaf volume, loaf height, biscuit width, and spread factor. Generally, on incorporation of a legume, the composite baked product tends to become darker as a result of Maillard reactions due to the relatively higher levels of lysine. Composite breads also tend to have lower volume and height and denser, more compact structure due to the reduction in levels of gluten.

Hallén *et al.* (2004) reported that wheat–cowpea composite breads became progressively darker (lower *L* values) with an increasing proportion of cowpea flour. Increasing proportion of cowpea in the composite appeared to somewhat decrease the specific volume of the bread due to a reduction in the wheat structure-forming proteins accompanied by a decrease in the ability of the dough to incorporate air. Increasing proportion of cowpea in the composite flours resulted in more compact bread with a denser structure and harder texture. Crust and crumb color of the breads became progressively darker with increasing proportions of cowpea, possibly due to increased Maillard reaction as a result of the high lysine content of the cowpeas. Similar results have been reported by Doxastakis *et al.* (2002) for breads made with wheat in composite with lupin, soya, or triticale flour. There were decreases in dough and bread yield and loaf volume on inclusion of lupin and soya in the composite but increases on inclusion of triticale. This was due to dilution of the wheat gluten structure on incorporation of the legumes. Triticale, on the other hand, augmented the gluten because it also contains gluten. For breads containing lupin and soya, crust color darkened, crumb became more yellow, and the crumb texture showed the presence of thickened cells. Overall, it was suggested that the composite flours produced acceptable bread in terms of weight, volume, texture, and crumb structure.

Oven spring and specific volume of wheat–plantain composite breads decreased with increasing proportion of plantain flour (Mepba *et al.*, 2007), whereas flow and break strength of composite biscuits decreased with increasing levels of plantain flour. Generally, the sensory scores of the wheat–plantain composite breads and biscuits decreased with increasing levels of substitution with plantain flour. Anton *et al.* (2008) reported that firmness and cohesiveness of wheat–bean composite tortillas decreased with increased substitution with bean flour. There was an increase in tortilla diameter but a reduction in thickness with an increase in substitution with bean flour due to the effect on the gluten network.

In a study on the quality of sponge cakes and layer cakes made from wheat–chickpea flour blends, layer cakes had decreased batter density and sponge cakes had increased batter density on incorporation of chickpea flour (Gómez *et al.*, 2008). There was a decrease in volume for both layer and sponge cakes on incorporation of chickpea flour. Addition of chickpea flour reduced the symmetry of the cakes, suggesting a decrease in gas retention capacity of the composite batters during baking. The crust of layer cakes became darker on incorporation of chickpea flour (Gómez *et al.*, 2008), presumably due to Maillard reactions and caramelization.

TECHNOLOGICAL ISSUES

Virtually no scientific research has been reported on the phytochemical quality and potential health benefits of legume composite baked goods. Some research suggests that antioxidant activity from phytochemicals such as phenolic compounds can be increased as a result of the baking process. With improved nutritional and phytochemical qualities of legume composite baked goods, there is the potential for increased use of nutrient-dense underutilized legumes such as cowpeas and marama beans.

SUMMARY POINTS

- Flours produced from protein-rich legumes can be used to improve the nutritional quality of cereals.
- Simpler technologies than that used for leguminous oilseed flour can be used for processing of flour from grain legumes such as dry cowpea seeds because of its low fat content.
- Unit operations used to produce legume flours impact both positively and negatively on the nutritional, physicochemical, and functional properties of the flours produced. This in turn influences the sensory quality of foods in which legume–cereal composite flours are used.
- Depending on the type of flours used when compositing, protein quality and/or fiber content can be improved.
- The ratio of flours in the composite may influence dough rheology and, consequently, physical and sensory characteristics of their baked goods. This directly influences consumer acceptability of final products.
- More research on the phytochemical quality of baked goods using composite flours is required.

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CHAPTER 19

Potential Use of Okra Seed (*Abelmoschus esculentus* Moench) Flour for Food Fortification and Effects of Processing

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CHAPTER OUTLINE

INTRODUCTION

Macro- and micronutrient malnutrition has worldwide endemic potential because of its impacts both in developing nations and in the industrialized regions of the world. Systemic malnutrition is increasingly becoming a serious threat to human health because it is a major risk factor for many diseases and it contributes to morbidity and even mortality. One way to curb the global problem is through food fortification of plant origin. Food fortification is broadly aimed at allowing people to obtain from their diet all the energy, including macro- and micronutrients, they need to enjoy healthy and productive lives (Allan *et al.*, 2006; Aminigo and Akingbala, 2004).

Most food fortification programs are targeted to augment levels of essential nutrients required in the diet, including protein and micronutrients such as iron, vitamin A, iodine, zinc, folate

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(vitamin B_9), cobalamin (vitamin B_{12}), thiamine (vitamin B_1), riboflavin (vitamin B_{12}), niacin (vitamin B_3), vitamin B_6, ascorbic acid (vitamin C), vitamin A, calcium, selenium, and fluoride. Okra is rich in some of the essential micronutrients, and the fact that no difference was observed in the protein efficiency ratio of its flour heated at 130°C compared with non-heated flour indicated the absence of anti-nutritional factors (Karakoltsidis and Constantinides, 1975). The okra seed meal contains more than 50% good quality protein on a fat-free, dry-weight basis, whereas most of the suitable amino acids in okra seed protein are present in amounts that are equal to or exceed the amounts in eggs, casein, and the United Nations Food and Agriculture Organization reference protein from okra source (Akingbala *et al.*, 2003). Table 19.1 lists the amino acid composition of okra products that makes okra-based products desirable for food consumption (Savello *et al.*, 1982) and makes okra a good source for food fortification strategies. The objective of this chapter is to appraise the level of food fortification using okra products to enrich select foods that are consumed in tropic and temperate regions of the world, including the impacts that selected processing techniques such as pretreatments may provide.

BREAD FORTIFICATION WITH OKRA FLOUR

In Egypt, the nutritive value of bread produced using composite flour that contained maize was increased by supplementing it with okra flour. The resulting bread was satisfactory and comparable to wheat-based bread because okra contains sticky gluten. Although bitter taste was observed after the consumption of bread that was produced from composite flour that included fenugreek seeds as supplement, which restricted its use because of consumer acceptability constraints, there are no such restrictions for bread that includes okra flour as supplement. Young rats were used as experimental animals, and it was found that maize meal containing okra flour supplement produced the highest growth in the rats (77 g) compared to other diets with fenugreek (72 g) and cottonseed flour (48 g). The protein content in the base okra flour for the bread production was determined to be 18% (Taha, 1947). The associated benefits of okra flour in bread fortification include its slippery characteristics, which facilitate water absorption in the colon and ensure bulk in stools; the fact that is acts as a probiotic while stabilizing blood sugar; and that it binds not only

TABLE 19.1 Amino Acid Composition of Okra Products for Food Fortification[a]

Amino Acid	Whole Seeds	Full-Fat Flour	Defatted Flour	Protein Concentration	Protein Isolate
Aspartic acid	11.57	11.17	10.88	10.89	12.12
Threonine	2.86	2.84	2.97	3.37	2.90
Serine	5.07	4.80	4.67	5.23	4.97
Glutamic acid	15.91	17.29	17.25	19.15	17.35
Proline	3.79	4.60	4.55	4.88	4.98
Glycine	4.78	4.54	4.46	4.77	4.16
Alanine	4.83	4.89	5.79	4.53	4.59
Cystine	3.63	2.86	1.88	1.89	1.90
Valine	4.24	4.27	4.76	4.42	4.33
Methionine	1.83	2.12	2.01	2.18	2.21
Isoleucine	2.96	3.00	2.98	3.06	3.13
Leucine	6.21	5.64	6.81	6.96	6.97
Tyrosine	3.46	4.09	3.95	5.15	4.03
Phenylalanine	4.41	4.86	4.59	4.80	4.85
Histidine	2.34	3.63	2.91	3.61	3.83
Lysine	6.22	6.79	6.93	6.19	6.47
Tryptophan	2.02	2.22	2.64	2.57	2.03
Arginine	11.17	9.82	10.42	10.02	9.98

Source: Adapted from Bryant *et al.* (1988).
[a]*Data represent mean of duplicate determinations in grams amino acids/100 g protein.*

cholesterol but also bile acid carrying toxins dumped into it by the filtering liver (Livera, 2009). Defatted okra flour and okra full-fat flour have also been found to have *in vitro* protein digestibility of 81.22 and 81.94%, respectively. These values are comparable to the *in vitro* protein digestibility obtained for soy defatted flour and soy full-fat flour of 75.57 and 84.71%, respectively. Calculated protein efficiency ratios of okra seed products vary between 2.16 and 2.22, comparable with a range of 2.14−2.19 obtained for soy products, suggesting similarity in protein quality (Bryant *et al.*, 1988).

CEREAL-BASED FOOD FORTIFICATION WITH OKRA FLOUR

Cereal-based foods are widely utilized in many developing nations as dietary staples for adults and weaning foods for infants. In Africa, cereal-based foods account for up to 77% of total caloric consumption. The major cereal-based foods in these regions are derived mainly from maize, sorghum, millet, rice, or wheat. Although cornmeal with okra is a frequently used staple food in Cameroon (Kana Sop *et al.*, 2008), other fermented cereal-based foods such as liquid porridge, including ogi, mahewu, and mahe, or stiff gels, including agidi, kenkey, bogobe, banku, injera, and kisra, are common and important sources of food in other developing countries (Chavan and Kadam, 1989; Osungbaro, 2009; Otunola *et al.*, 2007).

Maize is deficient in most essential nutrients, especially essential amino acids, vitamins, and minerals, but it constitutes more than 90% of the cereals consumed in many developing countries (Aminigo and Akingbala, 2004). It is reported to be low in tryptophan and lysine, which are essential amino acids needed in the diet (Akingbala *et al.*, 2003). The inclusion of okra flour in breakfast or complementary food contributes to the dietary intake of sodium (0.03%) and potassium (2.14%). Table 19.2 lists the various okra products that can be used for food fortification and some of the associated nutritional benefits. Fiber in okra ensures bulk in stool, preventing or improving constipation better than crops with harsh seed coat such as wheat bran, which may cause some irritation and injury to the intestinal tract. The implication is that the vegetable binds excess cholesterol and toxins (in bile acids) that may cause health disorders and subsequently passes it out of the body; thus, the crop is one of the cheapest available functional food crops in the tropics and temperate regions of the world (Jenkins, 2009; Livera, 2009).

TABLE 19.2 Nutritional Profile of Okra Products

Product	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Na (%)	K (%)	CHO (%)
Whole okra grain flour[a]	20.5	14.73	36.9	5.70	0.03	2.14	—
Dehulled okra grain flour[a]	34.2	25.6	8.72	5.91	0.05	0.81	—
Raw okra seed[b]	21.0	—	29.6	5.10	—	—	—
Okra seed meal[b]	35.3	—	21.6	5.22	—	—	—
Roasted okra seed meal[b]	30.3	—	9.47	6.30	—	—	—
Okra whole seeds[c]	24.24	16.22	23.43	4.79	—	—	25.44
Okra hulls[c]	4.04	1.43	43.44	3.00	—	—	39.69
Okra kernels[c]	41.03	29.95	1.50	6.07	—	—	17.12
Okra full-fat flour[c]	39.15	29.07	2.64	5.98	—	—	17.68
Okra defatted flour[c]	54.14	1.67	4.00	8.19	—	—	24.03
Okra protein concentrate[c]	69.08	0.88	0.02	5.42	—	—	19.16
Okra protein isolate[d]	90.13	1.08	0.05	3.09	—	—	0.00

[a]*Data from Ashaye* et al. *(2005).*
[b]*Data from Aminigo and Akingbala (2004).*
[c]*Data from Bryan* et al. *(1988).*
[d]*Data from Otunola* et al. *(2007).*

In Nigeria, maize is a staple cereal consumed in various forms, including processing such as boiled maize, roasted maize, maize flour, and ogi slurry (wet-milled maize gruels). Large amounts of nutrients such as fiber, protein, calcium, iron, phosphorous, and vitamins including thiamine, riboflavin, niacin, folic acid, and panthotenic acid are lost during the processing of cereals for ogi manufacture (Adeniji and Porter, 1978; Osungbaro, 2009). Ogi slurry is a major weaning and breakfast food in Nigeria. Attempts have been made to supplement the levels of both macro- and micronutrients in ogi with okra flour because the flour is reported to be rich in high-quality protein (Otunola *et al.*, 2007; Oyelade *et al.*, 2003) and to have considerable levels of most of the nutrients that are commonly lost during the manufacture of ogi (Chavan and Kadam, 1989). The proximate constitution of oil expressed from okra meal has also been found to include palmitic acid (27.2%), stearic acid (2.8%), arachidic acid (0.1%), oleic acid (43.7%), linolic acid (26.6%), and unsaponifiable matter (0.4%) (Jamieson and Baughman, 1920). Table 19.3 indicates the effect of using okra flour to supplement the nutrients lost during the processing of select cereal-based foods (maize gruel and ogi) in most developing countries.

Data on the chemical composition of okra flour–ogi mixes indicate substantial increases in the levels of protein, increasing from 6.50% in an unsubstituted sample to 12.40% in a sample that was fortified with 50% okra flour. There were also increases in fat, crude fiber, and ash contents (Otunola *et al.*, 2007). Also, okra seed fortification of ogi at the 20% level using defatted and roasted meals increased crude protein content by 122 and 106%, respectively. The ash contents in the mixes were also increased two- to fivefold, whereas fat increased by 1.5–2.2% (Aminigo and Akingbala, 2004).

The nutritional attributes of foods that have been fortified with okra flour have been found to increase in quantity and quality (see Table 19.3). The quantity of the nutrients in the resulting food components, and hence their quality, is determined by the primary nutrients in the okra flour, the quality of which is usually affected by processing. The effect of roasting and roasting time on the antioxidant activity of okra seeds including *in vitro* digestibility stability under gastric and intestinal phases was determined by Adelakun *et al.* (2009b). The protein and fat contents of the roasted okra were affected by the roasting time. There was an initial increase in protein content of okra flour, whereas the protein slightly decreased after 20 min. The fat content and antioxidant activity decreased, whereas there was an increase in the ash and fiber content and antioxidant activity. The study showed that roasting reduced the protein value in okra flour and that its consumption in this form has the potential to prevent chronic diseases because most antioxidative activities occur in the gastrointestinal tract. Adelakun *et al.* (2009a) studied the influence of pretreatment on yield and chemical and antioxidant properties of a Nigerian okra seed flour, and they found that pretreatment such as soaking and blanching resulted in increases in yield and protein content. The antioxidant activity was slightly

TABLE 19.3 Effect of Okra Flour Fortification on Cereal-Based Staples

Staple	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Na (mg/g)	K (mg/g)
Whole maize grain flour	9.3	3.99	3.31	1.70	0.08	0.35
Whole okra grain flour[a]	20.5	14.73	36.9	5.70	0.03	2.14
Dehulled okra grain flour[a]	34.2	25.6	8.72	5.91	0.05	0.81
Traditional maize gruel[a]	6.7	3.33	0.90	0.31	0.03	0.18
10% okra–maize gruel fortification[b]	13.6	8.72	5.98	1.64	0.04	0.24
40% okra–maize gruel fortification[b]	21.9	13.08	9.04	2.6	0.05	0.37
0% okra–ogi fortification[b]	6.50	1.66	0.27	0.05	0.01	—
20% okra–ogi fortification[b]	8.20	1.80	0.29	0.08	0.03	—
50% okra–ogi fortification[b]	12.40	2.34	0.34	0.11	0.06	—

[a]*Data from Ashaye* et al. *(2005).*
[b]*Data from Otunola* et al. *(2007).*

TABLE 19.4 Effect of Pretreatment on Yield, Protein, Fat, Ash, Fiber, Carbohydrates, and Antioxidant Levels in Okra Flour

Sample	Time (h)	Yield (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Carbohydrates (%)	Antioxidant Level (% Inhibition)
Untreated	0	23.5	41.1	31.0	3.4	3.5	10.6	48.3
S1	6	26.3	39.0	31.1	3.8	3.1	12.9	49.1
S2	12	30.3	41.1	30.1	3.9	3.6	11.1	48.1
S3	18	31.7	43.2	29.1	3.8	3.6	9.9	51.3
S4	24	37.6	43.5	29.0	3.9	3.7	9.4	46.9
S5	36	39.3	44.0	28.1	3.8	3.8	9.3	44.7
S6	48	40.9	43.4	28.1	4.0	3.8	10.6	45.5
B1	0.1	27.4	41.4	27.1	3.6	3.6	14.2	31.7
B2	0.2	35.5	42.1	27.1	3.7	3.6	12.7	32.5
B3	0.3	42.2	43.5	26.2	3.8	3.6	12.7	30.3
B4	0.4	39.2	46.1	25.2	3.8	3.7	11.3	27.4
B5	1.0	31.2	39.5	25.1	3.9	3.7	16.9	27.8

Source: Adapted from Adelakun *et al.* (2009a).

increased by soaking, whereas blanching reduced the component due to leaching. Table 19.4 presents the effect of soaking and blanching pretreatment on the yield of okra flour, protein, fat, ash, fiber, carbohydrates, and antioxidant attributes.

OKRA FLOUR AS SUPPLEMENT IN INFANT-WEANING FOODS

In our study on the influence of variety on protein, fat content, and some physical characteristics of okra seeds for some cultivars of okra available in Nigeria, the seeds of the following cultivars were found to be rich in protein: LD-88, VI-104, UI_4-30, 47-4, and V-35. The protein values ranged between 22 and 45% in the whole seed flour fractions of the different cultivars, whereas the fat content in the seed coat was between 4 and 10%, with 47-4 and LD-88 cultivars having the highest and least fat content, respectively (Oyelade *et al.*, 2003). It is evident that processing involving removal of seed coat from the whole seeds before milling enhanced the protein content in all okra lines (Figures 19.1 and 19.2). This attribute probably makes the seed of the vegetable a promising alternative component in the formulation of traditional weaning foods in developing nations because it is a potential source of enhanced protein-based foods for low-income earners. Okra seed meal as a component in weaning food increased the protein and fat contents. The preferred fractions of okra in the traditional Nigerian weaning meal have been found to be 70:30 (corn–okra blends) and 90:5:5 (corn–soybean–okra blends) (Jideani and Adetula, 1993).

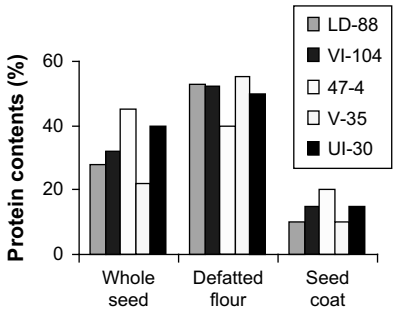

FIGURE 19.1
Effect of variety on the protein contents of various fractions of okra seeds. *Source:* Journal of Food Engineering, *Vol. 57, Oyelade, O. J., Ade-Omowaye, B. I. O., and Adeomi, V. F., Influence of variety on protein, fat contents and some physical characteristics of okra seeds, pp. 111–114, Copyright Elsevier (2003).*

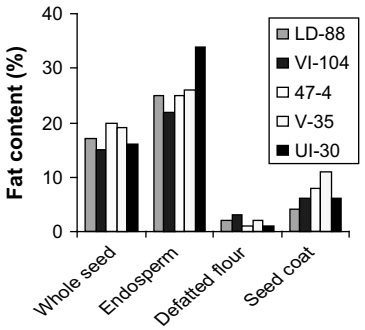

FIGURE 19.2
Fat contents of various fractions of okra seeds as affected by variety. *Source:* Journal of Food Engineering, *Vol. 57, Oyelade, O. J., Ade-Omowaye, B. I. O., and Adeomi, V. F., Influence of variety on protein, fat contents and some physical characteristics of okra seeds, pp. 111–114, Copyright Elsevier (2003).*

TECHNOLOGICAL ISSUES

Okra can be used to supplement nutrient intake for the vast majority of people in the temperate and tropic regions of the world. The seed can be ground and sifted to obtain high-protein and high-oil products of considerable nutritional value. Although the literature suggests that its oil may contain considerably less gossypol, if any, the meal is an adequate substitute for wheat flour. However, potential obstacles to the use of okra seed for fortification include the constituent gossypol or gossypol-like compounds and cyclopropenoid fatty acids in the oil. The concentration of these components in okra seed and the consequent effect of cultivars on the fraction require validated and standardized protocols because cyclopropenoid has been found to be toxic to humans (Martin and Rhodes, 1983).

Analysis of various components of okra indicates a significant influence on proximate constituents. Okra dehulling is a major technological operation for protein concentration or isolation in okra seeds. The protein derived from okra has been reported to be of high quality (Oyelade *et al.*, 2003), comparable to that obtained from soy products and cottonseed flour. Efforts must be amplified if the potential of okra seed is to become a reality because dehulling has been identified as a major constraint to effective utilization of okra in food fortification programs. Therefore, the use of commercial blenders to dehull okra, which has been reported by Bryan *et al.* (1988), merits further investigation in order to obtain high-quality okra products.

In many developing nations, including Nigeria, for mechanization of agriculture to succeed, it must be based on domestic engineering initiatives to design, develop, and manufacture locally most of the tools, equipment, and machines needed for all agricultural production field operations, postharvest processes, and other rural industrial activities (Odigboh, 1999). Investigation of the physicochemical properties of okra products could provide a suitable benchmark as an indication of the need to develop improvised processing equipment for the crop. Required equipment includes solar driers for dehydration, seed dehulling equipment, an indigenous canning line, blanching equipment, and mechanical press for oil extraction (Oyelade and Ade-Omowaye, 2009). Thus, okra, due to its enormous potential in tropic and temperate regions, including potential production of biomass from its foliage and fiber from the dried stems and matured pods, which may be used for paper pulp or in the textile industry or as fuel (Martin and Ruberte, 1981), should be an impetus to revitalize dwindling global indigenous technology and develop crop processing equipment in the geographical locations where it is grown. In addition, okra mucilage could also find useful application in jaggery production and paper sizing. Grated okra could be used as a thickener, binder, or flavoring agent (Oyelade and Ade-Omowaye, 2009).

Some basic engineering properties of food crops relevant to the design of crop processing equipment for okra seeds have been determined for the seeds of some preferred okra cultivars in Nigeria, with an average diameter of 3.99–4.71 mm, surface area of 28.27–88.92 cm^2, average masses of seeds varying between 0.05 and 0.06 g, and average density varying from 0.11 to 0.32 g/cm^2 (Oyelade *et al.,* 2003). Calisir *et al.* (2005) determined some physico-chemical properties of okra seeds that are commonly grown in Turkey and that have varying dimensional properties to those reported for some Nigerian okra. Therefore, efforts should be amplified to determine reliable physicochemical parameters of all known okra lines in various geographical locations in order to adequately design and construct functional equipment for okra on a global scale.

CONCLUSION

Okra flour has huge potential to be used to enrich foods in order to provide adequate nutrients for individuals for whom daily nutritional needs are not being met. Based on the nutrient profile of okra, including the amino profile and the effect of processing on this, human consumption of okra flour can be promoted because of its positive health effects. However, considerable effort needs to be directed at addressing associated technological issues regarding effective utilization of the food product in food fortification, including the following:

- The constituent gossypol or gossypol-like compounds and cyclopropenoid fatty acids in the oil
- The constraint of dehulling to produce high-quality okra products for effective utilization in food fortification programs
- Studies on the physicochemical properties of okra products to provide a suitable benchmark for the development of functional okra processing equipment
- Determination of the effect of variety and agronomical traits on nutritional and physicochemical parameters of all known okra lines to present okra to wider population groups as a vital food crop.

SUMMARY POINTS

- Okra is an important crop in temperate and tropical climates.
- The seed of okra is high in quality amino acids.
- Due to its high-quality protein, it can be used in flour fortification.
- Okra flour has the potential to be used to enrich foods in order to provide adequate nutrients for individuals for whom daily nutritional needs are not being met. These nutrients may be affected by various processing procedures.
- The seeds can be a source of antioxidant, which is essential in maintaining health.

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CHAPTER 20

Apricot Kernel Flour and Its Use in Maintaining Health

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

ALPA Anti-lipid peroxidative activity
KOH Potassium hydroxide
RP Reducing power
RSP Radical scavenging power
TPC Total phenolic content

INTRODUCTION

Apricot (*Prunus armeniaca* L.), because it is a good source of nutrients, is one of the most familiar crops worldwide (Baytop, 1999). Apricot kernel (Figure 20.1) is an important source of dietary protein as well as oil and fiber (Femenia *et al.*, 1995). The kernel is also reported to have high antioxidant and antimicrobial activities (Yigit *et al.*, 2009).

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FIGURE 20.1
Apricot kernels. Apricot kernels are mainly used in the production of oils and bezaldehyde. The kernel is also added to bakery products either whole or grounded, and it is also consumed as an appetizer.

Apricot kernels are mainly used in the production of oils and bezaldehyde, and the kernels are also added to bakery products either whole or grounded and also consumed as an appetizer. Kernel oil has been used in cosmetics and as a pharmaceutical agent (Alpaslan and Hayta, 2006). The apricot and its kernel have been used in folk medicine as a remedy for various diseases. For example, apricot kernel paste has been prescribed to heal vaginal infections. In very small amounts, the toxic hydrogen cyanide present in bitter apricot kernels has been recommended for asthma, cough, and constipation (Chevallier, 1996).

PHYSICAL PROPERTIES

The percentage of the kernel in the pit of apricot varies from 18.8 to 38.0%, calculated as [(pits)/(pits + kernels)] × 100. The average dimensions of apricot kernels are as follows: length, 14.0–19.17 mm; width, 9.99–10.20 mm; thickness, 3.3–6.27 mm; geometric mean diameter, 9.89–10.31 mm; and mass, 0.47–0.48 g. The 100-kernel weight range is 28.7–65.1 g (Alpaslan and Hayta, 2006).

CHEMICAL COMPOSITION

The reported protein, oil, and ash content of apricot kernel is 14.1–45.3, 27.7–66.7, and 1.7–2.9%, respectively (Alpaslan and Hayta, 2006).

PROTEINS

Apricot kernels contain a substantial amount of dietary protein. The protein content of apricot kernels ranges from 14.1 to 45.3% (Alpaslan and Hayta, 2006). A study found that apricot kernel proteins contain 84.7% albumin, 7.65% globulin, 1.17% prolamin, and 3.54% glutelin. Nonprotein nitrogen comprises 1.17%, and other proteins comprise 1.85% (Abd-El-Aal *et al.*, 1986). Research on the physicochemical properties of apricot kernel proteins revealed that proteins had ultraviolet absorption (λ_{max}) of 282 nm; a fluorescence spectrum (emission max) of 315 nm; and four subunits with molecular sizes of 58.6, 37.4, 25.2, and 16.5 kDa, respectively (El-Adawy *et al.*, 1994).

TABLE 20.1 In Vitro Protein Digestibility Values for Apricot Kernel Flour, Apricot Kernel Protein Isolate, and Casein[a]

		Digestible Protein(%)[b]	
Enzyme System	**Casein (%)**	**Kernel Flour**	**Kernel Protein Isolate**
Pepsin	33.4 ± 3.1	30.6 ± 2.5	32.8 ± 2.7
Trypsin	72.8 ± 2.5	30.7 ± 3.0	66.9 ± 2.9
Pancreatin	95.9 ± 1.8	35.5 ± 2.6	95.9 ± 2.4
Pepsin–Pancreatin	99.1 ± 0.3	96.4 ± 1.2	98.1 ± 1.5

Source: Reprinted from *Food Chemistry*, 19, Abd-El-Aal, M. H., Hamza, M. A., and Rahma E. H., *In vitro* digestibility, physico-chemical and functional properties of apricot kernel proteins, 197–211, Copyright 1986, with permission from Elsevier.
[a]*The data represent a number of cultivars. Data are mean ± SD.*
[b]*Protein digestibility of apricot kernel flour and protein isolates is high in the pepsin–pancreatin system and quite low when either pepsin or trypsin is used.*

Nutritive value of proteins

Essential amino acids in apricot kernel constitute 32–34% of the total amino acids (Femenia *et al.*, 1995). The major essential amino acids (mmol/100 g meal) are arginine (21.7–30.5) and leucine (16.2–21.6), and the predominant nonessential amino acid is glutamic acid (49.9–68.0) (Kamel and Kakuda, 1992). Table 20.1 indicates that protein digestibility of apricot kernel flour and protein isolates is high in the pepsin–pancreatin system and quite low when either pepsin or trypsin is used (Abd-El-Aal *et al.*, 1986).

Utilization and incorporation of detoxified apricot kernel flours in food products has been reported as completely safe with regard to toxicity (El-Adawy *et al.*, 1994). The suitability of apricot kernels as a source of protein was evaluated using (1) kernels from a sweet variety; (2) untreated bitter kernels; and (3) kernels debittered by boiling in 0.1% Na_2CO_3, soaking in water, and drying at 100°C. Protein efficiency ratios (vs. 2.81 for casein) were 1.64 for the sweet variety kernels, no growth (due to bitterness causing low food intake) for the untreated bitter kernels, and 1.2 for the debittered kernels. Net protein ratios were as follows: casein, 3.95; sweet variety kernels, 2.70; untreated bitter kernels, 2.81; and debittered kernels, 3.09 (Gabrial *et al.*, 1981).

CARBOHYDRATES

Carbohydrate content of apricot kernel has been variously reported as 25.5% (w/w), 17.3%, and 18.1–27.9% (Alpaslan and Hayta, 2006). Total sugar content has been reported as 4.10 and 7.76% and invert sugar content as 5.86% (Pala *et al.*, 1996).

OILS

The oil content of the kernels varies from 27.7 to 66.7%. Unsaponifiable matter ranges from 0.1 to 1.6, saponification number ranges from 187.3 to 199.0, iodine value is 90.0–104.8,

TABLE 20.2 Fatty Acid (%) Profile of Apricot Kernels[a]

Palmitic (16:0)	Palmitoleic (16:1)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	Reference
4.4	0.1	1.4	69.3–71.4	26.0	0.1	6
8.8	1.2	1.2–2.0	66.3	31.7	0.2	22
4.5–6.6	0.6–0.9	1.7	72.9	18.8–24.0	0.1–1.2	8
6.1–8.6	0.1	0.6	62.1	23.1–27.7	0.1	14

Source: Reprinted with kind permission from Springer Science+Business Media: *Journal of the American Oil Chemists' Society*, Apricot kernel: Physical and chemical properties, 83, 2006, 469–471, M. Alpaslan and M. Hayta.
[a]*The major fatty acids in apricot kernel are oleic and linoleic acids.*

specific gravity is 0.876–0.932, and the refractive index is 1.464–1.480 (Alpaslan and Hayta, 2006). Table 20.2 shows the fatty acid (FA) profile of apricot kernels. The contents of the major FAs were reported as oleic acid (58.3–73.4%) and linoleic acid (18.8–31.7%) (Alpaslan and Hayta, 2006). Sweet apricot kernels have been reported to contain more oil than that contained in bitter kernels, and oleic acid and linoleic acid correspond to approximately 92 g/100 g of the total FAs present (Femenia *et al.*, 1995).

The contents of unsaturated FA (91.5–91.8%) and saturated FA (7.2–8.3%) have been reported, as well as those of neutral lipids (95.7–95.2%), glycolipids (1.3–1.8%), and phospholipids (2.0%). The kernel oil contains 11.8 mg/100 g campesterol, 9.8 mg/100 g stigmasterol, and 177.0 mg/100 g sitosterol (Alpaslan and Hayta, 2006).

Four tocopherol and six phytosterol isomers were identified and quantified in apricot kernel oil; among these, γ-tocopherol (475.11 mg/kg of oil) and β-sitosterol (273.67 mg/100 g of oil) were predominant (Turan *et al.*, 2007).

VITAMINS AND MINERALS

The kernels contain thiamine, riboflavin, niacin, vitamin C, α-tocopherol, and δ-tocopherol (Slover *et al.*, 1983). The mineral content ranges of apricot kernel (mg/100 g dry matter) are as follows: Na, 35.2–36.8; K, 473–570; Ca, 1.8–2.4; Mg, 113–290; Fe, 2.14–2.82; and Zn, 2.33–3.15 (Alpaslan and Hayta, 2006).

AMYGDALIN

Depending on the variety, apricot kernels contain the cyanogenic glycoside amygdalin. Amygdalin (also known as laetrile or vitamin B_{17}) is commonly found in the kernels of almonds, apricots, cherries, peaches, and apples. The amount of cyanogenic glycosides in plants varies with plant species and environmental effects. Amygdalin contains a cyanide group between a glycoside and a benzene ring that can be released after hydrolysis (Figure 20.2; Cho *et al.*, 2006). The amygdalin content of apricot and bitter almond kernels (*Prunus armeniaca* L.) has been reported to be approximately 3 or 4% by weight (Niels, 1996). Apricot kernels contain approximately 20–80 μmol/g of amygdalin, and it is very high (5.5 g/100 g) in bitter apricot cultivars and not detected in the sweet ones.

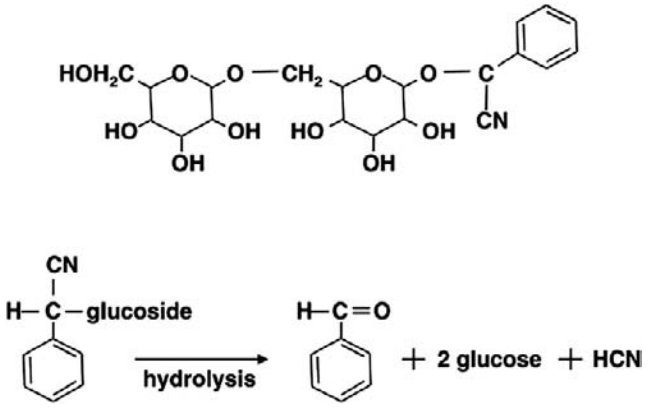

FIGURE 20.2
Molecular structure and hydrolysis of amygdalin to yield cyanide. Amygdalin contains a cyanide group between a glycoside and a benzene ring that can be releases after hydrolysis. *Source: Reprinted with permission from Cho, A. Y., Yi, K. Y., Rhim, J. H., Kim, K. I., Park, J. Y., Keum, E. H., Chung, J., and Oh, S. (2006). Detection of abnormally high amygdalin content in food by an enzyme immunoassay.* Mol. Cells *21, 308–313.*

On average, an apricot kernel contains approximately 0.5 mg of cyanide (Femenia *et al.*, 1995).

Hydrolysis of cyanide can be catalyzed by either endogenous enzymes contained within the kernels or exogenous β-glucosidase released from bacteria within the gastrointestinal tract or from ingested foods inside the intestine. Cyanogenic glycosides are nontoxic but can release free hydrogen cyanide (Cho *et al.*, 2006). The hydrogen cyanide content has been found to be 8.9–11.7 mg/100 g in bitter cultivars and 200 mg/100 g in the wild cultivars (Baytop, 1999).

TECHNOLOGICAL ISSUES

Health-promoting effects of amygdalin

Apricot kernels and their oil were historically used to treat tumors and ulcers (Rieger, 2006). Amygdalin has been reported to be used for preventing and treating migraine, hypertension, chronic inflammation, and other reaction source diseases and for the treatment of cancer (Toshiyuki *et al.*, 2003). Amygdalin has also been reported to improve cerebral function (Hiromi, 1995).

Adverse reactions

Amygdalin was first isolated in 1830. In 1845, it was used to treat cancer in Russia, and again in the 1920s in the United States, but it was considered too poisonous. In the 1950s, a reportedly nontoxic, synthetic form was patented for use as a meat preservative and later marketed as Laetrile for cancer treatment. Amygdalin, also referred to as laetrile or vitamin B_{17}, was popularized as a cancer cure (Milazzo *et al.*, 2006). However, a clinical trial of amygdalin carried out in 1982 found that "no substantive benefit was observed in terms of cure" and more than 2 of the 178 patients suffered from cyanide toxicity (Moertel,1982). Amygdalin is sometimes confused with laetrile; however, amygdalin and laetrile are different chemical compounds. Laetrile, which was patented in the United States, is a semisynthetic molecule sharing part of the amygdalin structure, whereas the "laetrile" made in Mexico is usually amygdalin, the natural product obtained from crushed apricot kernels, or neo-amygdalin. Excess consumption of apricot kernels (to produce more than 1 mg/l cyanide in blood) may cause poisoning. The fatal dose of hydrogen cyanide has been reported to be 0.5 mg/g (Stoewsand *et al.*, 1975). A systematic review in 2006 concluded that the claim that Laetrile has beneficial effects for cancer patients is not supported by data from controlled clinical trials (Milazzo *et al.*, 2006). Due to lack of evidence, laetrile has not been approved by the U.S. Food and Drug Administration.

Patents associated with apricot kernel

A health care tea for patients with dry mouth and tongue, dry excrement, anorexia, disturbed sleep, etc. has been proportionally prepared from apricot kernel, yam, tuckahoe, lily bulb, and crystal sugar (Jinyi, 2006).

A food for curing upper respiratory tract infection, acute and chronic bronchitis, asthma, pulmonary tuberculosis, etc. with a certain therapeutic effect was reported to be made by using sweet apricot kernel, peach kernel, and walnut kernel according to a mixing ratio of 1:1:2–3 and adding the auxiliary materials of cane sugar, fine flour, vegetable oil, and ginseng powder and adopting the production processes for Chinese Guangdong moon cake, Chinese Xiaogan sesame sweets, and amber sugar-coated walnut meat. It can be made into the respective forms of cake and sweets (Jiang, and Hai, 2002).

A disease-preventing black plum–apricot kernel liquor for suppressing thrombosis, relaxing cough and asthma, delaying senility, etc. was prepared from 10 Chinese medicinal materials, including black plum, apricot kernel, dried ginger, and tangerine peel (Lie, 2002).

An abrasive substance for skin cleansing has been proposed in which natural kernels, shells, fruit skins and/or seeds (walnut, hazelnut, almond shell flour, olive, apricot, peach, cherry or plum kernel flour, flours of palm kernels and coconut, jojoba fruits, macadamia nuts, pistachios and pine shells, corn cob flour, wheat bran, oat flour, or wood flours) are ground to give a flour of defined particle size of 50–2000 μm. The flour is treated with aqueous hydrogen peroxide solution, and the lightening and degreasing of the flour is brought about by the simultaneous metered addition of the remainder of the bleaching agent with an alkali solution in alkaline medium (Guenter and Friebel, 2008).

Processing and other uses

Apricot kernels can be obtained as a by-product from apricot processing plants. However, because of the lack of systematic collection and utilization of apricot kernels, this valuable product with a major industrial potential remains unexploited (Femenia *et al.*, 1995).

Both water and methanol extracts of sweet kernels have antioxidant potential. The highest percentage inhibition of lipid peroxidation (69%) and total phenolic content (7.9 ± 0.2 μg/ml) has been reported in the methanol extract of sweet kernels and in the water extract of sweet kernels, respectively (Yigit *et al.*, 2009; Table 20.3).

During storage, a significant decrease was observed in iodine (104.1–94.9 g I_2/100 g), whereas saponification (192.8–195.5 mg potassium hydroxide (KOH)/g), peroxide (6.2–10.0 mEq/kg), and acid (3.6–5.9 mg KOH/g) were significantly increased compared to initial levels of 192.8–195.5 mg KOH/g, 6.2–10.0 mEq/kg, and 3.6–5.9 mg KOH/g, respectively. Apricot kernel oil can be stored at ambient temperature (20°C) after adding 0.02% *tert*-butylhydroquinone, followed by packing in amber-colored glass bottles and polyethylene pouches (Gupta and Sharma, 2009).

The oil of apricot kernels has been used in Germany and the United States in preparing fixed oil and macaroon paste (Femenia *et al.*, 1995).

The detoxified apricot kernel flour and protein isolates appear to be good sources of protein for food products (Abd-El-Aal *et al.*, 1986).

Several studies have been conducted to remove amygdalin from apricot kernels. Apricot kernels were debittered by soaking in either water or 2% sodium hydroxide at 60°C with a ratio of kernel to soaking medium of 1:2 (Ibrahim *et al.*, 1977). A temper fermentation process was proposed to achieve total cyanide removal of 70 g/100 g kernel requiring elimination of bitter kernels containing antimicrobial substances by leaching and boiling prior to temper fermentation (Tuncel *et al.*, 1990).

TABLE 20.3 Total Phenolic Compounds in Methanol and Water Extracts of Sweet and Bitter Apricot Kernels

Extract	Absorbance (760 nm)	Gallic Acid Equivalent (μg/ml)
Control	0.003	–
Water extract of bitter kernels	0.11	0.4 ± 0.1
Methanol extract of bitter kernels	0.12	0.5 ± 0.0
Water extract of sweet kernels	2.04	7.9 ± 0.2
Methanol extract of sweet kernels[a]	1.45	5.7 ± 0.3

Source: Reprinted with permission from Yigit, D., Yigit, N., and Mavi, A. (2009). Antioxidant and antimicrobial activities of bitter and sweet apricot (*Prunus armeniaca* L) kernels. *Braz. J. Med. Biol. Res*. 42, 346–352.
[a]*Methanol extract of sweet apricot kernels has the highest percentage inhibition of lipid peroxidation and total phenolic content.*

TABLE 20.4 Effect of Apricot Kernel Flour (AKF) Addition on Sensory Properties of Noodles[a]

AKF (%)	Odor	Appearance	Firmness (Mouthfeel)	Taste	Total Acceptability
0 (control)	9.20[a]	7.45[a]	6.30[a]	8.85[a]	7.95[a]
5	7.70[b]	7.10[a]	5.05[b]	7.40[b]	6.81[b]
10	5.90[c]	6.05[b]	4.20[c]	7.00[c]	5.78[c]
15	4.70[d]	5.80[b]	4.10[c]	5.45[d]	5.01[d]
20	4.00[e]	5.85[b]	3.45[d]	4.35[e]	4.39[e]
LSD ($p = 0.05$)	0.621	0.575	0.430	0.372	0.277

LSD, Least significant difference.
Source: Reprinted with permission from Eyidemir, E., and Hayta, M. (2009). The effect of apricot kernel flour incorporation on the physicochemical and sensory properties of noodle. *Afr. J. Biotechnol*. 8, 85–90.
[a]*Different letters in the same column represent statistically significant* ($p < 0.05$) *differences between means. The noodles containing up to 15% apricot kernel flour are acceptable in terms of their sensory properties.*

Apricot kernels have been reported to play an important role in the industrial production of marzipan in some countries (Groves, 1983).

The extraction, characterization, and evaluation of apricot kernel oil for use in preparing biscuits and cakes has been investigated and its effect on the acceptability of the product has been evaluated (Abd-El-Aal *et al.*, 1986).

Apricot kernel yoghurt was made using apricot kernels, fresh milk, *L. bulgaricus* and *S. thermophilus*. Fresh and dried apricot kernels were boiled at 80°C for 10–15 min, defibrinated with hot water at 80–100°C, filtered, mixed with fresh milk, sterilized at 85–95°C for 5 min, cooled, inoculated with 3% starter, filled into containers, fermented at 41 ±1°C for 4–6 h, and stored at <4°C for 12 h. Optimal conditions for fermentation were as follows: ratio of apricot kernel thick liquid to milk, 3:7; 8% sugar; 3% mixed starter of *L. bulgaricus* and *S. thermophilus* at a ratio of 1:1; and temperature and time of 42°C and 5 h, respectively (Suping and Wenjuan, 2003).

Apricot kernel has been used in the enrichment of noodles. The properties of noodles were examined by adding apricot kernel flour to the noodle formulation on a 5, 10, 15, and 20% flour weight basis. As shown in Table 20.4, the results obtained in the study suggested that acceptable noodles in terms of sensory properties could be produced by incorporating apricot kernel flour into wheat flour up to the level of 15% flour weight basis (Eyidemir and Hayta, 2009).

The antioxidant properties of peeled, defatted, and roasted apricot kernel flours have been evaluated by determining radical scavenging power (RSP), anti-lipid peroxidative activity (ALPA), reducing power (RP), and total phenolic content (TPC). RSP, RP, and TPC did not increase linearly but showed a maximum for 10 min of roasting. Roasting reduced ALPA values; thus, unroasted samples showed the highest ALPA value (Durmaz and Alpaslan, 2007).

SUMMARY POINTS

- It has been claimed that bitter apricot kernels can help cure cancer. However, this claim has not been scientifically proven.
- Any products proposed to be used in the treatment of cancer must be carefully evaluated in terms of efficacy.
- Apricot kernels can be obtained at a relatively low cost as a by-product from the many food companies that process apricots.

- Due to the absence of strategies for the optimal utilization of apricot kernels, these kernels are a by-product with a major industrial potential that has not been sufficiently evaluated.
- To achieve the most economical and efficient utilization of apricot kernels, more knowledge on the varieties, properties, and composition is required.

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CHAPTER 21

Macadamia Flours: Nutritious Ingredients for Baked Goods

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

EAA Essential amino acid
MUFA Monounsaturated fatty acid
SFA Saturated fatty acid
wb Wet basis

INTRODUCTION

Macadamia is a nutritious tree nut indigenous to Australia, originating from the coastal rain forests in Queensland and New South Wales. Among the existing species, *Macadamia integrifolia*, or the smooth-shell macadamia, is commercially grown for harvesting edible nuts (Stephenson, 2005). As shown in Table 21.1, the top three producers of macadamia nuts are

Flour and Breads and their Fortification in Health and Disease Prevention. DOI: 10.1016/B978-0-12-380886-8.10021-2

TABLE 21.1 Annual Production of Macadamia Nut-in-Shell and Kernel in Selected Countries[a]

	Production of Nut-in-Shell (Metric Tons)			Estimated Production of Kernel (Metric Tons)				
Year	Australia	United States (Hawaii)	South Africa	Australia	United States (Hawaii)	South Africa	Others	Total
2004	43,700	24,040	17,785	12,600	4,750	3,063	4,750	25,163
2005	35,500	25,628	14,540	10,000	5,200	4,205	6,085	25,490
2006	43,900	24,494	18,625	12,200	5,500	4,480	5,860	28,040
2007	41,800	26,308	20,287	11,600	3,750	4,902	6,348	26,600
2008	35,000	18,597	23,112	10,500	3,750	5,600	6,273	26,123

Source: Data from Australian Macadamia Society (2009), Bekker and Lee (2008), and Landgraf *et al*. (2009).
[a]*From 2004 to 2008, annual production of macadamia in both forms tended to decrease for Australia and the United States, whereas an increasing trend is shown for South Africa.*

Australia, the United States, and South Africa (Australian Macadamia Society, 2009; Bekker and Lee, 2008; Landgraf *et al*., 2009). The total production of these three countries accounts for more than 70% of the global production. The other macadamia producers are located in southern Africa, including Kenya and Malawi, as well as South and Central America, such as Guatemala, Brazil, and Costa Rica (Bekker and Lee, 2008). The United States is not only one of the world's leading macadamia producers but also the largest market for the nuts. The import volume of macadamia nuts in shelled or unshelled, blanched, pickled, and other forms was 7337 metric tons in 2008 (Landgraf *et al*., 2009). In comparison with other tree nuts, macadamia was ranked eighth in terms of the global production volume during the 2006 and 2007 crop years, whereas the first to fifth were almond, hazelnut, cashew, walnut, and pistachio, respectively (Alasalvar and Shahidi, 2008).

Macadamia kernels provide a high amount of calories because approximately 70% of the kernel weight is lipids. Nevertheless, the lipids are rich in monounsaturated fatty acids (MUFAs), which may help reduce serum cholesterol and thus lower the risk of cardiovascular disease (Munro and Garg, 2008). In addition, macadamia kernels are good sources of proteins, dietary fibers, vitamins, and minerals. Defatted or reduced-fat macadamia flours obtained as by-products of macadamia oil production can be nutrient-rich ingredients for foods and beverages.

This chapter provides a review of the production and nutritional quality of macadamia nuts and flours. Details on functional aspects as well as possible applications of macadamia flours in food products are also provided.

PRODUCTION OF MACADAMIA NUTS, FLOURS, AND OTHER MACADAMIA-DERIVED PRODUCTS

Overall processing steps

Mature macadamia nuts consist of 69% by weight of outer green husk or pericarp (2–4 mm thick) and inner brown shell or testa (2 or 3 mm thick) covering the remaining 31% of round kernel (Axtell and Fairman, 1992; Munro and Garg, 2008). After harvesting, dehusking should be done within 24 h to retard respiratory heat generation, microbial growth, and other biochemical reactions related to quality deterioration (Munro and Garg, 2008). The husk waste can be processed to mulch. The resulting nut-in-shells still have a high moisture content—up to 30% on a wet basis (wb). Therefore, drying is the next crucial step for extending shelf life and increasing kernel yield after cracking. To avoid browning of the kernel core, especially after roasting, incremental drying has been commercially adopted. Wet-in-shell nuts are air-dried in the shade, bins, or aerated silos for up to 4 weeks to reduce the moisture content to 10–15% wb. The nuts are then moved to bin- or silo-type hot air dryers operated under multiple drying temperatures (40–60°C).

TABLE 21.2 Grading and Application of Macadamia Kernel[a]

Grading of Kernel	Lipid Content (% Wet Basis)	Criteria	Application
Grade I	>75	Float in water bath (specific gravity 1.00)	Edible nuts
Grade II	65–75	Float in saline bath (specific gravity 1.024)	Edible nuts
Grade III	45–65	Float in saline bath (specific gravity 1.150)	Raw material for oil extraction

Source: Data from Axtell and Fairman (1992) and Macfarlane and Harris (1981).
[a]*Grading is based on specific gravities of macadamia kernels. Higher grade kernels have lower specific gravity due to greater oil content.*

This process may require up to 6 days in order to acquire approximately 3.0% wb moisture of the nut-in-shells or 1.5% wb moisture of the kernels (Munro and Garg, 2008; Silva *et al.*, 2005; Walton and Wallace, 2008). A major disadvantage of this drying process is the long operating time required. Alternative drying methods have thus been developed to shorten the drying time. These methods include combined hot air and microwave drying (Silva *et al.*, 2005) and hybrid heat pump–hot air drying (Borompichaichartkul *et al.*, 2009). The dry-in-shell nuts are further cracked manually or using machines with rotating rubber or steel rollers (Axtell and Fairman, 1992). The shell may be used as a fuel source, as filler in the plastic industry, or processed to activated carbon. Whole kernels are graded via sink–float separation technique based on their specific gravity. Examples of grading procedure and product characteristics are listed in Table 21.2. Grade I and II kernels may be further roasted and seasoned to produce snacks. Lower grade or broken kernels may be used as an ingredient in confectionery, desserts, and baked goods or even used for oil and paste production. Due to the kernels' high lipid content, mechanical extraction of the oil using expeller or screw press can be commercially applied. The refined, cold-pressed oil can be used for food and cosmetic purposes. The pressed cake is normally ground into meal and used as a protein supplement in animal feed (Axtell and Fairman, 1992; Munro and Garg, 2008). Pulverization of full-fat macadamia kernels results in nut paste, which can be used as an ingredient in nut spreads, desserts, baked goods, or even savory sauces. The flavor and aroma of the paste can be enhanced by using the roasted kernels as raw materials. The overall processing of macadamia is depicted in Figure 21.1.

Production of macadamia flours

Studies have shown that macadamia oil cake, which may be used alone or mixed with soybean meal, can be an efficient protein source in the feed for cattle and fish without adverse effects (Acheampong-Boateng *et al.*, 2008; Balogun and Fagbenro, 1995). This macadamia by-product may also be a source of nutrients for humans, with an insignificant amount of anti-nutrients. Production of flour from macadamia oil cake or meal is simple. Further drying and defatting may not be required because the oil cake has less than 10% moisture and approximately 13% lipid content (Macfarlane and Harris, 1981). The remaining lipid content in this low-fat macadamia flour is comparable with that found in full-fat soy flour (approximately 20% lipid content) (U.S. Department of Agriculture (USDA), 2009). However, removal of lipids from the flour may provide several benefits, including improvement of some functional properties of the flour (Jitngarmkusol *et al.*, 2008) and alleviation of rancid flavor development in the flour during storage.

NUTRIENT COMPOSITION OF MACADAMIA NUTS AND FLOURS

Overall nutrient composition

The amount of nutrients in macadamia kernels may vary according to their cultivars, level of maturity upon harvesting, and cultivating location and conditions (Munro and Garg, 2008).

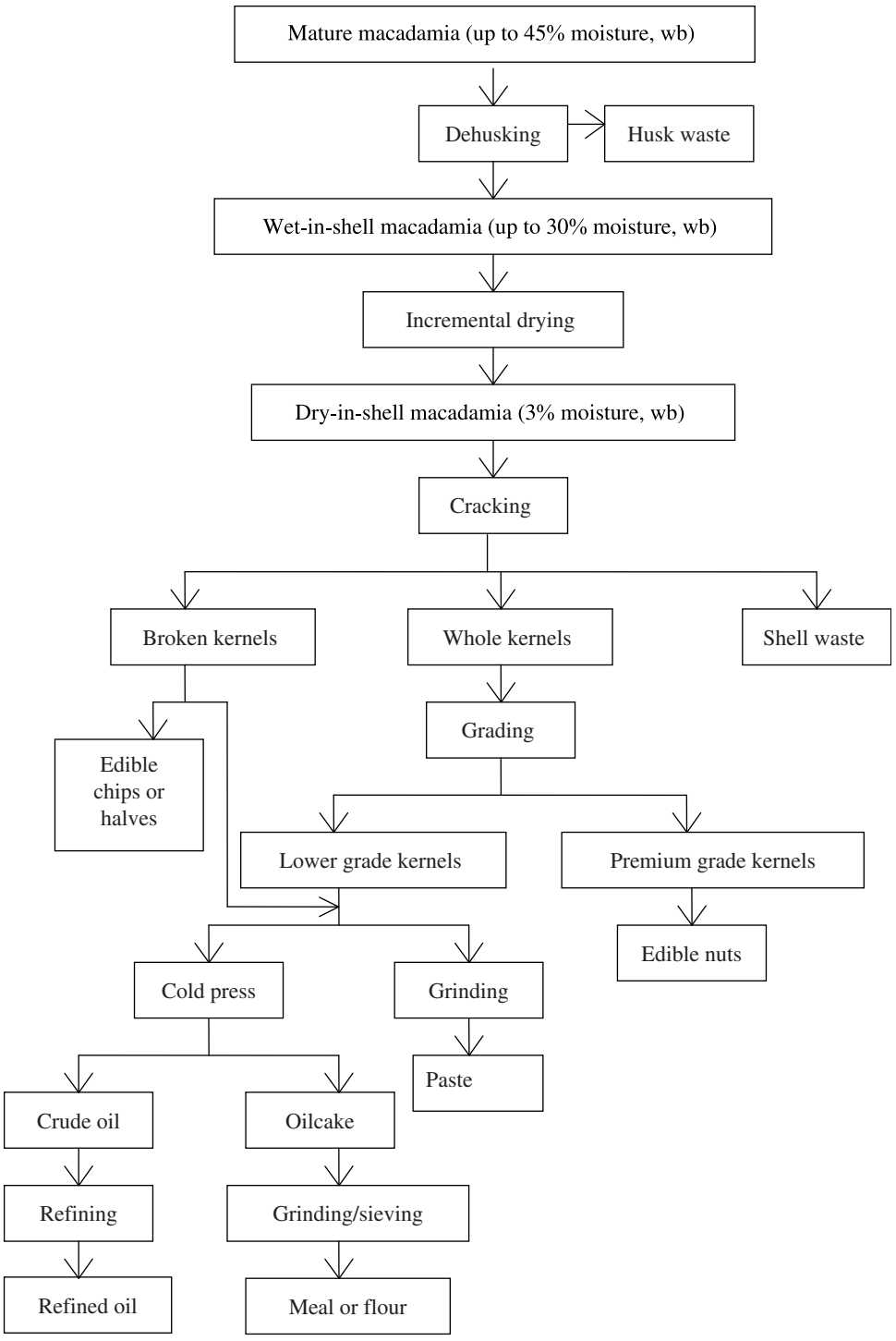

FIGURE 21.1
Overall processing of macadamia. Critical steps of macadamia processing include incremental drying of the nut-in-shell, cracking, and grading. Premium-grade macadamia kernels are main products, whereas macadamia flours are by-products of the macadamia oil production.

Sathe *et al.* (2008) performed an extensive review of the chemical composition of macadamia kernels. According to the review, moisture, lipid, protein, ash, and sugar contents in the edible nuts are 1.4–2.1, 66.2–75.8, 7.9–8.4, 1.1–1.2, and 1.4–4.6%, respectively. Munro and Garg (2008) stated that dietary fiber content of macadamia kernels cultivated in Australia (6.4%) is

TABLE 21.3 Nutrient Composition of Full-Fat and Defatted Nuts[a]

	Macadamia		Almond		Hazelnut		Cashew	
Composition (g/100 g)	**Full Fat**	**Defatted**	**Full Fat**	**Defatted**	**Full Fat**	**Defatted**	**Full Fat**	**Defatted**
Moisture	1.36	5.61	4.70	9.29	5.31	13.53	5.20	9.26
Protein	7.91	32.65	21.22	41.95	14.95	38.09	18.22	32.45
Essential amino acids	4.02	16.59	8.67	17.14	6.95	17.71	9.15	16.30
Lipids	75.77	—	49.42	—	60.75	—	43.85	—
Saturated fatty acids	12.06	—	3.73	—	4.46	—	7.78	—
Monounsaturated fatty acids	58.88	—	30.89	—	45.65	—	23.80	—
Polyunsaturated fatty acids	1.50	—	12.07	—	7.92	—	7.85	—
Carbohydrate	13.82	57.04	21.67	42.84	16.70	42.55	30.19	53.77
Sugars	4.57	18.86	3.89	7.69	4.34	11.06	5.91	10.53
Dietary fiber	8.60	35.49	12.20	24.12	9.70	24.71	3.30	5.88
Starch	1.05	4.33	0.74	1.46	0.48	1.22	23.49	41.83
Ash	1.14	4.70	2.99	5.91	2.29	5.83	2.54	4.52
Calcium	0.09	0.37	0.26	0.51	0.11	0.28	0.04	0.07
Magnesium	0.13	0.54	0.27	0.53	0.16	0.41	0.29	0.52
Phosphorus	0.19	0.78	0.48	0.95	0.29	0.74	0.59	1.05
Potassium	0.37	1.53	0.71	1.40	0.68	1.73	0.66	1.18

Source: Data from USDA (2009).
[a]*All data are from raw (unroasted) nuts. Data of defatted nuts are estimated from those of the full-fat nuts, recalculated on defatted basis. Essential amino acids consist of tryptophan, threonine, isoleucine, leucine, lysine, methionine, phenylalanine, valine, arginine, and histidine. Carbohydrate is calculated by difference.*

slightly lower than that of the kernels cultivated in the United States (8.6%). Nevertheless, nutrient composition of the U.S. macadamia kernels and other nuts reported by the USDA (2009) is used as the representative data set for the following discussion.

Nutrient content of whole and defatted macadamia nuts, in comparison with those of the top three nuts consumed on a global basis, is shown in Table 21.3. Whole macadamia kernel has the highest lipid content but provides the lowest amount of proteins and carbohydrates. However, protein and carbohydrate contents of defatted macadamias are comparable to those of the other defatted nuts. Note that the proximate composition of the defatted nuts in Table 21.3 is estimated from the nutrient content of the whole nuts on a fat-free basis. These values also represent the estimated chemical composition of the flours from defatted nuts. In the case of macadamia, the speculated data for the defatted nuts are in accordance with the values determined from the defatted flours in a previous study. Jitngarmkusol *et al.* (2008) reported the proximate composition of totally defatted flours (0.5–0.9% fat, wb) from three macadamia cultivars grown in northern Thailand. Protein, carbohydrate, and ash contents of those defatted flours are 30.00–32.85, 45.66–51.84, and 4.38–5.53%, wb, respectively.

Proteins

Defatted macadamia nuts and flours can be a potential source of proteins in human diets. The quality of the macadamia proteins, in terms of essential amino acid (EAA) content, is also comparable to that of the other nut proteins (see Table 21.3). EAAs including histidine and arginine account for 50% of the total macadamia proteins, which is a similar percentage to that found in cashew nut proteins (50%) but slightly higher than those in almond (41%) and hazelnut proteins (46%). The main EAA present in all of the four nuts is arginine (23–35% of the total EAAs). However, tree nut proteins, similar to other plant proteins, are inferior to animal proteins because they are incomplete proteins. Lysine is the first limiting amino acid in macadamias, whereas the first limiting amino acid in almonds, hazelnuts, cashew nuts, and most of the tree nuts is tryptophan (Sathe *et al.*, 2008; USDA, 2009).

Carbohydrates

According to the carbohydrate composition, dietary fiber is the major form of carbohydrates found in whole and defatted macadamias, almonds, and hazelnuts, whereas starch is the main carbohydrate in both forms of cashew nuts (see Table 21.3). Therefore, defatted macadamia flours are also good sources of dietary fibers. Because this type of macadamia flour contains approximately 3.4% wb of crude fiber (Jitngarmkusol *et al.*, 2008), soluble fiber is possibly present as the major dietary fiber in the flours. Sugar is another category of carbohydrates found in tree nuts. More than 90% of the total sugars in macadamias as well as the other three nuts is sucrose. The sugar content in macadamia kernels may affect the quality of the kernels, and thus flours, in terms of color development during drying and the roasting process. Wall and Gentry (2007) indicated that macadamia kernels with higher sucrose and reducing sugar contents are prone to greater browning development, particularly after roasting. Hydrolysis of sucrose can occur during thermal processing, resulting in an increased amount of reducing sugars available for the Maillard reaction. Variation in sugar content of macadamia kernels may be caused by the differences in cultivars and levels of maturity. For instance, immature kernels have higher sucrose and reducing sugar contents; thus, they are more likely to become defected brown kernels after roasting.

Lipids

If macadamia flours are partially defatted, residual lipids also enhance the nutritional qualities of the resulting flours. Macadamia oil contains the greatest amount of MUFAs (81%) compared to the MUFAs in almond oil (66%), hazelnut oil (79%), and cashew nut oil (60%). The major MUFA in all of the nuts listed in Table 21.3 is oleic acid. This fatty acid accounts for 99% of the total MUFA content in almonds, hazelnuts, and cashew nuts. The composition of MUFAs in macadamias, however, is slightly different. The nuts contain 74% oleic acid and 22% palmitoleic acid in the MUFA fraction. Regular consumption of MUFA-rich macadamia kernels or its oil has been shown to reduce serum total and low-density lipoprotein cholesterol; however, its effects on serum triglyceride and high-density lipoprotein cholesterol are still inconclusive in clinical studies (Munro and Garg, 2008). Palmitic acid and linoleic acid are the major saturated and polyunsaturated fatty acids, respectively, in the four types of tree nuts. Saturated fatty acid (SFA) fraction in macadamia oil (17%) is higher than that found in almond and hazelnut oil (8%) but slightly lower than the SFA fraction in cashew nut oil (20%). On the contrary, macadamia oil contains the smallest proportion of polyunsaturated fatty acids (2%) in comparison to that in the other three nuts (14−26%) (USDA, 2009).

Micronutrients

Significant amounts of micronutrients are also present in defatted macadamia nuts and flours. Ash or total mineral content in all defatted nuts shown in Table 21.3 is similar. Potassium, phosphorus, magnesium, and calcium are major minerals found in tree nuts. Defatted macadamia nuts and flours contain moderate amounts of these four minerals in comparison with the other three nuts. With regard to vitamins, macadamias, almonds, hazelnuts, and cashew nuts are good sources of thiamine, tocopherols, folate, and phylloquinone, respectively (USDA, 2009). Phenolic compounds are also present in macadamias and other tree nuts. Wu *et al.* (2004) reported the total phenolic content of macadamias, almonds, hazelnuts, and cashew nuts as 1.56, 4.18, 8.53, and 2.74 mg of gallic acid equivalent per gram of the whole nut, respectively. The authors also determined oxygen radical scavenging capacity of lipophilic and hydrophilic extracts of the tree nut samples. Greater antioxidant capacity has been reported for the hydrophilic extracts of the nuts. Defatted macadamia nuts and flours may thus contain significant amount of hydrophilic antioxidants.

FUNCTIONAL PROPERTIES OF MACADAMIA FLOURS AND PROTEINS

Macadamia flours

Functional properties of flours, including water and oil absorption capacities, emulsification/stabilization, and foam formation, arise from interactions between their chemical composition and other food components. Proteins and carbohydrates are key substances because they contain both hydrophilic and hydrophobic portions, as well as charged functional groups. Variation in qualitative and quantitative aspects of these macromolecules may contribute to different functional properties of the flours. Because functional properties of food ingredients greatly influence the overall qualities of the food products, data on these aspects are required in order to effectively use the flours. Jitngarmkusol *et al.* (2008) determined the functional properties of partially defatted and totally defatted macadamia flours prepared from three macadamia cultivars grown in northern Thailand. Overall results from this study are shown in Table 21.4. Greater removal of lipids from the flours results in enhanced water and oil absorption capacities, as well as foaming capacity. The authors proposed that after extraction of the total lipids, proteins and carbohydrates of the macadamia flours may become more soluble and may interact more with the surrounding water or oil. Foaming stability of all macadamia flours is inversely related to foaming capacity of the flours. However, alteration in chemical composition after complete removal of lipids apparently does not affect emulsion activity and emulsion stability of the flours. Variation in the functional properties of the flours from different cultivars has been proposed to be related to the qualitative and quantitative differences in their chemical composition, especially proteins and carbohydrates.

Macadamia proteins

Because macadamia proteins play an important role in the functional properties of the flours, research on the functional properties of the isolated proteins under different conditions may provide insight into the functional properties, and thus the application, of

TABLE 21.4 Chemical Composition and Functional Properties of the Partially and Totally Defatted Macadamia Flours[a]

Chemical Composition/ Functional Property	Macadamia Flour					
	Partially Defatted			Totally Defatted		
	HAES 741	HAES 344	HAES 800	HAES 741	HAES 344	HAES 800
Lipid (%)	12.15 ± 0.22	12.80 ± 0.29	14.90 ± 0.33	1.03 ± 0.01	0.58 ± 0.01	0.61 ± 0.01
Protein (%)	30.96 ± 0.12	30.40 ± 0.21	31.92 ± 0.17	36.45 ± 0.29	35.32 ± 0.78	33.12 ± 0.22
Carbohydrate (%)	49.29 ± 0.33	49.94 ± 0.79	46.49 ± 0.68	52.23 ± 0.21	55.74 ± 0.80	57.09 ± 0.18
Water absorption capacity (g water/ g dry flour)	5.59 ± 0.35	5.40 ± 0.07	3.68 ± 0.16	6.72 ± 0.14	4.71 ± 0.17	4.48 ± 0.09
Oil absorption capacity (g oil/g dry flour)	3.39 ± 0.04	3.16 ± 0.06	3.05 ± 0.09	4.40 ± 0.79	4.93 ± 0.06	4.65 ± 0.17
Emulsion activity (%)	56.21 ± 1.08	51.94 ± 0.60	50.99 ± 1.53	50.81 ± 0.71	50.47 ± 0.23	49.05 ± 2.79
Emulsion stability (%)	51.68 ± 1.10	53.22 ± 0.59	50.44 ± 2.44	54.20 ± 2.03	53.52 ± 0.32	54.26 ± 0.73
Foaming capacity (%)	22.67 ± 0.58	31.00 ± 1.00	33.67 ± 2.52	126.00 ± 3.46	62.33 ± 3.06	65.67 ± 3.21
Foaming stability (%)	91.85 ± 0.78	86.52 ± 1.11	84.07 ± 2.76	56.27 ± 2.00	73.53 ± 1.77	75.07 ± 1.28

HAES, Hawaii Agricultural Experiment Station, a systematic nomenclature of macadamia.
Source: Data from Jitngarmkusol *et al.* (2008).
[a]*Lipid removal improves water and oil absorption capacities and foaming capacity of macadamia flours. Data are shown as mean ± SD of triplicate analyses and calculated on a dry basis.*

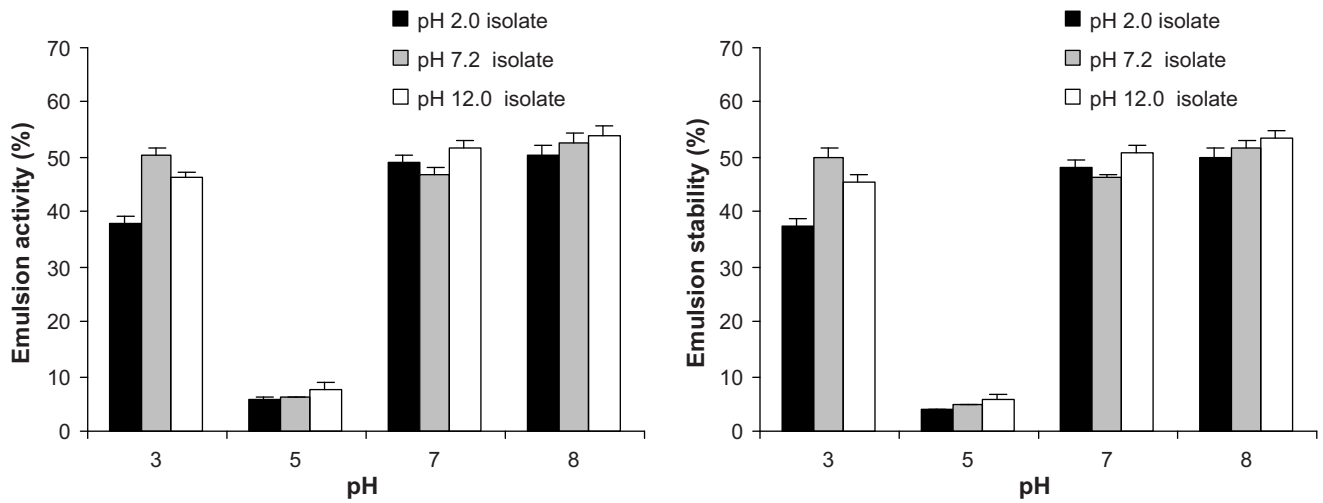

FIGURE 21.2

Emulsion activity and stability of macadamia protein isolates at different pH. Lowest emulsion activity and stability of macadamia protein isolates are evidenced at pH 5.0, the isoelectric pH, due to limited protein solubility. *Source: Adapted from Bora, P. S., and Ribeiro, D. (2004). Note: Influence of pH on the extraction yield and functional properties of macadamia (Macadamia integrofolia) protein isolates.* Food Science and Technology International, *10(4), 263–267.*

the flours in food products. Bora and Ribeiro (2004) determined the effects of extraction pH on yield and functional properties of macadamia protein isolates. Macadamia proteins were extracted three times from the defatted flours by solubilizing the proteins at pH 2.0, 7.2, and 12.0. The soluble proteins at each extracting pH were isoelectrically precipitated at pH 5.0. The results show that 83% of the macadamia proteins can be extracted at pH 7.2 and 12.0, providing 69% yield of the pH 5.0 precipitated proteins. However, at pH 2.0, only 52% of the proteins are extracted; thus, the lowest yield of the precipitated proteins (34%) is obtained. Water and oil absorption capacities of the proteins isolated at pH 2.0 (1.0 ml water or oil/g protein) are slightly lower than those of the remaining two isolates (approximately 1.6 ml water/g protein and approximately 1.2 ml oil/g protein). The pH-dependent emulsion activity and stability of the three isolates have been reported (Figure 21.2). Both properties of all three isolates are minimal at pH 5.0, the condition at which the proteins are least soluble. Gradual increase in these emulsion-related properties is evidenced above and below this isoelectric pH. The results from this study emphasize the importance of pH of the food matrix on functional properties of the isolated macadamia proteins and also the flours. Utilization of macadamia proteins and flours at acidic pH, especially at approximately pH 5.0, may be limited due to reduced protein solubility and inferior functional properties.

POSSIBLE APPLICATIONS OF MACADAMIA FLOUR IN BAKED GOODS

Nutritional aspects

Due to their high protein and dietary fiber contents, low-fat or defatted macadamia flours may be applied as additional protein and dietary fiber sources in food products, including baked goods. However, the limited amount of lysine is a crucial problem in utilizing macadamia flours as potential sources of proteins. Fortification of lysine content may be obtained by using the combination of macadamia flours with other types of lysine-rich flours, such as other tree nut flours and soy flours. The resulting composite flours may be used to replace wheat flours in bakery products. For instance, specialty breads with

enhanced proteins and dietary fibers may be formulated from the composite flours, vital wheat gluten, and lipid emulsifier. Additional studies are warranted to determine appropriate formulas of the macadamia-containing composite flours as well as to evaluate their applications in baked goods.

Functional aspects

Apart from nutrient fortification, functionality-related applications of macadamia flours can also be considered. Despite the difference in protein content, water absorption (or holding) capacity of partially and totally defatted macadamia flours (4–7 g/g dry flours; see Table 21.4) is comparable to those of soy flours, soy protein concentrates, and soy protein isolates (3–6 g/g solids) (Boyacioglu, 2006). Therefore, similarly to soy flours and soy proteins, small amounts of macadamia flours may be used to enhance moisture retention in breads and to help prolong freshness of the products. Water and oil absorption capacities, together with emulsification and foaming properties of macadamia flours, may aid in improving cake qualities. In the case of the defatted soy flours containing approximately 47% protein (USDA, 2009), adding 3–6% of the flours to cake batter results in smoother texture of well-emulsified batters, more even distribution of air cells, as well as softer crumb (Endres, 2001). Partial or total substitution of the defatted soy flours with macadamia flours in cake batter may be applicable. With oil absorption capacity of the macadamia flours, flavor entrapment within cakes, cookies, and other flavor-rich baked goods may be enhanced. Another possible use of macadamia flours, or in the form of combined macadamia–soy flours, is as a milk and/or egg replacer in baked goods. The appropriate amount of macadamia flours in bakery formula depends on the type of product. A higher amount of macadamia flours may be added to chemically leavened products rather than yeasted products, and likewise for soy flours (Amendola and Rees, 2003). Although a number of in-house formulations have been developed, technical papers on the application of macadamia flours in bakery products are scarce. More research is needed to verify these presumable applications.

TECHNOLOGICAL ISSUES

According to the information on nutritional and functional properties of low-fat and defatted macadamia flours, these tree nut flours are promising ingredients in foods, particularly bakery products. However, commercial production of macadamia flours is still limited. Scientific research on the application of these flours in baked goods as well as other foods and beverages is still scant. Current information and the suggestions in this chapter may help expand the possibility to create well-designed experiments on the utilization of macadamia flours and may eventually lead to an increase in the production volume of these value-added products from macadamia.

ADVERSE REACTIONS

Allergic reactions to macadamia nuts have been reported. Such adverse food reactions include, although not life threatening, gastrointestinal hypersensitivity, respiratory symptoms, and skin manifestations. However, a few cases of anaphylaxis, which may cause death, have also been reported (Lerch *et al.*, 2005). Hence, to protect consumers, utilization of macadamia nuts and flours must be clearly identified on food labels.

SUMMARY POINTS

- Macadamia is a tree nut indigenous to Australia, whose kernels can be processed into a wide variety of products, such as nut snacks, oil, and flours.

- Macadamia and its products are highly nutritious. Full-fat macadamia kernels have high amounts of MUFAs, whereas defatted macadamia flours are good sources of proteins and dietary fibers.
- A limited amount of lysine is a significant problem in using macadamia flours as potential protein sources. Mixtures of macadamia flours and other lysine-rich flours are thus recommended as ingredients for protein fortification in baked goods.
- A higher degree of lipid extraction improves functional properties, including water and oil absorption capacities and foaming capacity, of macadamia flours.
- At a pH near the isoelectric point of macadamia proteins (5.0), protein solubility and functional properties may be compromised.
- Due to their outstanding functional properties, macadamia flours may be used to replace egg and dairy ingredients, as well as to enhance the texture and flavor of baked goods.

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CHAPTER 22

Banana and Mango Flours

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

AS Available starch
DF Dietary fiber
EPP Extractable polyphenols
GI Glycemic index
HI Hydrolysis index
IDF Insoluble dietary fiber
RDS Rapidly digestible starch
RS Resistant starch
SDF Soluble dietary fiber
SDS Slowly digestible starch
TDF Total dietary fiber

INTRODUCTION

During approximately the past decade, nutrition science has studied the relationship between dietary habits and disease risk, and the concept that the diet has a significant role in the modulation of various functions in the body has been supported. This implies that the diet and/or its components could contribute to an improved state of well-being, a reduction of risks related to certain diseases, and even an improvement in quality of life. It is generally accepted that plant-derived foods, such as wine, fruits, nuts, vegetables, grains, legumes, and

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spices, have some beneficial effects on human health, particularly on age-related diseases such as cardiovascular and neurodegenerative diseases, type II diabetes, and several types of cancer. As a result, there is currently major interest among consumers and members of the food industry regarding products that can promote health and well-being. These foods have been generically termed functional foods (Table 22.1).

Important items in this review are the nutraceutical ingredients of fruits, in which polyphenols (flavonoids, anthocyanins, tannins, etc.) that show antioxidant capacity and indigestible carbohydrates in the fraction called "dietary fibers" are present. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and other associated substances. Dietary fibers have beneficial physiological effects, including laxation, blood cholesterol attenuation, and blood glucose attenuation (Anonymous, 2000).

The presence of significant amounts of bioactive compounds, such as flavonoids and carotenoids, in dietary fiber from fruit indicates considerable nutritional value. The food industry is searching for new sources of dietary fiber to use as ingredients. This is a recent trend within the industry. The most widespread consumed dietary fiber products are those derived from whole grain. However, during approximately the past decade, high dietary fiber materials from fruits (citrus, apple, and others) have been steadily introduced in the occidental world markets.

In most cases, the dietary fiber present in some fruits, including unripe fruits, has better nutritional quality than that in whole grain. These fruits have significant amounts of associated bioactive compounds (flavonoids, carotenoids, etc.) and a more balanced composition (higher overall fiber content, greater soluble dietary fiber (SDF)/insoluble dietary fiber (IDF) ratio, greater water- and fat-holding capacities, lower metabolic energy value, and higher phytic acid content) (Jiménez-Escrig *et al.*, 2001). Resistant starch (RS) is a dietary fiber component with important physiological effects.

TABLE 22.1 Examples of Functional Foods and Their Possible Effects on Human Health

	Functional Food	Functional Ingredients or Bioactive Compounds	Potential Health Benefit
FDA-approved health claims[a]	Whole oat products	β-Glucans	Lower cholesterol levels
	Psyllium	Soluble fiber	
	Special fortified margarine or salad dressing	Plant stanol or sterol esters	
	Sugarless chewing gums and candies	Sugars alcohols	Do not promote tooth decay
Do not have FDA-approved health claims[b]	Fatty fish	Ω-3 fatty acids	Reduce risk of heart diseases
	Cranberry juices	Proanthocyanidins	Reduce urinary tract infections
	Chocolate	Flavonoids	Reduce low-density lipoprotein cholesterol
	Garlic	Organosulfur compounds	Lower cholesterol levels
	Green tea	Catechins	Reduce risk of some types cancer
	Cruciferous	Glucosinolates, indoles	
	Tomatoes and tomato products	Lycopene	
	Dark green leafy vegetables	Lutein	Reduce risk of age-related macular degeneration
	Fermented dairy	Probiotics	Support gastrointestinal tract health

[a]*The functional foods described have all been tested in clinical trials and all carry Food and Drug Administration (FDA)-approved health claims.*
[b]*The foods described do not have FDA-approved health claims. However, their potential health benefits are currently being investigated.*

AGRONOMIC CHARACTERISTICS

Banana

Bananas (*Musa paradisiaca* spp.) are considered among the first fruits to be harvested by primitive agricultures and have been present in diverse cultures and civilizations for centuries. Bananas originated in Southeast Asia, including northern India, Cambodia, Sumatra, Java, the Philippines, and Taiwan. In the sixteenth century, the banana cultivar was introduced in the islands of Santo Domingo and Cuba. At the end of the nineteenth century, the first commercial plantations were established in Jamaica, and thereafter in diverse countries of Central America and Mexico.

Banana is a climateric fruit cultivated in many countries, primarily those located in the tropical and subtropical regions (approximately 120–130 countries), and it represents a major staple. Annual world production of banana is approximately 104 millions tons. The main producers of banana are Brazil, China, Ecuador, the Philippines, and India. The leading exporters of banana are Ecuador, Colombia, Costa Rica, and the Philippines.

Edible *Musa* plants are classified into AA, AB, BB, AAA, AAB, ABB, AAAA, AAAB, and ABBB genomic groups. In general, dessert banana cultivars are AA or AAA, with this last group including almost all bananas sold. Cooking bananas, also named plantains, are predominantly AAB, ABB, or BBB. The great biodiversity of banana plants provides potential for diverse uses and applications (Aurore *et al.*, 2009).

Unripe banana contains large amounts of starch, cellulose, hemicellulose, and lignin in the pulp. The nutritional/nutraceutical potential of unripe banana starch and fiber has been reported (Langkilde *et al.*, 2002).

Mango

The mango (*Mangifera indica* L.) is the most important member of the Anarcadiaceae family. This fruit is native to southern Asia, especially Burma and eastern India. It spread early into Malaysia, eastern Asia, and eastern Africa.

The genus *Mangifera* includes approximately 50 species, but only 3 or 4 produce edible fruits. The fruit typically called "mango" weighs between 150 g and 2 kg, has an ovoid–oblong shape, and is 4–25 cm long and 1.5–10 cm thick.

The mango tree has adapted to many tropic and subtropic regions of the world. Conversely, its fruit is fragile, and significant postharvest wastage occurs in producing countries due to insufficiently established practices of handling, transporting, storing, and ripening. Ripe fruit is consumed raw as a dessert fruit, whereas the rest of production is processed into different products such as nectar, juice powder, and canned mango slices in syrup chutney (Wu *et al.*, 1993).

Mango fruits are harvested at a mature green stage and stored for ripening. When fruit is fully grown and ready for picking, the stem snaps easily. Depending on the variety and environmental conditions, the fruit takes 6–10 days to ripen under ambient conditions (temperature and relative humidity), and it becomes overripe and spoiled within 15 days after harvest so that the fruit must be eliminated and not consumed.

CHEMICAL COMPOSITION

Banana

The chemical composition of banana varieties depends of the ripening state; however, agronomic traits, the type of soil, and climatic conditions alter the major and minor components of the fruit. In one study, lower moisture content was found in the unripe pulp of banana (69%) than in ripe banana pulp (74%), the carbohydrate content was higher in the former sample (28.7%) than in ripe banana (21.8%), but an inverse pattern was

obtained for fiber. The unripe pulp had 2.0% and the ripe pulp had 0.5%; this pattern might be related to the higher pectin levels present in the ripe state of the pulp (Aurore *et al.*, 2009).

Starch is the main carbohydrate in the unripe banana (73−77%, dry basis), and it is replaced by simple sugars such as sucrose, glucose, and fructose when the fruit begins the maturing process. Starch present in diverse food crops such as unripe banana is consumed after cooking and preparation of foods in which the starch is gelatinized. It was demonstrated that a fraction of the starch consumed in the diet escapes digestion and absorption in the small intestine of healthy people and is fermented in the large intestine with the production of short-chain fatty acids. This fraction was called RS, and its consumption has been associated with reduction of the glycemic index, low absorption of cholesterol, and prevention of colon cancer. RS was found in unripe banana; in the mature stage, this fruit is considered to have the highest RS content of all natural products (Faisant *et al.*, 1995).

Dietary fiber (DF) is a nutritional component also present in the unripe banana. From a strict nutritional standpoint, DF is not a nutrient because it does not directly contribute to the basic metabolic processes of the body. The role of DF is more physiologic because it stimulates the intestinal peristalsis and intestinal evacuation. As such, DF is not digested in the small intestine; it is fermented in the large intestine and promotes the growth of the beneficial intestinal flora while also binding diverse substances such as cholesterol. The physiological properties of DF are important in the prevention and treatment of obesity, coronary heart disease, colon cancer, and diabetes.

Other compounds in the banana fruit that are associated with the prevention of diverse health problems are the bioactives such as polyphenols that show antioxidant properties due to the ability to trap free radicals that damage the biomolecules and cause cellular aging. The bioactive compounds join to DF and can exert their function in the large intestine, where the level of free radicals is important.

Mango

The main components of mango are water and carbohydrates, with small amounts of DF, protein, lipid, and vitamins. The amounts of these components depend on the variety of mango; for example, the amount of carbohydrate ranges between 90.1 and 93.6%, and the amount of DF ranges between 3.85 and 12.64%. Mango is a good source of vitamin C, with values between 27 and 80 mg/100 g of fresh pulp. In the unripe state, the mango's main carbohydrate is starch, which in the mature fruit is replaced by monosaccharides and disaccharides such as glucose, fructose, and sucrose (Wu *et al.*, 1993). For this reason, the isolation and characterization of mango starch using the pulp has been reported as an alternative source with industrial potential (Bello-Pérez *et al.*, 2005).

The parenchymatous tissues and cell walls are the DF supply for fruits and vegetables. The DF of mango pulp depends on the variety of mango and ripening stage, ranging between 12.64 and 3.85% (Limonta-Carvalho *et al.*, 2004). Due to the high amount of peel produced in diverse industries, mango peel as a DF source has been studied. This DF is a rich source of indigestible polysaccharides, principally IDF. DF content in mango peel ranges between 65 and 71 g/100 g of dry sample, with a total soluble polyphenols level between 44 and 70 mg/g of dry sample (Larrauri *et al.*, 1996). Mango dietary fiber was obtained from the unripe whole fruit (pulp and peel), presenting a DF content of 28.1 g/100 g of dry sample, with a good balance of SDF (14.25%) and IDF (13.8%), which is important from a nutritional standpoint (Vergara-Valencia *et al.*, 2007). The polysaccharide pectin is present in mango and provides firmness; when the fruit is unripe, the pectin concentration is high, and the pectin level decreases during ripening of the tissue.

TABLE 22.2 Commercial End Uses of Mango and Banana

Product	Mango	Banana
Juice	X	—
Concentrate	X	—
Frozen pulp	X	X
Marmalade	X	—
Snacks	X	X
Dried pulp (powder)	X	X
Pieces in syrup	X	—

END USE OF THE FRUITS

Table 22.2 shows the commercial end uses of mango and banana. Both fruits are preferentially consumed as fresh products; however, due to their seasonality and their commercialization in various regions of the world, they are processed in various locations with the objective of year-round availability. Mango is consumed in industrialized products as juice, concentrate pulp, and nectar, whereas banana has minor application in industrialized products—it is found in the market as frozen pulp, snacks, and dried pulp.

UNRIPE FLOUR

The agronomic problems of banana and mango are similar. A significant amount of the cultivates is lost due to pests such as black sigatoga (banana). Inflorescence and vegetative malformation of mango, caused by the fungal pathogen *Fusarium subglutinans*, is one of the most important diseases of this crop, occurring in most mango-growing countries worldwide. In addition, hurricanes, cyclones, and tropical storms cause damage to the fruits during the growing of cultivate. When the fruits reach mature size and physiological maturity, they are cut. Due to insufficiently established practices of handling, transporting, storing, and ripening during the postharvest stage, significant amounts of the fruits are lost.

Ideas to diversify the final use of fruits such as banana and mango include the development of functional ingredients, including those with a low amount of glycemic carbohydrates and with slow digestible starch. Interest in foods with a low amount of glycemic carbohydrates has increased during approximately the past decade. A high intake of food products rich in nondigestible carbohydrates has been related to several physiological and metabolic effects. In the digestive tract, the nondigestible carbohydrates exert a buffering effect that links excess acid in the stomach, increases the fecal bulk, and stimulates intestinal evacuation. In addition, it provides a favorable environment for the growth of the beneficial intestinal flora. Consumption of food products with a high amount of nondigestible carbohydrates such as fibers, particularly highly viscous SDFs, is usually associated with moderate postprandial glycemic responses, a property of importance in the dietetic treatment of diabetes.

Unripe fruits are a rich source of starch. It has been reported that starch-rich products vary in digestibility (Han and BeMiller, 2007). The rate and extent of starch digestion are reflected in the magnitude and duration of the glycemic response. Most starch-rich food products contain a portion of starch that is rapidly digestible (RDS), a portion that is slowly digestible (SDS), and a portion that is resistant to digestion in the small intestine. The nutritional quality of a food is related to its glycemic index (GI). Differences in glycemic and insulinemic responses to dietary starch are directly related to the rate of starch digestion. The benefits of starchy food products that have a high level of SDS have been reported; a product that provides the nutritional benefits of starch (a supply of glucose) but that does not produce the postprandial hyperglycemic and hyperinsulinemic spikes associated with the RDS is most desirable. For example, people with type II diabetes benefit from the consumption of foods with high

amounts of SDS because it does not produce hyperglycemia followed by hypoglycemia; in addition, SDS may prolong satiety. Although not all low-GI foods are rich in SDS, most SDS-rich foods have a low GI (Han and BeMiller, 2007).

Mango flour

Unripe mango flour was prepared with the objective to produce a DF-rich powder. Total starch content in the DF-rich powder was 29.8%. This level is important during the processing of food products with this functional ingredient because it can contribute to the formation of RS, as has been shown in mango starch extrudates (Agustiniano-Osornio *et al.*, 2005). Total dietary fiber (TDF) content in DF-rich powder was 28.1% (Vergara-Valencia *et al.*, 2007), and in mango peels the fiber content was 65−71% (Larrauri *et al.*, 1996). TDF in DF-rich powder could be considered low. This value might be related to its high starch content (29.9%). In some applications as an ingredient, DF-rich powder's starch levels might be of importance, given the additional functional properties imparted by this polysaccharide. DF-rich mango powder presents a balance between SDF (14.3%) and IDF (13.8%). This characteristic might also be important from a nutritional standpoint. Bioactive compounds are considered to be important to good health; in this group, polyphenols are included. The content of extractable polyphenols (EPPs) or total soluble polyphenols in DF-rich mango powder is 16.1 mg/g. EPPs appear to be absorbed in the digestive tract, exerting systemic effects (Saura-Calixto, 1998). Anti-radical efficiency was tested in EPPs, and a value of 15×10^{-3} was obtained, which is considered suitable for antioxidant DF production (Jiménez-Escrig *et al.*, 2001).

Banana flour

Traditionally, bananas are used to prepare regional food products and dishes and sometimes to produce vinegar and spirits. Some varieties or cultivars are used in small rural communities as special diets and for traditional medicine. For example, they may be used for infants, elderly people, and patients with stomach problems (antidiarrheal and intestinal disorders), gout, and arthritis. In some countries of South America (e.g., Colombia and Ecuador), precooked banana flour is used to produce an elaborate regional dish called "empanadas". The use of unripe banana to prepare flour might be advantageous because the fruits with damage on the peel or those that fall as a result of cyclones and hurricanes can be used for this purpose. Diversifying the end uses of banana can be beneficial (economic) for farmers. Unripe banana can be used to produce functional flour because of the high levels of starch and nonstarch polysaccharides (DF) present in the fruit during this stage. It has been reported that unripe banana has the highest resistant starch content (Faisant *et al.*, 1995) and consequently is a good source of indigestible and functional carbohydrates. In addition, the polyphenol content increases the functional character of unripe banana.

Table 22.3 shows the diverse procedures used to prepare banana flour using the pulp. The different flours all have a high starch content and an important amount of DF, but the variety "Prata" has the lowest DF content (1.17%). The procedure and variety used in our laboratory produced unripe banana flour with a high DF level. Precooking of banana produced flour with higher starch content, although DF was not tested. To increase the indigestible carbohydrates of unripe banana flour and decrease the starch content, enzymatic (Rodríguez-Ambriz *et al.*, 2008) and chemical (Aguirre-Cruz *et al.*, 2008) treatments were carried out (see Table 22.3). The enzymatic treatment was produced with α-amylase for 3 h at 70°C after starch gelatinization. The total dietary fiber in this fiber-rich powder increased from 10.4% in the unripe banana flour to 31.8%, whereas the total starch and available starch decreased. The amount of total indigestible fiber was high (approximately 70 g/100 g of the dry fiber-rich powder), indicating that a large amount is not digestible and can be used as a functional ingredient in the preparation of foodstuffs (Rodríguez-Ambriz *et al.*, 2008). A fiber-rich powder was prepared with acid treatment using unripe banana flour that was prepared with the pulp and peel. The unripe banana flour presented a total dietary fiber content of 17.14% and starch level of 73.01%. The diverse

TABLE 22.3 Chemical Composition of Unripe Banana and Mango Flours

Sample	Flour Production	Chemical Composition				Reference
		Protein	Fat	Fiber	Starch	
Green banana "Prata"	Dehydrated	4.73 ± 0.84	0.70 ± 0.03	1.17 ± 0.02	75.20 ± 0.47	Borges *et al*. (2009)
Unripe banana (*Musa paradisiaca*)	Dehydration by freeze-drying	2.92 ± 0.10	0.83 ± 0.01	9.67 ± 0.05	74.65 ± 2.08	Pacheco-Delahaye *et al*. (2008)
	Dehydration by double drum dryer	3.30 ± 0.25	0.5 ± 0.05	9.01 ± 0.19	63.50 ± 0.55	
	Dehydration by irradiation microwave	3.12 ± 0.18	0.17 ± 0.15	9.43 ± 0.20	64.52 ± 0.25	
	Conventional dehydration	3.08 ± 0.08	0.31 ± 0.01	9.37 ± 0.45	74.30 ± 2.32	
Unripe banana (*Musa paradisiaca*)	Peeled and cut sliced (1 cm), rinsed in citric acid solution (0.3%), and dried (50°C) and ground	3.4 ± 0.3	ND	10.4 ± 1.4	76.8 ± 1.0	Rodríguez-Ambriz *et al*. (2008)
	Liquefaction	5.3 ± 0.6	ND	31.8 ± 2.1	52.4 ± 1.1	
Unripe banana (*Musa paradisiaca*)	Cut sliced (1 cm), rinsed in citric acid solution (0.3%), and dried (50°C) and ground	4.03 ± 0.06	3.24 ± 0.03	17.14 ± 0.19	73 ± 0.06	Aguirre-Cruz *et al*. (2008)
	Acid treated (1.6 M HCl, 38°C, 11 days)	ND	ND	60.75 ± 0.25	ND	
Unripe banana (*Musa paradisiaca*)	Peeled and cut sliced (1 cm), rinsed in citric acid solution (0.3%), and dried (50°C) and ground	3.3 ± 0.4	2.7 ± 0.38	14.5 ± 0.46	73.4 ± 0.92	Juarez-García *et al*. (2006)
Unripe cooking bananas	Treatment in water (85°C, 5 min) and dried (65°C, 48 h)	3.77	3.49	ND	87.07	Yomeni *et al*. (2004)
Dwarf Kalapua		2.84	3.29	ND	86.42	
Bluggoe		2.88	1.13	ND	90.19	
Plantain French somber						

Continued

TABLE 22.3 Chemical Composition of Unripe Banana and Mango Flours—continued

Sample	Flour Production	Chemical Composition				Reference
		Protein	Fat	Fiber	Starch	
Green banana	Sliced pulp and extraction buffer (ascorbic acid and EDTA) homogenized, freeze-dried, and sieved (60 mesh); residue washed with ethanol and acetone and then dried at room temperature	2.8 ± 0.13	0.33 ± 0.35	8.95	65.7 ± 2.8	da Mota *et al*. (2000)
Ouro da mata		2.8 ± 0.19	0.78 ± 0.51	7.76	76.5 ± 2.9	
Nanica		2.6 ± 0.20	0.82 ± 0.47	6.28	76.1 ± 3.7	
Nanicao		2.9 ± 0.04	0.47 ± 0.29	8.86	68.2 ± 4.0	
Prata ana		2.5 ± 0.02	0.58 ± 0.48	10.46	72.4 ± 3.6	
Prata comun		2.6 ± 0.06	0.42 ± 0.24	15.56	61.3 ± 6.9	
Mysore		3.3 ± 0.10	0.52 ± 0.28	11.28	64.9 ± 2.5	
Maca		3.1 ± 0.3	1.4 ± 0.2	11.4 ± 1.2	71.3 ± 2.8	Suntharalingam and Ravindran (1993)
Green cooking banana “Alukehel”	Pressure-cooked (15 lb/in., 5 min), slices (0.5 cm) dipped in sodium metabisulfite solution (1% w/v, 5 min) and dried	3.3 ± 0.9	1.3 ± 0.9	12.6 ± 1.4	68.4 ± 4.2	
“Monthan”		4.2 ± 0.11	2.4 ± 0.02	28.0 ± 0.2	29.8 ± 0.06	Vergara-Valencia *et al*. (2007)
Unripe mango	Cut sliced (1 cm) and dried (50°C)	5.2	2.5	34.4	ND	Larrauri *et al*. (1996)
Mango peel	Peels blanched (3 min), wet milled, washed at 95°C (5 min), pressed, dried, and milled (particle size, 15 mm)					

ND, not determined.

acid treatment increased the DF content in the unripe flour from 19.8% to 60%. The authors concluded that this fiber-rich powder could be used in food and medical applications because of the increased consumption of fiber-rich products (Aguirre-Cruz *et al.*, 2008).

USE OF UNRIPE FLOUR

Mango flour

Unripe mango flour has been tested in the preparation of cookies and bread. In these formulations, mango flour was added to substitute the wheat germ used in the control sample. In the cookies, the wheat flour:mango flour ratio was 25:75, and for bread it was 60:40. Both products with added mango flour presented an increase in TDF level. Cookies with mango flour had a TDF of 17.4%, and the control had a TDF of 13.3%. Bread with mango flour showed a TDF of 16.6%, and the control had a TDF of 14.2%. However, depending on the type of product, the increases in SDF and IDS were different.

Bakery products with mango flour presented similar available starch (AS) content. A larger difference in AS was evident between the two breads because AS content in control is approximately 50% more than that of the mango flour-added products, suggesting it can be used as an alternative for products with reduced digestible starch contents. The starch hydrolysis index (HI) of products "as eaten" showed that control bread exhibited a 34.7% hydrolysis at 180 min, which was higher than in cookies with mango flour and its control. Starch HIs and derived pGIs for products containing mango flour were lower than those determined for their respective control samples, indicating that this fiber exerts a significant effect on the rates of digestion and absorption of the starch component of the meals. Bakery products with mango flour concentrate may be an alternative for people with special caloric requirements (Vergara-Valencia *et al.*, 2007).

Banana flour

Unripe banana flour was added to bread in which the wheat flour had been totally replaced. The unripe banana flour bread showed an increase in DF up to 100% higher than that of the control sample. Resistant starch content was also higher (6.7%) in unripe banana flour bread than in the control bread (1.0%). The insoluble indigestible fraction was higher in the bread with unripe banana flour (22.3%) than in the control (12.4%). These results had a beneficial effect on the HI of starch and GI; HI and GI were higher (65.1 and 64.3%, respectively) in the bread with unripe banana flour than in the control bread (81.9 and 78.8%, respectively) (Juarez-Garcia *et al.*, 2006).

In our laboratory, studies have been conducted on the use of unripe banana flour in cookies with whole grains. The control cookies with whole grains of maize, barley, and oat presented a DF content of approximately 11%, which may be higher in the product with unripe banana flour. In addition, tortillas with unripe banana flour had a lower amount of available starch and higher resistant starch content when storage time increased.

The results obtained from the application of unripe banana flour in foodstuffs indicate that people with low caloric requirements may use these kinds of products as dietary aides without restrictions to consumer preferences.

TECHNOLOGICAL ISSUES

Banana and mango flours can be prepared using single procedures in order to minimize production costs. The idea is to design small factories close to plantations where the products that did not meet the quality control to be commercialized as fruit can be used in the process to elaborate flours. The end use of the fruit flours in bakery products has been demonstrated, with functionality and nutritional characteristics in their carbohydrate content. The level of indigestible carbohydrates was increased in products with the fruit flours, decreasing the glycemic

response. In addition, they showed antioxidant capacity. The results obtained from the application of unripe fruit flours in foodstuffs indicate that these kinds of products may be used as dietary aides by people with low caloric requirements without restrictions to consumer preferences.

SUMMARY POINTS

- Flours obtained from unripe fruits such as mango and banana are an important source of indigestible carbohydrates and polyphenol compounds.
- Mango and banana flours can reduce the glycemic response.
- Unripe banana flour can be modified by enzymatic and chemical treatments to increase the amount of indigestible carbohydrates.
- Mango and banana flours can be added to diverse bakery products, and evaluation of their nutraceutical potential is necessary.
- Unripe banana has the highest resistant starch content.
- Bioactive compounds, such as flavonoids and carotenoids, are present in dietary fiber of fruits.

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CHAPTER 23

Use of Potato Flour in Bread and Flat Bread

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

η' Dynamic viscosity
DSC Differential scanning calorimetry
G' Storage modulus
G'' Loss modulus
PPC Potato protein concentrate
tan δ Loss factor
WAI Water absorption index

INTRODUCTION

Potato flour is the oldest commercial processed potato product (Willard and Hix, 1987). It is a highly versatile raw material that can be used in several processed food products. Potato flour

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is produced in large quantities in the United States and several European countries. It is a major processed product in global trade. The Netherlands, Germany, the United States, and Belgium are the top exporting countries and combined exported 0.27 million tons of potato flour in 2007. Potato flour has been associated with the baking of bread for a long time, and it is known to reduce staling and improve toasting properties of bread. Bakers have traditionally used peeled, cooked, and mashed potatoes to impart potato flavor and improve retention of freshness in bread (Willard and Hix, 1987). Potatoes have long been recognized as an excellent yeast food because of the presence of adequate amounts of minerals such as potassium, magnesium, and phosphorus required for vigorous fermentation. Potato constituents have the unique ability to stimulate growth of yeast cells and activate fermentation of sugar. Potato flour is also reported to provide a distinctive flavor, reduce product firming and staling, and assist in the leavening of the product (Pyler, 1982). Flat bread, also known as poor man's bread, is more common than bread in many countries. Flat bread processing is simple, and flat breads are made from flour, water, and salt. Flat breads are normally unleavened, although some leavened flat breads are also made. Flat breads can range from 1 mm to a few centimeters in thickness, and they were known in ancient Egypt. Flat breads are generally made with wheat flour, although flour from corn, rye, rice, or barley is also used. Potato flour is also used in the preparation of flat bread. The lefse (a Scandinavian potato flat bread) is quite popular in Europe. Potato is the major ingredient in potato flat bread, in addition to other flours. For example, rye flour is used in Norwegian and Iceland flat breads, whereas barley and wheat flour are used in Finland flat bread. Potato is used in different forms, such as mashed or flakes or flour, in the preparation of flat bread. In Slovakia, potato bread contains 1–5% boiled potatoes. In countries such as India, Pakistan, Turkey, and Saudi Arabia, wheat flour is used for making flat breads. The addition of potato flour in small quantities to wheat flour has been found to improve the quality of flat breads in India (Singh *et al.*, 2005b), Pakistan (Anjum *et al.*, 2008), and Saudi Arabia (Al-Mane, 1991). In India, flatbread is known as "chapati," "naan," or "paratta."

POTATO FLOUR

Composition

The composition of potato flour generally reflects the composition of the potato tubers from which it is prepared. The composition (average values) of potato varieties from the United Kingdom (Paul and Southgate, 1978), the United States (Treadway *et al.*, 1950), Nigeria (Okorie *et al.*, 2002), and India (Chandra Shekara and Shurpalekar, 1983) is given in Table 23.1. The values given for potato varieties from the United Kingdom and the United States are similar. However, differences were observed in the other two cases. These differences could be due to differences in variety, growing location, environmental conditions during crop growth, and analytical methods used. Furthermore, the table shows that potato is a good source of potassium and ascorbic acid.

Physicochemical properties

The physicochemical properties of flours made from potatoes vary with genotypes and method of preparation (Kamal *et al.*, 2002). Flour from boiled tubers (gelatinized) showed higher water absorption and total and reducing sugars compared to flour prepared from raw tubers (ungelatinized). Pant and Kulshrestha (1995) determined the physical characteristics of potato flour made from six potato cultivars and observed a significant positive correlation between water absorption and particle size index. Singh *et al.* (2003) determined the physicochemical properties of potato flour from three potato cultivars. The amylose content of potato flours ranged from 9.1 to 10.8%. Significant cultivar differences were observed, with Kufri Jyoti flour showing the highest amylose content and Kufri Pukhraj flour the lowest. The water absorption index (WAI) was also higher for Kufri Jyoti flour (6.6) and lowest for Kufri Pukhraj flour (5.6). Potato flour with higher amylose

TABLE 23.1 Composition of Potato Flour (per 100 g)

	Potato Flour			
Moisture (%)	7.6[a]	7.4[b]	10.0[c]	—
Carbohydrate (g)	79.9	79.0	78.4	87.3[d]
Crude fiber (g)	1.6	1.6	2.9	1.3
Crude protein (g)	8.0	7.6	3.9	8.1
Fat (g)	0.8	1.0	1.3	—
Ascorbic acid (mg)	19	—	—	—
Thiamin (mg)	0.4	—	—	—
Riboflavin (mg)	0.1	—	—	—
Niacin (mg)	3.4	—	—	—
Ash (g)	3.7	3.3	3.3	2.5
Calcium (mg)	33	34.3	—	—
Phosphorus (mg)	178	176	—	—
Iron (mg)	17.2	13.9	—	—
Sodium (mg)	—	41.3	—	—
Potassium (mg)	—	1373	—	—

[a]*All data in this column from Paul and Southgate (1978).*
[b]*All data in this column from Treadway* et al. *(1950).*
[c]*All data in this column from Okorie* et al. *(2002).*
[d]*All data in this column from Chandra Shekara and Shurpalekar (1983).*

content showed higher WAI and water solubility index. Singh *et al.* (2005b) determined the physicochemical properties of flours made from six potato cultivars and found bulk density, ash content, and amylose content of 0.77–0.92 g/ml, 2.98–4.08%, and 5.9–8.88%, respectively.

Pasting properties

Pasting properties of the potato flour provide a unique functional fingerprint for each cultivar flour, which embodies structural and molecular components of the native potato tissue. The Rapid Visco Analyzer and Brabender Viscoamylograph are used to measure pasting behavior of flours and also time of gelatinization, pasting temperature, peak viscosity, breakdown, setback, and final viscosity. These properties were identified as potential characteristics for cultivar differentiation. Singh *et al.* (2005a) determined pasting properties of flours prepared from six cultivars and found the pasting temperatures to be approximately 1°C lower than their respective conclusion temperatures of gelatinization measured using differential scanning calorimetry (DSC). They concluded that potatoes with higher sensory mealiness scores resulted in flours having lower transition and pasting temperatures, setback, and peak and final viscosity (Table 23.2). Pasting curves of native and gelatinized flours of three potato cultivars are illustrated in Figure 23.1. Peak, breakdown, and final and setback viscosity of native flours from different potato cultivars were 3020–3660, 140–512, 4164–6237, and 1655–2180 cP, respectively, compared to 140–384, 12–22, 210–588, and 83–226 cP, respectively, for gelatinized flours. The lower values for pasting parameters of gelatinized flours compared to their native flours may be due to the presence of disrupted granules and loss of the molecular organization. Pasting properties of potato flours prepared from two potato cultivars differing in texture—Russet Burbank with a mealy texture and IdoRose with a waxy texture—were studied by Higley *et al.* (2003). It has been shown that potato flour viscosity can be improved through a specific expression of a low-molecular-weight glutenin (LMW-GS-MB 1) gene in tuber (Benmoussa *et al.*, 2004). The mean viscosity value of flour from the field-grown tubers of a transgenic line exhibiting high expression of LMW-GS-MB1 mRNA showed a threefold increase in viscosity at 23°C compared to flour from nontransgenic tubers.

TABLE 23.2 Pasting Properties of Freeze-Dried Flours from Different Potato Cultivars[a]

Cultivar	Pasting Temperature (°C)	Peak Viscosity (RVU)	Trough (RVU)	Breakdown (RVU)	Setback (RVU)	Final Viscosity (RVU)	Setback Ratio
Kufri Bahar	66.5[a]	343[a,b]	177[b]	166[b]	140[b]	317[b]	1.8[b]
Kufri Ashoka	67.6[b]	328[a]	145[a]	183[c]	107[a]	252[a]	1.7[a,b]
Kufri Kanchan	68.1[c]	346[a,b]	158[a]	188[c]	108[a]	266[a]	1.7[a]
Kufri Kunden	66.7[a]	419[c]	245[d]	144[a]	207[c]	482[d]	2.0[c]
Kufri Dewa	66.9[a,b]	351[a,b]	202[c]	149[a]	156[b]	358[c]	1.8[b]
Kufri Lalima	67.2[b]	376[b]	221[c]	155[a,b]	168[b]	389[c]	1.8[b]

Source: Reprinted from Singh, N., Kaur, L., Ezekiel, R., and Guraya, H. S. (2005). Microstructural, cooking and textural characteristics of potato (*Solanum tuberosum* L.) tubers in relation to physicochemical and functional properties of their flours. *Journal of the Science of Food and Agriculture*, 85, 1275–1284.
[a]*Values with the same letter in a column do not differ significantly ($p < 0.05$).*

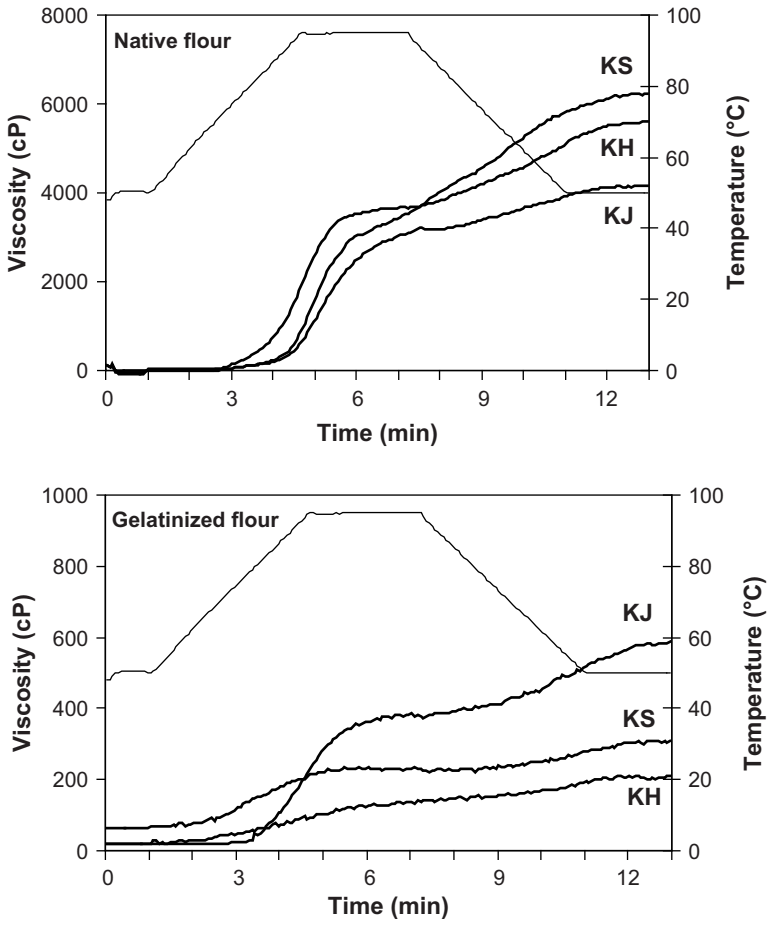

FIGURE 23.1
Pasting properties of native and gelatinized flour prepared from three potato varieties. KH, Kufri Himalini; KJ, Kufri Jyoti; KS, Kufri Shailja. *Source: Unpublished data.*

TABLE 23.3 Thermal Properties of Freeze-Dried Flours from Different Potato Cultivars[a]

Cultivar	T_0 (°C)	T_p (°C)	T_c (°C)	$\triangle H_{gel}$ (Jg^{-1})
Kufri Bahar	55.6[a]	61.6[a]	67.5[a]	11.3[c]
Kufri Ashoka	57.8[b]	63.7[b]	68.8[b,c]	9.5[a]
Kufri Kanchan	61.6[c]	65.4[c]	69.3[c]	9.6[a]
Kufri Kunden	55.7[a]	61.9[a]	67.3[a]	9.7[a]
Kufri Dewa	56.0[a]	62.2[a]	67.0[a]	11.6[c]
Kufri Lalima	55.8[a]	62.4[a]	67.9[a,b]	10.4[b]

T_c, conclusion temperature; T_0, onset temperature; T_p, peak temperature; $\triangle H_{gel}$, enthalpy of gelatinization (dry weight basis, based on starch weight).
Source: Reprinted from Singh, N., Kaur, L., Ezekiel, R., and Guraya, H. S. (2005). Microstructural, cooking and textural characteristics of potato (*Solanum tuberosum* L.) tubers in relation to physicochemical and functional properties of their flours. *Journal of the Science of Food and Agriculture*, 85, 1275–1284.
[a]*Values with the same letter in a column do not differ significantly* ($p < 0.05$).

Thermal properties

Significant variations in onset temperature, peak temperature, conclusion temperature, and enthalpy of gelatinization of flours from different potato cultivars have been reported (Table 23.3; Singh *et al.*, 2005a). Starch granules from the mealier potatoes gelatinized at significantly lower temperatures compared with those from the less mealy potatoes.

Rheological properties

Significant differences were observed in the rheological parameters of potato flour prepared from three cultivars (Singh *et al.*, 2003). The rheological parameters—that is, storage modulus (G'), loss modulus (G''), loss factor (tan δ), and dynamic viscosity (η')—showed significant variation among potato flours when subjected to frequency testing ranging from 0.1 to 20 Hz at different temperatures (Figure 23.2). Among the potato flours, Kufri Badshah flour showed the highest G', G'', and η', whereas these were lowest for Kufri Pukhraj flour. Tan δ of potato flours decreased with increase in frequency (Table 23.4). The rheological properties of potato starches have been reported to depend on granular structure, the amylose:amylopectin ratio, and the presence of phosphate esters, which may affect the rheological properties of the potato flours. Consistency coefficients and flow behavior indices of potato flours were observed to be 5.89–14.94 Pa s^n and 0.27–0.35 n, respectively. Generally, flours with lower amylose content and higher water absorption exhibit higher consistency coefficients.

Retrogradation properties

When the cooked flours are cooled, amylose and amylopectin chains realign to a crystalline structure, resulting in gel formation. This process is known as retrogradation. The retrogradation of pastes prepared from potato flours is measured by determining water expelled during storage at 4°C, known as syneresis (Singh *et al.*, 2003). The syneresis (%) value of cooked pastes from the potato flours differed significantly (Table 23.5). Kufri Jyoti and Kufri Badshah potato flour pastes showed more syneresis. The syneresis of cooked pastes from potato flours increased progressively during storage. Potato flours containing large-sized starch granules showed higher syneresis values, whereas those containing small-sized starch granules showed lower syneresis.

POTATO–WHEAT COMPOSITE FLOUR

Unleavened flat breads called chapaties are made from the whole wheat flour in India and are the staple food of many other countries as well. The desired quality characteristics for chapaties are soft texture, light creamish brown color, and baked wheat aroma (Haridas Rao, 1993).

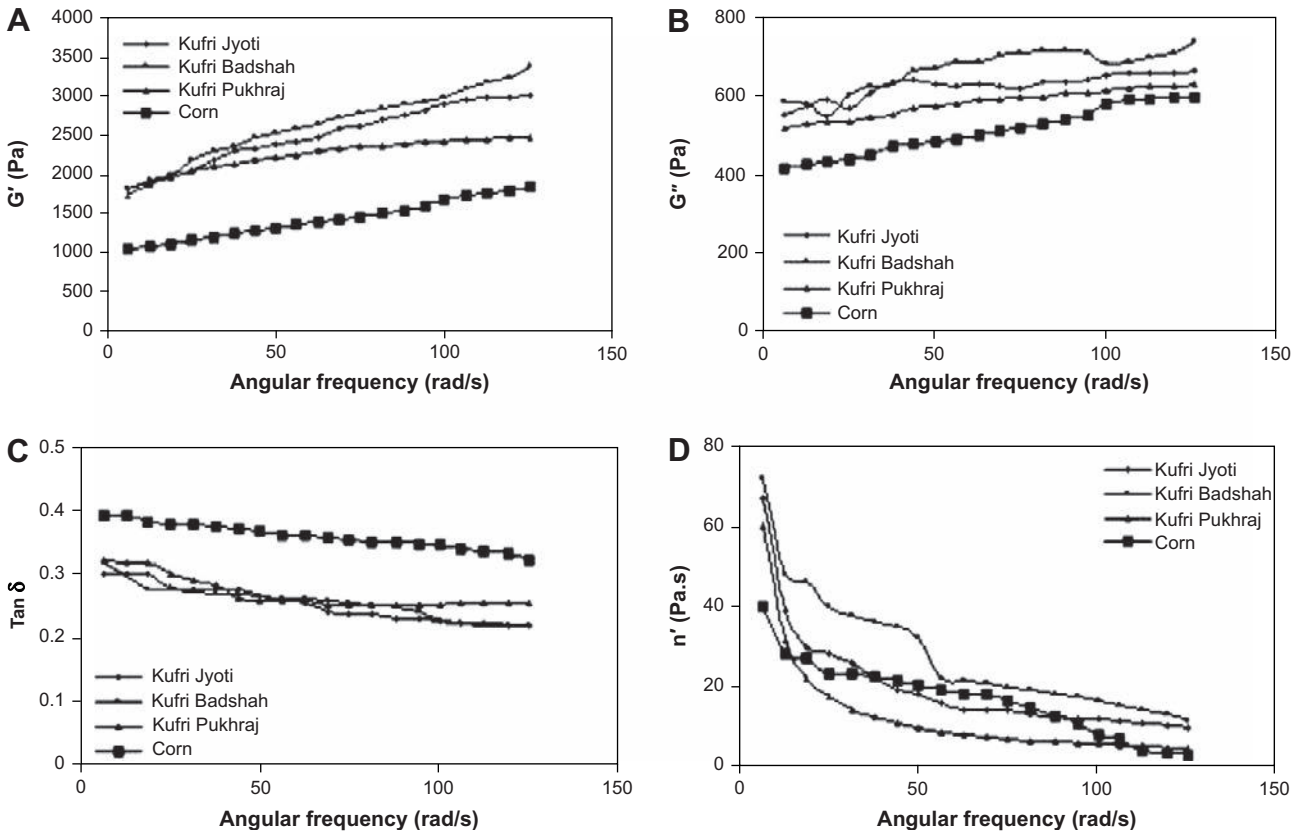

FIGURE 23.2
(A) Storage modulus (G'), (B) loss modulus (G''), (C) loss factor (tan δ), and (D) dynamic viscosity (η') of corn (for comparison) and potato flours at 40°C. *Source: Reprinted with permission from Singh, J., Singh, N., Sharma, T. R., and Saxena, S. K. (2003). Physicochemical, rheological and cookie making properties of corn and potato flours.* Food Chemistry, *83, 387–393.*

Flours from cereals other than wheat are incorporated into chapaties to improve protein content and extensibility. Potato flour can also be incorporated into the whole wheat flour to make chapaties with, for example, improved water absorption and softness.

Physicochemical properties

The addition of potato flour up to 4% to wheat flour increased water absorption by as much as 15.6% (Al-Mane, 1991). The dough development time (peak time) and dough stability decreased, whereas the arrival time increased. When industrial potato fiber preparation was used as a supplement of wheat flour (1.5%), it enhanced the swelling capacity of starch

TABLE 23.4 Rheological Properties of Potato Flours at 20°C (Angular Frequency of 125.7 rad/s)[a]

Cultivar	G' (Pa)	G'' (Pa)	Tan δ	η' (Pa s)
Kufri Badshah	3383[d]	739[c]	0.2185[a]	11.15[c]
Kufri Jyoti	3010[c]	662[b]	0.2279[a,b]	9.856[b]
Kufri Pukhraj	2467[b]	628[b]	0.2540[b]	4.582[a]

G', storage modulus; G'', loss modulus; η', dynamic viscosity.
Source: Reprinted with permission from Singh, J., Singh, N., Sharma, T. R., and Saxena, S. K. (2003). Physicochemical, rheological and cookie making properties of corn and potato flours. *Food Chemistry*, 83, 387–393.
[a]*Values with the same letter in a column do not differ significantly* ($p < 0.05$).

TABLE 23.5 Retrogradation (Syneresis %) Properties of Potato Flour[a]

Cultivar	Day 4	Day 6	Day 8
Kufri Badshah	9.14^{c}	12.20^{c}	16.23^{c}
Kufri Jyoti	10.01^{d}	13.90^{c}	18.45^{d}
Kufri Pukhraj	8.25^{b}	9.56^{b}	12.85^{b}

Source: Reprinted with permission from Singh, J., Singh, N., Sharma, T. R., and Saxena, S. K. (2003). Physicochemical, rheological and cookie making properties of corn and potato flours. *Food Chemistry*, 83, 387–393.
[a]*Values with the same letter in a column do not differ significantly* ($p < 0.05$).

granules in the dough and favored gelling as well as mechanical and molecular behavior of water in the bread structure (Soral Smietana *et al.*, 2003). The microstructure proofs of bread crumbs indicate an indirect participation of potato fiber components in the fibrous construction of gluten network formation.

Pasting properties

Maximum viscosity showed a considerable increase with the addition of 4% potato flour, and even 1% potato flour caused a noticeable increase. A decrease in both transition temperature and temperature of maximum viscosities with an increase in the percentage of potato flour has been reported (Al-Mane, 1991). Compared to wheat flour, a wheat–potato flour (85:15) blend resulted in decreased peak viscosity, hot paste viscosity, cold paste viscosity, and breakdown viscosity (Chandra Shekara and Shurpalekar, 1983).

Thermal properties

Potato starches separated from three potato cultivars and differing in amylose content were blended with wheat flour at 10–50% potato starch and DSC studies were performed (Zaidul *et al.*, 2008). The gelatinization peak temperature of the potato–wheat flour mixture was found to remain almost identical up to 30% but decreased at 40 and 50% potato starch due to significant dilution of wheat flour. The gelatinization peak temperature of the mixtures was slightly higher than that of the control samples due to the influence of the wheat gluten in the mixtures.

Rheological properties

Sarker *et al.* (2008) studied dough characteristics of mixtures of wheat flour and potato starch prepared from three cultivars and found that dough stability significantly increased with an increase in potato starch. Wheat–potato flour blends yield sticky dough at a consistency of 500 BU (Chandra Shekara and Shurpalekar, 1983). When potato flour was used at 15–20%, a consistency of 600 BU was required for proper handling of the dough, whereas at 25%, even a high consistency of 700 BU resulted in a sticky dough with poor handling property. Water absorption increased with the increase in the proportion of potato flour in the blend. The band width of the farinogram, an index of mobility, narrowed from 50 to 15 BU as the level of potato flour caused highly significant changes in extensograms by lowering the resistance to extension and increasing the extensibility. Al-Mane (1991) observed that incorporation of potato flour (2–4%) to dough decreased the maximum resistance to extension and the relative resistance but decreased the dough extensibility only slightly.

Textural properties

The blending of potato flour with wheat flour did not alter the compressibility and cohesiveness of doughs (Chandra Shekara and Shurpalekar, 1983). However, the addition of potato flour increased the adhesiveness. Even at 5% level of potato flour, the adhesiveness increased by 100%. The increase in adhesiveness with potato flour can be attributed to the partial gelatinization of starch in potato flour.

EFFECT OF POTATO FLOUR ON BREAD QUALITY

The use of potato in bread making can be traced back to ancient times. Two levels of potato flour are used in bread. In ordinary white bread, 2 or 3% potato flour is used. A higher level of 6% potato flour is used in "potato bread." The addition of larger quantities of potato flour was found to be undesirable. In regular whole wheat and rye breads, 2 or 3% potato flour is used to preserve freshness, due to the increased water absorption afforded by the potato flour. The addition of 2–4% potato flour does not affect the exterior quality of the bread, but it improves interior qualities such as texture, aroma, and flavor. Kaack *et al.* (2007) reported that the enzymatic solubilized potato fiber with a high concentration of soluble fiber and a low concentration of cellulose and lignin could be used for substitution of at least 12% wheat flour for baking of bread with an attractive color, texture, and delicious flavor. For bread preparation, 7 or 8% of damaged starch is desirable in the panary fermentation. During the preparation of potato flour, a high percentage of starch is damaged, and this is helpful in bread preparation when wheat flour–potato flour blends are used (Chandra Shekara and Shurpalekar, 1984). Bread containing cooked potato mash was softer (compressibility, 10.8 kg/V) than bread containing flour prepared from drum dried potato (compressibility, 15.0 kg/V) or potato flour prepared from dried potato slices (compressibility, 12.8 kg/V) at the 15% level of substitution of wheat flour. The overall acceptability of bread quality was satisfactory when potato was blended either as cooked and mashed or as potato flour from dried slices. However, when potato flour prepared from drum dried potato flakes was incorporated in bread, its quality was unacceptable, with an excessively dark crust and a somewhat brownish crumb. Potato flour offers low reducing sugar content, with antioxidants, and with emulsifiers for improved baking properties (Willard and Hix, 1987).

EFFECT OF POTATO FLOUR ON FLAT BREAD QUALITY

Potato flour and flakes are the second solid ingredient of potato flat breads commonly produced in the Scandinavian countries. Nevertheless, the effects of potato flakes and flour on a variety of flat breads have not been thoroughly investigated. Al-Mane (1991) did not observe a significant difference in texture and flavor of Saudi Arabian flat bread made from wheat flour and wheat flour blended with potato flour (up to 4%). No significant differences were observed in the exterior quality attributes, such as crust color and symmetry, and interior quality attributes, such as crumb color, grain and texture, and flavor, between the control wheat flour flat bread and the potato flour blends up to the 4% level. However, significant differences in break and shred, and in the symmetry of the two layers of flat bread were observed. Al-Mane concluded that the addition of up to 4% potato flour to wheat flour does not have any significant adverse effects on the quality of Saudi Arabian flat bread.

Potato flour up to 20% can be mixed with wheat flour for the preparation of the leavened flat bread naan. Naan made from wheat–potato composite flour is highly acceptable (Anjum *et al.*, 2008). The addition of 2% potato flour to wheat flour caused a marked increase in the extensibility of Indian flat bread chapaties (Singh *et al.*, 2005b), and it increased progressively with increasing levels of potato flour up to 80%. Wheat flour chapaties showed an extensibility of 3.38 mm after 30 min of baking, and it increased to 11.21 mm with the addition of 2% potato flour. The addition of potato flour to wheat flour also affected the maximum force (force to rupture), modulus of deformation, and energy to rupture (Table 23.6). It was concluded that the properties of chapaties made from wheat flour are significantly affected by the addition of potato flour up to 8%, resulting in chapaties with higher extensibility, lower peak force, and lower energy to rupture.

GLUTEN-FREE POTATO BREAD

The incidence of celiac disease or other allergic reactions/intolerances to gluten is increasing. Worldwide, the number of people who suffer from celiac disease has been predicted to increase

TABLE 23.6 Textural Properties of Flat Bread ("Chapaties") Prepared with the Addition of Potato Flours from Different Cultivars to Wheat Flour

Cultivar	Potato Flour (%)	Extensibility (mm)	PLR (kN)	MD (MPa)	ER (J)
Control	0	3.38	0.0044	7.91	0.032
Kufri Chandramukhi	2	11.21	0.0016	1.25	0.010
	4	12.20	0.0015	1.08	0.005
	6	12.53	0.0012	1.05	0.004
	8	12.72	0.0010	1.03	0.002
Kufri Badshah	2	6.60	0.0029	1.98	0.019
	4	6.77	0.0027	1.37	0.018
	6	6.81	0.0022	1.24	0.016
	8	10.71	0.0018	1.18	0.011
Kufri Jawahar	2	3.78	0.0036	4.28	0.050
	4	4.43	0.0031	3.42	0.031
	6	4.98	0.0023	2.18	0.029
	8	8.43	0.0020	1.92	0.015

ER, energy to rupture; MD, modulus of deformation; PLR, peak load to rupture.
Source: Reprinted with permission from Singh, N., Kaur, S. P., Kaur, L., and Sodhi, N. S. (2005b). Physico-chemicals, rheological and chapatti making properties of flours from some Indian potato cultivars. *Journal of Food Science and Technology*, 42, 344–348.

by a factor of 10 during the next few years, resulting in a growing market for gluten-free products (Gallagher *et al.*, 2005). The replacement of gluten, particularly in a bread formulation, presents a major technological challenge because it is an essential structure-building protein that is necessary for formulating high-quality breads. Potato and potato products such as potato flour and potato starch have been found to be useful in preparing gluten-free bread formulation. A formulation based on rice and potato starch supplemented with fish surimi (as a structure enhancer) at the 10% level produced loaves with higher volumes and a softer crust and crumb texture than those of the control (Gallagher *et al.*, 2005). A survey conducted in Alberta, Canada, showed that potato flour along with rice flour and maize starch were used most frequently in baking gluten-free breads. Many gluten-free bread mixes available on the market include either potato starch or potato pulp.

HEALTH BENEFITS OF USING POTATO

Potato is one of the most important and versatile foods from a nutritional standpoint. Potato protein has a particularly favorable lysine content in comparison with cereal proteins, whose amino acid scores, on the basis of human requirements, are much lower (Woolfe, 1987). In terms of quantities required to maintain nitrogen balance in adult humans, the potato protein has better nutritive value than the protein of wheat flour or rice or corn. The unleavened flat bread called chapaties, a staple food in northern India, is generally prepared from wheat flour, which has 8–12% protein but is limited in certain essential amino acids. Partial substitution with other flours can help improve the nutritional quality of chapaties. Lysine contents in potatoes are similar to those in animal protein, and potato flour can be utilized to overcome protein and calorie malnutrition in the general population (Anjum *et al.*, 2008). Substituting wheat flour with potato flour at 20% was found acceptable for the preparation of the leavened flat bread naan. A high intake of potatoes prevents weight gain in men (Halkjaer *et al.*, 2004). A study carried out in Australia found that one of the foods contributing to resistant starch intake was potatoes. In a study performed in Sweden, potato products and bread were found to be the main sources of resistant starch in the Swedish diet. Boiled potato has a low level of resistant starch (1.2%) and a high level of digestible starch (78.2%), and it is therefore considered a high glycemic index food (Garcia Alonso and Goni, 2000). However, retrograded potato flour has higher resistant starch (10.4%) and lower digestible starch (68.9), and therefore it has a lower glycemic index. Potatoes contain antioxidants, predominantly vitamin C and certain

carotenoids and anthocyanins. Potatoes are a rich source of vitamin C, and even after losses during processing, a considerable quantity remains in potato flour (Woolfe, 1987). Attempts are being made to increase the carotenoid content of potatoes.

HEALTH CONCERNS REGARDING THE USE OF POTATO

Acrylamide, a known neurotoxin and carcinogen, was reported to be present in several foods in 2002. It was originally detected in potato products such as French fries and potato chips and later was reported in several cereal-based products. Acrylamide is formed during frying or baking at high temperatures (>100°C) in breads prepared from wheat flour or wheat and potato composite flours. It is not formed in boiled potatoes because the temperature during boiling does not exceed 100°C. Acrylamide is formed as a result of the Maillard reaction mainly between the amino acid asparagine and reducing sugar glucose. This reaction causes the formation of acrylamide and browning of fried (flat bread) or baked (bread) products. However, the amount of acrylamide consumed in the form of different foods is several hundred times lower than the amount required to cause any toxic effects to humans. To date, no studies have reported any carcinogenic effects of acrylamide ingestion from foods. The addition of glycine during dough making significantly reduced acrylamide in both flat breads and bread crusts (Brathen *et al.*, 2005). In bread crusts, the reduction of acrylamide ranged from 50 to 90%, and in flat breads the reduction ranged from 60 to 95%. By the addition of the enzyme asparaginase to the dough, it was possible to obtain a significant reduction in acrylamide in bread crusts.

FORTIFICATION

Attempts to utilize potato protein concentrate (PPC) for human consumption by enriching bakery products have had limited success due to the undesirable taste, smell, and texture of the dried coagulate. Researchers are trying to reduce the undesirable flavor, improve the texture, and increase the solubility. Use of PPC to replace part of the wheat flour in bread has also been tried (Knorr, 1979), and it was found that up to approximately 10% of wheat flour could be replaced by PPC without changing the volume of the bread.

ADVERSE REACTIONS

Toxicity

Potato has some toxic or potentially toxic constituents, such as glycoalkaloids, proteinase inhibitors, and lectins. The major glycoalkaloids in most cultivated potato species are α-solanine and α-chaconine, both of which are derived from the alkaloid aglycone solanidine. Glycoalkaloid content in cultivated potatoes is 6.4 mg/100 g tuber fresh weight (f.wt). When the glycoalkaloid content exceeds 20 mg/100 g tuber f.wt, it becomes unsafe for human consumption. This happens mostly in potatoes that become green due to exposure to light. Accidental consumption of potatoes containing high levels of glycoalkaloids has caused severe illness. Glycoalkaloids may have two toxic effects: inhibition of cholinesterase, thus affecting the nervous system, and disruption and injury to membranes in the gastrointestinal tract (Morris and Lee, 1984). Solanine poisoning among London schoolboys who consumed peeled and boiled potatoes containing 25–30 mg α-solanine/100 g potatoes has been reported. However, dried potato products are reported to contain lowered glycoalkaloid levels. For example, a study carried out in Russia reported a glycoalkaloid content of 31 mg/100 g f.wt. in tubers of "Loshitskii" variety (Woolfe, 1987). After cleaning and boiling, the level dropped to 7 mg/100 g. Drying reduced it further to 5.25 mg/100 g. Glycoalkaloids and their metabolites have been reported to have beneficial effects for human health, such as inhibiting the growth of human colon and liver cancer cells (Lee *et al.*, 2004). Potatoes contain high concentrations of proteinase inhibitors, which inhibit or prevent the activities of the major

animal pancreatic digestive proteinases, including trypsin, chymotrypsin, elastase, and carboxypeptidases A and B (Woolfe, 1987). However, processes such as boiling, microwave heating, or baking denature most inhibitors, and they become normal protein nutrients. Potato also contains lectins or hemagglutinins, which are carbohydrate-binding cell-agglutinatory proteins. Very little is known about the function of the potato lectin and its nutritional significance for humans.

Allergy

Sodium bisulfite is normally used as a source of SO_2 to prevent enzymatic browning in dehydrated potatoes. Despite the fact that at SO_2 levels normally used (200–400 ppm), no significant problems of toxicity have been reported (Willard and Hix, 1987), some occurrences of allergenic reactions to sulfited raw vegetable have prompted legislation in the United States requiring labeling of SO_2 in finished products with levels higher than 10 ppm. To overcome this problem, a considerable amount of potato flakes used industrially are produced with no added sulfur dioxide.

TECHNOLOGICAL ISSUES

Large-scale manufacture of potato flour involves dehydration of peeled, cooked potatoes on a single-drum drier equipped with applicator rolls and grinding of a thin dried sheet of potato to the desired fineness. By spreading the mash into a thin sheet, extremely rapid evaporation of water is achieved, and there have been no major changes to this method of potato flour preparation. The most simple and widely used procedure for the production of potato flour consists of preparing slices, drying them, and then grinding to get flour of desired fineness. For the preparation of potato flour on a smaller scale, potato slices are solar dried, and dried slices are ground to get flour. However, using this method, the flour can become contaminated with dust gathered during sun drying. In composite breads, relatively higher levels of non-wheat flour could be incorporated in bread by adopting highly capital-intensive processes such as mechanical dough development in place of conventional processes. Because processes such as mechanical dough development involve the use of imported machinery and high energy, they may not be practical in developing countries. Incorporation of non-wheat flours such as potato at levels of approximately 10% by appropriate modifications to conventional methods would be much more useful. Increasing the proportion of potato flour beyond 10% can make the dough sticky due to increased adhesiveness, which limits its use for blending at higher levels with wheat flour.

SUMMARY POINTS

- Potato flour has been associated with the baking of bread for a long time and is known to help retain the freshness of bread and provide a distinctive flavor.
- Potato flour has a high viscosity compared to other flours, and its addition to wheat flour increases water absorption and consistency, and decreases dough development time.
- The addition of potato flour (2–4%) improves the interior qualities of bread, such as texture, aroma, and flavor, without significantly affecting exterior attributes.
- Potato flour up to 20% can be mixed with wheat flour to prepare highly acceptable leavened flat bread called naan.
- The addition of 2% potato flour to wheat flour increases the extensibility of Indian unleavened flat bread called chapaties and reduces the energy required to rupture.
- Potato flour has been found to be useful in preparing gluten-free bread.
- In terms of quantities required to maintain nitrogen balance in adult humans, the potato protein has better nutrition value than the protein of wheat flour or rice or corn.
- Retrograded potato flour has higher resistant starch and lower digestible starch and, therefore, a lower glycemic index.

- The addition of glycine during dough making significantly reduces acrylamide in both flat breads and bread crusts.
- Potato flour contains a much lower level of glycoalkaloids than do the fresh tubers, which have a quite low level, with an average content of 6.4 mg/100 g tuber f.wt. Therefore, glycoalkaloid toxicity is not a health concern.

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SECTION 2

Fortification of Flour and Breads and their Metabolic Effects

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CHAPTER 24

Mineral Fortification of Whole Wheat Flour: An Overview

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

$FeSO_4$ Ferrous sulfate
NaFeEDTA Sodium iron ethylenediaminetetraacetate
NTDs Neural tube defects

INTRODUCTION

During the twentieth century, nutritional scientists developed dietary standards, dietary guidelines, food pyramids, recommended dietary allowances, and reference daily intakes. However, during the past few decades, nutritionists have been more concerned with food security and safety, malnutrition, and diet–health linkages. They have emphasized that ample quantities of vital nutrients should be available to everyone throughout the year. Broadly, these nutrients are categorized into macronutrients and micronutrients. Among macronutrients, proteins, fats, and carbohydrates are important, whereas vitamins and minerals constitute the group of chemical compounds known as micronutrients. Micronutrients are responsible for

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regulating various metabolic pathways, and their deficiencies lead to drastic health disparities (McGuire and Beerman, 2007).

Food security is often conceptualized in the context of food energy or calorie intake; however, it is now recognized that a large segment of the world's population, especially in developing countries, consumes food that is deficient in micronutrients such as iron and iodine. Reduction in micronutrient deficiencies can contribute significantly to improving health, productivity, and the well-being of humans. Micronutrient deficiency, also known as "hidden hunger," is an aggravating factor for health status and quality of life, affecting more than half of the world's population (Long *et al.*, 2007; Mayer *et al.*, 2008). Micronutrient deficiency disorders exert drastic effects in pregnant and lactating women due to their higher demands for nutrients. These disorders can be lethal for neonates as well (Adu-Afarwuah *et al.*, 2008).

The current situation calls for immediate action, especially in developing countries, to address these micronutrient deficiencies before they reach epidemic proportions. They can be overcome by following diverse strategies, such as food-based dietary modules. However, these strategies require thorough knowledge of the nutrient sources and their accessibility/availability for humans. Among these strategies, food fortification is practically more applicable and has drawn considerable attention from governmental and health agencies. The success of food fortification programs lies in the selection of vehicle and fortificant. Ideally, a food consumed by most of the population should be chosen as a vehicle, and the programs should be designed in such a way as to include high-risk groups. In developing economies, fortification of cereal products is popular because of high population coverage (World Health Organization (WHO), 1999). Multiple fortification of whole wheat flour with novel iron sources is gaining popularity to overcome micronutrient malnutrition in developing countries. Economic and nutritional aspects of such fortification programs require that the fortificants added to the flour be evaluated for their effect on the native components of the food, sensory attributes of the food, and their availability in the final product during processing of the food (Akhtar *et al.*, 2010).

Cereals are the best vehicle for fortification in most developing countries because 95% of the population consumes cereals as a dietary staple. These are also relatively inexpensive to grow and are consumed worldwide by all economic classes. However, versatility in preparation and uses makes them more effective in these strategies (Ranum, 2001). Mineral deficiencies can be overcome with the use of fortification programs. Globally, whole wheat flour serves as a dietary staple for millions of people, especially in Asian countries. Thus, mineral fortification of whole wheat flour should be mandatory to overcome the problem of hidden hunger in the developing world.

MICRONUTRIENT DEFICIENCIES

Micronutrient malnutrition affects more than half of the world's population, particularly in developing countries. Iron and iodine deficiencies are global problems, and manifestations of such micronutrient deficiencies are mainly anemia and goiter (Table 24.1). Moreover, these are the most important risk factors for illness and death, affecting 2 billion people throughout the world (Mayer *et al.*, 2008).

Worldwide, iron deficiency is associated with approximately 861,000 deaths and approximately 35 million disability-adjusted life years lost (Stoltzfus *et al.*, 2003). Diet is a major factor because many communities that rely on their dietary staples lack adequate iron intake, thus resulting in iron deficiency anemia (IDA). Populations in developing countries are at higher risk due to poverty and hunger. They are also at higher peril due to parasitic infections, whose occurrence varies from 10 to 50%, because these problems increase the prevalence of IDA (Hallberg *et al.*, 1998).

TABLE 24.1 Characteristic Features of Mineral Deficiencies

Deficiency	Health Consequences
Iron deficiency	Anemia
	Increased susceptibility to infection
	Impaired growth
	Muscle cramps
	Impaired cognitive function
	Defects in thermoregulation
	Increased risk of pregnancy complications
	Increased risk of low birth weight
	Heart attack
Iodine deficiency	Aids in metabolism regulation
	Fetal development
	Component of the hormone thyroxin

Source: Gibson and Ferguson (1999).

The consequences of IDA are reduced psychomotor and mental development in infants, adverse pregnancy outcome, premature delivery, and decreased immune function. IDA reduces a person's ability to perform physically demanding tasks, and anemic laborers have demonstrated impaired productivity. Furthermore, individuals with IDA require greater oxygen consumption to maintain body temperature during cold exposure. This could lead to negative caloric balance in individuals whose dietary intake is already restricted (Walter *et al.*, 1986).

INTERVENTION STRATEGIES

Food-based strategies are the most effective and sustainable approach to combat these deficiencies. Food diversification involves consumption of a variety of foods to meet nutrient requirements. Communities in the developing world rely on regional specific sources of foods that are often deficient in some minor constituents. Under such conditions, there is a need to add some other food components to balance nutrient intake. The effectiveness of supplementation programs depends on the level of coverage and compliance.

In comparison to these strategies, food fortification is a socially acceptable, economical, and flexible approach that is used to improve nutrition in a number of developed countries. For example, fortification of milk, margarine, and cereals has greatly reduced the occurrence of mineral deficiencies. To be successful, the following points need to be considered before the implementation of any fortification project:

1. The deficiency of key micronutrients should be evaluated through population-based studies.
2. The extent of deficiency and influence of dietary factors should be analyzed, making up for dietary deficiencies by balancing the dietary requirements.
3. Selection of appropriate vehicle for fortification purposes should be made carefully, with consideration of the fact that the majority of the population should have easy access to the vehicle and the product should be affordable.
4. Selection of appropriate fortificants is another important factor for a successful fortification program. In this regard, safety, storage stability, and consumer acceptability should be studied well in advance of program implementation.
5. The levels of fortificants are critical, and special consideration should be given for indigenous concentration of the specific micronutrients available through the food to be fortified. Moreover, issues pertaining to bioavailability should be addressed due to the presence of antinutritional factors such as phytic acid.

TABLE 24.2 Provisional Recommendations for the Addition of Iron to Fortified Flour Based on Type of Flour and Fortificant as Well as Per Capita Flour Intake

			Per Capita Wheat Availability (g/Day)[a]			
	Flour Type (Extraction)	Fortificants	<75[b]	75–149	150–300	>300[c]
Iron	Low	NaFeEDTA	40 ppm	40 ppm	20 ppm	15 ppm
		Sulfate/fumarate[d]	60 ppm	60 ppm	30 ppm	20 ppm
		Electrolytic powder	NR[e]	NR[e]	60 ppm	40 ppm
	High	NaFeEDTA	40 ppm	40 ppm	20 ppm	15 ppm

Source: Flour Fortification Initiative (2008).
[a]*Proxy for wheat flour intake.*
[b]*Per capita consumption of <75 g/day does not provide a sufficient level of fortificant to cover micronutrients for women of childbearing age. Fortification of additional food vehicles should also be considered.*
[c]*Few countries have per capita consumption of >300 g/day.*
[d]*Ferrous fumarate is the preferred compound for maize flour after nixtamalization.*
[e]*Not recommended because the very high levels of electrolytic iron needed would negatively affect sensory properties of fortified flour.*

6. In circumstances in which multiple micronutrient fortification is recommended, interactive effects of different nutrients need to be studied through controlled animal modeling.
7. Programs should be designed such that success does not require changes in the dietary habits of the population nor any personal contact with recipients. However, public education is still required to create a demand for the fortified products.
8. The proper mixing of fortificants in respective products should be very well studied. Furthermore, fortified products should be available to all communities year-round.
9. Trade harmonization is often desirable because many countries have different standards for fortification according to their requirements.
10. Follow-up is essential for determination of outcomes of fortification programs.

In countries in which wheat is a staple diet of the population, emphasis has been placed on fortifying the wheat flour with multiple micronutrients in order to enhance intake and overcome mineral deficiencies. International organizations such as WHO/Eastern Mediterranean Regional Office, the U.S. Agency for International Development, and the Micronutrient Initiative have established guidelines for vitamins and minerals fortification of wheat flour. They consider it as the most feasible approach to overcome micronutrient deficiency, especially in developing countries (Flour Fortification Initiative (FFI), 2008) (Table 24.2).

WHEAT FLOUR FORTIFICATION: ECOLOGICAL STUDIES

Approximately 600 million metric tons of wheat are commercially milled annually and consumed in nearly every nation of the world (FFI, 2008). Whole wheat flour is a staple food of the population in the Indian subcontinent and provides more than 50% of the total energy intake. Similarly, in countries such as Syria, Algeria, Turkey, and Iran, wheat consumption is approximately half the total caloric intake and ranges up to 600 g/day. In Egypt and Saudi Arabia, where wheat products provide slightly more than one-third of the caloric intake per capita, consumption is approximately 300–400 g/day (Ranum, 2001). Consequently, wheat flour and its products are good vehicles for fortification with micronutrients such as iron, zinc, and vitamin A.

Wheat flour fortification with micronutrients was started in the United States and Canada in the 1940s. In the 1950s, Chile started fortifying wheat flour, and during the 1960s some Latin American countries passed legislation encouraging fortification of foods. Accordingly, the addition of iron and B vitamins began on a voluntary basis by some millers. Several other countries in the Middle East and North Africa are implementing fortification programs for

wheat and corn flour with iron and other micronutrients. Central Asian countries—that is, Azerbaijan, Kazakhstan, Kyrgyz Republic, Mongolia, Tajikistan, and Uzbekistan—have started flour fortification (WHO, 1999). Vietnam, Thailand, the People's Republic of China, Pakistan, and Indonesia have also signed the consensus statement on wheat flour fortification. These countries are of the view that addition of micronutrients to flour is an efficacious method to reduce the prevalence of key deficiencies, especially iron. Jordan has also initiated a flour fortification program to benefit a larger sector of the population. Studies in Guatemala and Thailand have shown that village-level fortification can be operationally feasible. Chile has an iron deficiency prevalence of less than 1%, which most observers attribute to a strong flour fortification program (Opportunities for Micronutrient Interventions, 1996).

In Chile, the Ministry of Health legislated to add folic acid to wheat flour (2.2 mg/kg) to reduce the risk of neural tube defects (NTDs). That fortification policy resulted in a 40% reduction in NTDs. Moreover, folic acid fortification of wheat flour substantially improved folate status in women of reproductive age.

These developments have led to an increase in the worldwide percentage of wheat flour fortification from 18% in 2004 to 27% in 2007. The estimated number of people with access to fortified wheat flour increased by approximately 540 million, and the annual number of newborns whose mothers had access to fortified wheat flour during pregnancy increased by approximately 14 million. The number of countries with mandatory wheat flour fortification programs increased from 33 in 2004 to 54 in 2007 (Maberly *et al.*, 2008). Despite these successes, more than two-thirds of the world's population still lacks access to fortified wheat flour and its benefits, including millions of women of childbearing age. Regular access of the population to fortified flour may be effective in reducing the burden of micronutrient deficiencies (FFI, 2008). Programs continue to expand coverage of wheat flour fortification as a strategy to increase micronutrient consumption (WHO, 1999). However, emphasis has to be placed on implementing multiple micronutrient fortification because single micronutrient addition to an appropriate food vehicle is a less effective approach in food fortification programs. Multiple fortification of foods is a possible means of addressing deficiencies of two or more micronutrients at the same time in a cost-effective manner (Darnton Hill *et al.*, 1999).

IRON FORTIFICATION OF WHOLE WHEAT FLOUR

Iron fortification is mandatory in 52 countries, and such strategies have already shown effectiveness in reducing the extent of deficiency syndromes. The selection of an appropriate fortificant is the most important factor for the success of any wheat flour fortification program; for example, use of appropriate levels of the most bioavailable forms of iron can improve the iron status of populations with very little risk of adverse effects. A successful food fortification program includes the use of soluble iron such as ferrous sulfate ($FeSO_4$), the addition of ascorbic acid as an absorption enhancer, or the use of sodium iron ethylenediaminetetraacetate (NaFeEDTA) to overcome the negative effect of phytic acid. Therefore, better absorbed alternative compounds for cereal fortification include encapsulated $FeSO_4$ and NaFeEDTA (Hurrell, 2002) (Table 24.3). According to the recommendations of FFI (2008), NaFeEDTA, $FeSO_4$, and ferrous fumarate are preferred for whole wheat flour fortification. The amount of iron that should be added to cereal food staples (wheat flour, maize meal, and white rice) should be between 25 and 80 ppm, depending on the severity of the need for additional iron in the population and the estimated amount of consumption of the cereal to be fortified (Ranum, 2001).

However, due to its high bioavailability and low cost, Food and Chemical Codex-grade $FeSO_4$ is often considered as the best iron source. It can be used in bakery flour, semolina, and other types of low-extraction wheat flours. Large particle size or hydrated $FeSO_4$ can cause color and spotting problems. The use of $FeSO_4$ may not be appropriate in products stored for extended periods due to its promotion of oxidative rancidity of native or added fats, which reduces

TABLE 24.3 Relative Bioavailability of Iron Compounds Used in Food Fortification

Fortified Foods	Relative Bioavailability	Iron Compound
Infant formulas	100	Ferrous sulfate
Infant cereal	100	Ferrous fumarate
Drink powders	74	Ferrous saccharate
Infant cereal, rice	21–74	Ferric pyrophosphate
Infant cereals	25–31	Ferric orthophosphate
Wheat flour	5–90	Elemental iron
Wheat flour	12–90	NaFeEDTA

Source: Hurrell (1992).

TABLE 24.4 Relative Bioavailability of Iron from $FeSO_4$ and NaFeEDTA in Different Foods

Food	$FeSO_4$ (A)	NaFeEDTA (B)	Relative Bioavailability (B/A)
Refined sugar	38	11	0.3
Sugarcane syrup	33	11	0.3
Sweet manioc	14	17	1.2
Wheat	6	15	2.3
Egypt flatbread	2	5	2.5
Rice	4	12	2.9
Maize porridge	4	7	2.1
Soybeans	3	7	2.3

Source: International Anemia Consultative Group (1993).

acceptable shelf life. It can also produce changes in color and flavor over time, which would reduce consumer acceptance (Sharing United States Technology to Aid in the Improvement of Nutrition, 2001). The presence of phytates, polyphenols, and calcium is known to adversely affect the bioavailability of non-heme iron fortificants. Evidence indicates that in such cases, NaFeEDTA may prove to be a better choice of fortificant because iron from the EDTA complex remains bioavailable even in the presence of iron absorption inhibitors. The Joint Expert Committee on Food Additives has tentatively approved NaFeEDTA for use as a fortificant (Food and Agriculture Organization, 1996).

The valuable properties of NaFeEDTA as an iron fortificant have been described in the literature for approximately 25 years. The efficacy of NaFeEDTA for improving iron status has been demonstrated in several community trials. When mixed with food at fortification levels, not only is the iron in NaFeEDTA absorbed better than other non-heme forms of iron but also it has the advantage of making the total non-heme iron pool 2.5 times higher than that of $FeSO_4$ added to foods (Viteri *et al.*, 1995). Moreover, it is not sensitive to many food iron inhibitors, and it is particularly of interest for populations whose staple foods are cereal or legume based. Furthermore, due to its better absorption capacity and chemical stability, it does not cause fat to become rancid. Therefore, it is suitable for use in other (not previously fortified) foods (Akhtar *et al.*, 2008).

In Guatemala, NaFeEDTA was tested as a sugar fortificant (Viteri *et al.*, 1995). At a concentration of 1.0 g NaFeEDTA/kg sugar, fortification increased iron stores significantly, an effect that was apparent by 8 months and was still increasing 32 months after the intervention started (Ballot *et al.*, 1989).

Concern has been raised regarding the consumption of NaFeEDTA based on the consensus that dietary EDTA levels are high in developed nations due to the utilization of EDTA, NaEDTA, and CaNaEDTA as preservatives. However, acceptable daily intake of EDTA is 150 mg/person/day or 2.5 mg/kg/day. In 1992, the mean overall exposure to EDTA in the United States was

15 mg/person/day, which suggests that the use of NaFeEDTA as a fortificant may be possible in the United States and especially in developing countries in which usual intakes are lower (International Anemia Consultative Group, 1993) (Table 24.4). Huang *et al.* (2009) studied the fortification of wheat flour with electrolytic iron, $FeSO_4$, and NaFeEDTA at 60, 30, and 20 mg Fe/kg, respectively. They concluded that both NaFeEDTA and $FeSO_4$ fortified wheat flour have positive impacts on iron status in anemic students; however, NaFeEDTA was more effective.

STORAGE STABILITY, BIOAVAILABILITY, AND CONSUMER ACCEPTABILITY OF MINERAL FORTIFIED FLOUR

The success of any fortification program depends on the stability of micronutrients and the food to which they are added. Exposure of the fortificants to any of the physical and chemical factors, including heat, moisture, air, or light, and acid or alkaline environments during food processing, packaging, distribution, or storage affects their stability. The stability of the fortified flour remained acceptable during storage of fortified cereals as well as in bread using fortified flour. A marginal difference in proximate composition of fortified wheat flour was observed during storage for 60 days. Mineral fortification also exhibited an inhibitory effect on mold growth during storage in fortified flour for 60 days (Akhtar *et al.*, 2008). Factors affecting the choice of the forms of iron to use include bioavailability, functional and stability properties, commercial availability of food-grade materials, and cost. Unfortunately, the forms that show the greatest functional stability are often less absorbable, whereas those forms that show the greatest bioavailability have the greatest potential for damaging product quality (Mehansho *et al.*, 1999).

The addition of iron fortificants to whole wheat flour did not exhibit any critical alterations in the basic composition of the flours, and marginal differences in various flour attributes concerning rheology were detected in some studies. Higher water absorption as a result of mineral fortification was observed by Akhtar *et al.* (2009).

BIOAVAILABILITY OF FORTIFIED WHEAT FLOUR

If micronutrients are to be provided together, it is important to determine how they interact biologically because they have chemically similar absorption and transport mechanisms and are thought to compete for absorptive pathways. For example, interaction between zinc and iron is primarily antagonistic. Extreme levels of dietary zinc impair iron metabolism directly or indirectly, such as the direct interaction between zinc and iron in the intestine and indirectly in the lumen at an intracellular location distal to the site of the regulation of iron absorption (Lonnerdal, 2002).

Trace element interactions are generally antagonistic, and when two chemically similar ions are present in the intestinal lumen, the one with a greater ratio tends to exclude the other. The ingestion of excess amounts of zinc induces anemia and depresses tissue iron levels in rats and chicks (Bafundo *et al.*, 1984), but this interaction has received little attention with regard to humans. Previous studies have reported a variety of results concerning the interactive effect of dietary minerals on the iron content of organs and tissues. NaFeEDTA is thought to be highly bioavailable, and iron in it is chelated with EDTA, a commonly used food additive that prevents the iron from being bound with phytic acid. NaFeEDTA is better absorbed and not sensitive to many food iron inhibitors compared with other iron fortificants. The lower bioavailability of elemental iron compared to that of NaFeEDTA has been confirmed (Hurrell, 2002; Viteri *et al.*, 1995).

Awareness of these interactions, combined with a balanced evaluation of the dietary intake of the population with regard to absorption-promoting and -inhibiting substances and the risk for multiple deficiencies, could lead to more effective strategies to improve micronutrient status (Long *et al.*, 2007).

SUMMARY POINTS

- Macronutrient deficiencies adversely affect the health of large populations and contribute significantly to reduced quality of life in vulnerable groups.
- Although elimination of micronutrient deficiency has long been a priority of international organizations, it remains a major issue in developing countries.
- Accurate estimation of the prevalence of micronutrient deficiency in specific regions, policy decisions and their implementation, choice of mineral fortificants, levels of fortification, selection of a suitable vehicle, and feasibility studies are some of the key issues that need to be addressed for fortification programs to be successful.
- Wheat flour fortification with minerals seems to have a greater influence compared to many other vehicles in the Indian subcontinent, where the wheat flour is mainly consumed as a dietary staple to meet energy requirements.
- The fortificants added should not impart undesirable characteristics to the food, such as changes in color, taste, smell, and texture.
- Atmospheric conditions and lack of modern storage facilities for fortified flours demand more consideration than stability and acceptability issues. Mineral fortification should not unduly curtail the shelf life of the fortified whole wheat flour.
- Bioavailability of mineral fortified flour has long been debated and highlights the fact that use of novel iron sources in the presence of phytic acid in wheat flour could be a better choice for fortification.
- Overall, fortification of whole wheat flour can effectively be used as a means to control mineral deficiencies, especially in developing countries.

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CHAPTER 25

Iron Particle Size in Iron-Fortified Bread

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

Caco-2 Colonic adenocarcinoma cells
CO-reduced Carbon monoxide-reduced iron
H-reduced Hydrogen-reduced iron
NaFeEDTA Sodium iron ethylenediaminetetraacetate
RBV Relative bioavailability

INTRODUCTION

Mass fortification of wheat flour with iron holds promise as a major intervention to deliver iron in an absorbable form to large populations on a permanent and sustainable basis (World Health Organization/Food and Agricultural Organization (FAO), 2006). Unfortunately, several key issues on advancing fortification policy are still poorly addressed. First, despite international consensus about iron fortificants selection, many countries in which flour is fortified still use elemental iron as fortificants (i.e., some forms of hydrogen-reduced (H-reduced) iron and atomized iron) that are poorly absorbed. Second, fortification programs lack quality control and assurance systems at the mill level, and countries have failed in implementing monitoring and surveillance systems. Third, when industrially produced, flour is not regularly consumed by large population groups in a country. If these key issues are not resolved, the result will be slow progress toward effective flour fortification in a number of countries.

Available data (Flour Fortification Initiative, 2008) indicate that currently in the majority of countries, the type of iron used in wheat flour fortification is not specified, corresponding most likely to H-reduced iron or other forms of reduced iron (atomized iron) that are poorly

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absorbable. Other forms in use are ferrous sulfate, ferrous fumarate, electrolytic iron, and sodium iron ethylenediaminetetraacetate (NaFeEDTA).

Based on available data from the Food Balance Sheets of FAO and World Bank-supported Household Income and Expenditure Surveys, it was proposed that four wheat flour average consumption ranges be considered in designing flour fortification programs: >300, 150–300, 75–150, and <75 g/day.

Table 25.1 presents the recommended levels and types of iron fortificants based on ranges of per capita flour consumption and extraction of wheat flour with the purpose to optimize the efficacy of iron fortification. The preferred order of iron fortificants is ferrous sulfate, NaFeEDTA, and ferrous fumarate. If these fortificants cannot be used, then electrolytic iron powder is the only alternative iron compound recommended, provided flour consumption is sufficiently high. Atomized reduced and H-reduced elemental iron powder should not be used in flour/food fortification programs (Zimmermann *et al.*, 2005).

Estimated per capita consumption of greater than 75 g/day does not allow for addition of a sufficient level of fortificant to cover micronutrient needs of women of childbearing age. Fortification of additional food vehicles and other interventions should be considered. Because electrolytic iron has half the bioavailability of ferrous sulfate with less than 150 g/day consumed daily, use of this form of iron is not recommended; the very high levels of electrolytic iron needed would negatively affect sensory properties of fortified flour.

In high extraction flours, iron is only poorly absorbed because of the presence of phytates and other inhibitory factors (Hurrell, Lynch, *et al.*, 2002). NaFeEDTA is the recommended iron compound because its bioavailability is less affected by phytates. In addition, in high extraction flours, the content of any type of iron needs to be lower because the presence of fats causes rancidity and changes in color.

Clearly, with respect to iron fortification of wheat flour, the main challenge is to find a suitable iron fortificant. The dilemma is that iron compounds that are water soluble and highly bioavailable, such as ferrous sulfate, often react with other components in the food to which they are added, causing off-flavors, color changes, fat oxidation, or all three. On the other hand, compounds that are less soluble and therefore more stable in foods (elemental iron powders) are poorly absorbed (Hurrell, Bothwell, *et al.*, 2002). Addition of iron must be low to prevent undesirable changes in the sensorial properties of flours. Thus, ferrous sulfate can be added in amounts no higher than 40 mg of iron per kilogram in low extraction wheat (highly refined and low in fat). Dried ferrous sulfate of low particle size is preferred because the heptahydrate compound can cause color and spotting problems (International Anemia

TABLE 25.1 Recommendations for the Addition of Iron to Fortified Wheat Flour Based on Extraction, Fortificant Compound, and Estimated Per Capita Flour Availability[a]

		Nutrient to Be Added (mg/kg) Based on Estimated Per Capita Wheat Flour Consumption (g/Day)			
Flour Extraction Rate	**Compound**	**<75 g/Day**	**75–149 g/Day**	**150–300 g/Day**	**>300 g/Day**
Low	Ferrous sulfate	40	40	20	15
	NaFeEDTA	60	60	30	20
	Ferrous fumarate	60	60	30	20
	Electrolytic iron	NR[b]	NR[b]	60	40
High	NaFeEDTA	40	40	20	15

Source: Modified from Flour Fortification Initiative (2008).

[a] *Recommended levels and types of iron fortificants based on ranges of per capita flour consumption and extraction of wheat flour with the purpose of optimizing the efficacy of iron fortification.*

[b] *Not recommended because the very high levels of electrolytic iron needed could negatively affect sensory properties of fortified flour.*

Consultative Group (INACG), 2002). Less reactive iron compounds (ferrous fumarate or electrolytic iron) can be incorporated in amounts up to 60 mg/kg (INACG, 2002).

Ferrous fumarate has a relative bioavailability (RBV) similar to that of ferrous sulfate, the reference against which the bioavailability of all other iron compounds is measured (Hurrell, 1985). Because it is less soluble in water than ferrous sulfate, it causes fewer organoleptic problems. Ferrous fumarate is, as is ferrous sulfate, subject to the inhibitory effects on iron absorption of phytates present in cereal flours (Hurrell, Lynch, *et al.*, 2002). NaFeEDTA is a chelated iron compound in which the iron is protected from the inhibitors of iron absorption. It has a bioavailability two to four times that of ferrous sulfate, especially in meals with high phytate content (Bothwell and MacPhail, 2002). It is used in China (5% of refined wheat flour) and Pakistan (10% of unrefined wheat flour). Although both ferrous fumarate and NaFeEDTA thus seem to be good alternatives for electrolytic iron, the matrix of the food vehicle will determine the amount that can be used and hence its efficacy as a fortificant.

EFFECT OF PARTICLE SIZE OF ELEMENTAL IRON POWDERS

Several countries utilized elemental iron powders in their wheat flour fortification programs because of their low reactivity and high stability (Hurrell, Bothwell, *et al.*, 2002). There are considerable variations between the different types of elemental iron not only in particle size but also in dissolution rate and reactive surface (Hurrell, Bothwell, *et al.*, 2002). There is a negative relationship between dissolution rates in 0.1 M HCl acid and the median particle size and a stronger positive relationship between the reactive surface area and the dissolution rate (Hurrell, Bothwell, *et al.*, 2002; Swain *et al.*, 2003). On the other hand, these physicochemical characteristics of the iron powder influence its bioavailability, with iron absorption being high when the particle size is small, uniform, and more soluble in diluted acid (Hurrell, Bothwell, *et al.*, 2002).

Few studies on the bioavailability of iron from wheat flour fortified with elemental iron powders have been performed in humans. However, there is concern regarding the extrapolation of some of these results to the current iron absorption from wheat flour fortified with bakery-grade elemental iron powders because the iron compounds used in the studies do not have the same physicochemical characteristics as the ones employed in wheat fortification. For instance, several of these studies have used very low particle size of elemental iron, which has better solubility than the compounds used by the bakery industry, which usually have a nonhomogeneous particle size less than 45 μm (325 mesh size). Iron absorption studies in humans are summarized in Table 25.2.

Björn-Rasmussen *et al.* (1977) observed a better RBV (82−90%) of small particle size H-reduced iron compared with the 66 or 13% RBV observed when the same iron compound had a coarser particle size or a lower solubility, respectively.

Studies performed in rats with commercial electrolytic iron (<45 μm particle size) have shown a mean RBV of 44% (range, 16−70%), whereas the mean value for H-reduced powders was 30% (range, 13−54%) (Hurrell, Bothwell, *et al.*, 2002). Coarse H-reduced powders (<149 μm particle size) have a mean RBV of approximately 20% (Hurrell, Bothwell, *et al.*, 2002). Reported mean RBV values for commercial carbon monoxide-reduced iron (CO-reduced) and carbonyl iron (<45 μm particle size) were 19% (range, 12−32%) and 47% (range, 27−66%), respectively (Hurrell, Bothwell, *et al.*, 2002). Swain *et al.* (2003) studied the RBV of six elemental iron powders in rats (Table 25.3).

On the other hand, for each elemental iron type, smaller particle size iron has a better RBV value than that of coarse powders (Figure 25.1) (Hurrell, Bothwell, *et al.*, 2002). Furthermore, Arredondo *et al.* (2006) observed that iron bioavailability for bread fortified with H-reduced iron, using the *in vitro* digestion/human colonic adenocarcinoma (Caco-2) cell model, was 68.2 and 31.1% for particle sizes of 8 and <45 μm, respectively.

TABLE 25.2 Relative Bioavailability (RBV) of Elemental Iron Powders in Humans[a]

Reference	Iron Powder	Particle Size (μm)	RBV (%)
Höglund and Reizenstein (1969)	Reduced (Amersham and Studsvik)	48%, >30	15.2
		97%, ~5	45.5
Cook *et al*. (1973)	H-reduced (Abbott Laboratories, USA)	5–10	95
Björn -Rasmussen *et al*. (1977)	H-reduced (Elektrokemiska, Sweden)	?	13–90[b]
Hallberg *et al*. (1986)	Carbonyl (BASF, Germany)	?	5.5–17.9[b]
Forbes *et al*. (1989)	Electrolytic	10-30	74.6[c]
Walter *et al*. (2004)	H-reduced (Trace Sciences, Canada)	Average, 15	65
Hoppe *et al*. (2006)	Atomized, Atomet 95SP (Canada)	<45	36[d]
	H-reduced, Hi-Sol (USA)	<45	50[d]
	H-reduced, AC-325 (USA)	<45	56[d]
	Carbonyl, Ferronyl (USA)	<45	58[d]
	Carbonyl, CF (Germany)	<45	37[d]
	Electrolytic, A-131 (USA)	<45	65[d]
	Electrolytic, Electrolytic (India)	<45	59[d]
Swain *et al*. (2006)	Electrolytic, A-131 (USA)	<45	4–15[c]

[a]*Bioavailability of elemental iron powders relative to ferrous sulfate (RBV) in low-extraction wheat bread rolls; studies in humans with stable or radioactive isotopes. Results are expressed as mean.*
[b]*Bioavailability relative to intrinsic iron.*
[c]*Wheat farina.*
[d]*RBV was obtained by comparing the increase in serum iron concentration induced by the elemental iron with the increase induced by $FeSO_4$.*

TABLE 25.3 Relative Bioavailability (RBV) of Commercial Elemental Iron Powders in Humans[a]

Elemental Iron Powder[b]	RBV (%)
Carbonyl, Ferronyl (USA)	64
Electrolytic, A-131 (USA)	54
Electrolytic, Electrolytic (India)	46
H-reduced, AC-325 (USA)	42
Atomized, Atomet 95SP (Canada)	24
CO-reduced, RSI-325 (Sweden)	21

Source: Modified from Swain *et al*. (2003).
[a]*Bioavailability of commercial elemental iron powders relative to ferrous sulfate (RBV) in rats. Results are expressed as mean.*
[b]*Less than 45 μm particle size.*

TECHNOLOGICAL ISSUES

The selection of the iron fortificant is challenging because several factors need to be considered, including the cost, chemical reactivity, and bioavailability of the iron compound; the quantity of iron added; possible sensory changes in wheat flour during time at temperature and humidity conditions of storage; and the phytate content of wheat flour.

H-reduced powders and atomized iron, the most used forms of fortificant iron in wheat fortification, have the lowest cost, followed by electrolytic iron, ferrous sulfate, and ferrous fumarate. Microencapsulation of ferrous sulfate or ferrous fumarate overcomes organoleptic problems without modifying iron bioavailability, but it increases considerably the cost of these iron compounds. NaFeEDTA is two or three times more expensive than ferrous sulfate.

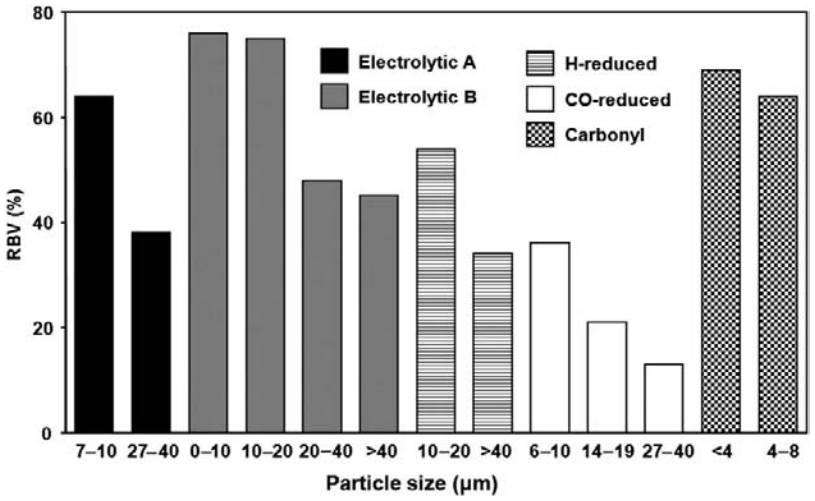

FIGURE 25.1
Effect of iron particle size on relative bioavailability (RBV) to ferrous sulfate of elemental iron powders. Smaller particle size elemental iron powders have better relative bioavailability to ferrous sulfate than that of coarse iron powders. Results are expressed as mean. *Source: Based on data from Hurrell* et al. *(2002).*

ENHANCING THE IRON ABSORPTION OF FORTIFIED WHEAT FLOUR

One of the recommended strategies to improve the bioavailability of iron is to add ascorbic acid. This vitamin increases iron absorption of both native food iron and fortification iron; however, there is evidence that this promoter effect may be less marked on elemental iron compounds (Swain *et al.*, 2006). Nevertheless, this vitamin is thermolabile, and it is destroyed during the process of baking. In contrast, ascorbyl palmitate, a synthetic ester composed of palmitic acid and L-ascorbic acid, is thermostable, and its reductive and vitamin properties are maintained even when exposed to baking temperatures. Pizarro *et al.* (2006) have demonstrated that the proportion of fortification iron absorbed as ferrous sulfate in bread is increased when this food is co-fortified with this compound at a molar ratio to iron $\geq$2:1 (Figure 25.2). Iron absorption may also be increased by promoting the consumption of wheat flour fortified products with foods rich in organic acids such as citric acid. Iron absorption from wheat flour noodles fortified with ferrous sulfate is increased 1.8 times when this product is consumed with lemonade (Olivares *et al.*, 2007). Another strategy is to add EDTA, a compound that protects fortification iron from the effects of iron absorption inhibitors such as phytates and

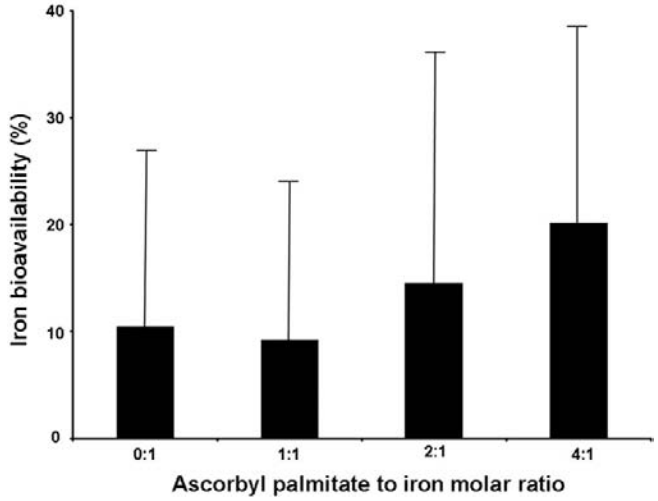

FIGURE 25.2
Iron absorption from iron-fortified wheat flour with or without ascorbyl palmitate. Ascorbyl palmitate at molar ratios to iron $\geq$2:1 significantly increased iron absorption of bread fortified with ferrous sulfate (ANOVA for repeated measures < 0.001). Results are expressed as mean $\pm$ SD, $n = 14$. *Source: Based on data from Pizarro* et al. *(2006).*

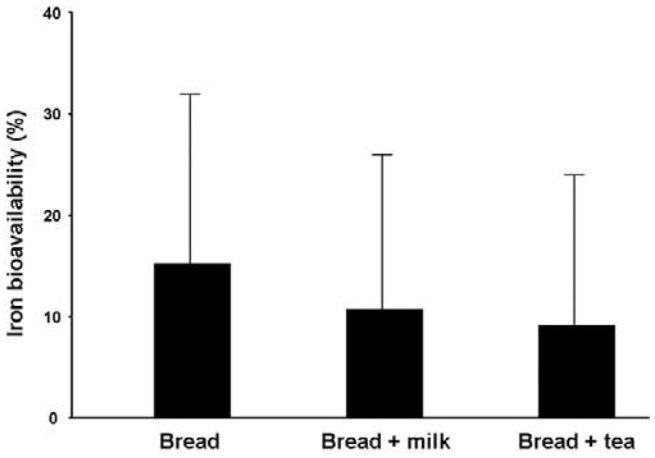

FIGURE 25.3
The effect of milk and tea on iron absorption from iron-fortified bread. Milk or tea significantly decreased iron absorption of bread fortified with ferrous sulfate ($p < 0.01$). Results are expressed as mean ± SD, $n = 9$. *Source: Based on data from Peña et al. (1991).*

polyphenols. A further approach is to avoid the consumption of fortified wheat flour products with foods rich in inhibitors of iron absorption (Peña *et al.*, 1991). Milk and tea decrease iron absorption of fortified bread with ferrous sulfate by 26 and 35%, respectively (Figure 25.3).

SUMMARY POINTS

- Studies in humans and rats demonstrate that iron from commercially elemental iron powders is 12−70% as bioavailable as iron from bakery-grade ferrous sulfate.
- Electrolytic and carbonyl iron has better bioavailability (~50% that of ferrous sulfate) than H-reduced, atomized, or CO-reduced iron.
- There is a negative relationship between dissolution rates in 0.1 M HCl acid and the median particle size of elemental iron powder, and there is a stronger positive relationship between the reactive surface area and the dissolution rate.
- Iron absorption of elemental iron powder is high when the particle size is small, uniform, and more soluble in diluted acid.
- Small particle size elemental iron powders, usually employed at the laboratory level due to their high solubility, have a bioavailability that is similar to that of ferrous sulfate. However, this iron absorption represents the maximum possible rather than the value that is obtained when food-graded elemental iron powders are utilized in flour fortification.

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CHAPTER 26

Iodine Fortification of Bread: Experiences from Australia and New Zealand

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CHAPTER OUTLINE

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LIST OF ABBREVIATIONS

EAR Estimated Average Requirement
MUIC Median urinary iodine concentration
UL Upper Level of Intake

INTRODUCTION

Internationally, iodine deficiency remains a leading cause of preventable mental disability (International Council for Control of Iodine Deficiency Disorders (ICCIDD)/United Nations Children's Fund (UNICEF)/World Health Organization (WHO), 2007). Unlike most nutrient deficiencies, which predominantly affect less affluent societies and individuals, iodine deficiency is found in both economically poor and affluent regions. Population iodine status is influenced by the amount of iodine in the environment, which determines the amount in locally grown foods.

WHO recommends Universal Salt Iodization (the iodization of all salt added to human and animal food) as the preferred approach to addressing iodine deficiency in human populations (ICCIDD/UNICEF/WHO, 2007). This has been effective in developing countries in which the majority of food consumed is prepared from raw ingredients in the household. However, there are substantial barriers to implementing such an approach in developed countries with complex food economies, considerable international trade, and a strong demand for consumer choice.

Foods ideally suited for fortification to address population-wide deficiencies include those widely and regularly consumed and that also support other nutritional health objectives. Bread and flours are ideal vehicles for fortification in cultures that rely on them as staples.

Mandatory fortification of bread with iodine was introduced in Australia and New Zealand in 2009. All salt used in bread making is required to be iodized at 25–65 mg/kg salt with the exception of bread represented as organic (Food Standards Australia New Zealand (FSANZ), 2008a,b). This chapter outlines the steps in the selection of bread as the food vehicle for iodine and the associated challenges.

FUNCTIONS AND REQUIREMENTS OF IODINE AND CONSEQUENCES OF DEFICIENCY

Iodine is an essential component of the thyroid hormones thyroxine and triiodothyronine, which regulate metabolism throughout life and influence fetal and childhood physical and cognitive development (ICCIDD/UNICEF/WHO, 2007). Hence, adequate iodine nutrition for pregnant women and children, especially young children, is particularly important.

Australia and New Zealand have a common set of nutrient intake recommendations for iodine (National Health and Medical Research Council/Ministry of Health, 2006), including Estimated Average Requirement (EAR; a daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group), Recommended Dietary Intake (the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97 or 98%) healthy individuals in a particular life stage and gender group), and Upper Level of Intake (UL; the highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population) (Table 26.1).

Cretinism and large deficits in intelligence are the most serious consequences of severe iodine deficiency (ICCIDD/UNICEF/WHO, 2007). The more subtle consequences of mild and moderate iodine deficiency have been demonstrated. In children, mild deficiency can cause small cognitive deficits and impaired motor control (Santiago-Fernandez *et al.*, 2004), as well as reduced growth (Zimmerman *et al.*, 2007). Providing sufficient iodine to school-aged children can partially reverse these (Gordon *et al.*, 2009; Zimmerman *et al.*, 2007).

TABLE 26.1 Iodine Nutrient Reference Values for Australia and New Zealand by Age and Physiological State

Age (Years)	Estimated Average Requirement (μg/Day)	Recommended Dietary Intake (μg/Day)	Upper Level of Intake (μg/Day)
1–3	65	90	200
4–8	65	90	300
9–13	75	120	600
14–18	95	150	900
Adults 19+	100	150	1100
Pregnant women			
14–18	160	220	900
19–50	160	220	1100
Lactating women			
14–18	190	270	900
19–50	190	270	1100

Source: National Health and Medical Research Council/Ministry of Health (2006).

ASSESSMENT AND CATEGORIZATION OF IODINE DEFICIENCY

Population iodine status is normally determined by measuring urinary iodine concentration, which closely reflects recent iodine intake (ICCIDD/UNICEF/WHO, 2007). The median urinary iodine concentration (MUIC) of schoolchildren is often used as an indicator of overall population status, with a MUIC of 100–200 μg/l considered optimal (ICCIDD/UNICEF/WHO, 2007). As shown in Table 26.2, pregnant women have higher cutoffs due to greater iodine requirements.

THE RE-EMERGENCE OF IODINE DEFICIENCY IN AUSTRALIA AND NEW ZEALAND

Following a series of reports suggesting iodine deficiency had re-emerged in New Zealand and areas of Australia, national surveys in both countries confirmed both populations were mildly deficient (Table 26.3) (Li *et al.*, 2006; Parnell *et al.*, 2003). The Australian survey revealed that iodine deficiency was concentrated in the southeast, where the majority of the population resides (Li *et al.*, 2006).

TABLE 26.2 International Council for the Control of Iodine Deficiency Diseases Criteria for Assessing Iodine Status in Different Groups

		Iodine Status by Group		
MUIC (μg/l)	Status of Population	School-Aged Children and Adults	Children <2 Years and Lactating Women	Pregnant Women
<20	Severe deficiency	Insufficient	Insufficient	Insufficient
20–49	Moderate deficiency	Insufficient	Insufficient	Insufficient
50–99	Mild deficiency	Insufficient	Insufficient	Insufficient
100–<150	Optimal	Adequate	Adequate	Insufficient
150–199		Adequate	Adequate	Adequate
200–299	Risk of iodine-induced hyperthyroidism in susceptible groups	More than adequate	No specific classifications for these groups	Above requirements at 250 (μg/l)
>300	Risk of adverse consequences	Excessive		In excess of requirements

MUIC, median urinary iodine concentration.
Source: ICCIDD/UNICEF/WHO (2007).

TABLE 26.3 Iodine Status of Different Groups in Australia and New Zealand

State/Region	Group	Sample Size	Median Urinary Iodine Concentration (μg/l)	Iodine Status
Australia				
New South Wales	Children (8–10 years)	427	89	Mild deficiency
Victoria	Children (8–10 years)	348	74	Mild deficiency
South Australia	Children (8–10 years)	317	101	Borderline
Western Australia	Children (8–10 years)	323	143	Adequate
Queensland	Children (8–10 years)	294	137	Adequate
Tasmania	Boys (4–17 years)	126	84	Mild deficiency
Tasmania	Girls (4–17 years)	99	81	Mild deficiency
New South Wales	Pregnant women	796	85	Insufficient
Victoria	Pregnant women	752	52–61	Insufficient
New Zealand				
Nationwide	Children (7–14 years)	1793	66	Mild deficiency
Otago	Breast-fed infants	43	44	Insufficient
Otago	Formula-fed infants	51	99	Insufficient

Source: FSANZ (2008a,b).

Fortification of bread with iodine in Tasmania

The Australian island state of Tasmania was the first region to identify the re-emergence of population mild iodine deficiency in the late 1990s (Hynes *et al.*, 2004). Aware of mild iodine deficiency re-emerging in other Australian states and New Zealand, the Tasmanian government wrote to the binational food regulatory body in July 2000 requesting an investigation into possible solutions for both countries.

An expert committee advised the Tasmanian government of its concern regarding the ongoing impact of mild iodine deficiency on fetal and infant brain development. The Tasmanian government recognized that achieving a binational solution required agreement from all jurisdictions in Australia and New Zealand and would take time to achieve. Therefore, an interim program to address iodine deficiency was adopted within Tasmania.

A number of interim strategies were considered, including fortifying milk, bread, flour, cattle feed, and/or the water supply; using iodine-enriched fertilizer for food crops; restricting the sale of non-iodized salt; and, providing iodine tablets.

Voluntary replacement of regular with iodized salt in bread was selected to address iodine deficiency. Bread is a nutritious locally produced dietary staple of much of the population. Key bread industry partners were supportive, and no legislative change was required. It was anticipated that previous experience of bread fortification via iodized bread improver in the 1970s would translate into broad acceptance of fortification by consumers.

Dietary intake modeling indicated a 30–60 μg/day median increase in iodine intake, and intakes among high consumers (95th percentile for males aged 19–24 years) were well within safe ranges (Seal, 2007). Testing by a leading bakery indicated no changes to taste, texture, product quality, or any technical problems from using iodized salt in bread. A comparison of standard and iodine-fortified bread consumption in 22 volunteers found a 14 μg/slice increase in urinary iodine excretion with fortified bread; consistent with predicted increases (Seal, 2007).

In September 2001, the Tasmanian health department developed a memorandum of understanding with local bakeries in lieu of formal regulation. Signatories agreed to use salt iodized in accordance with Australian food regulations in place of regular salt in bread for the Tasmanian market and also to advise the health department if they chose to resume manufacturing bread using non-iodized salt. In return, the Tasmanian health department

agreed to continue monitoring population iodine status, conduct random bread sampling to test compliance, actively promote bread as a source of iodine, liaise with local and national government, and review the program after 6 months.

Outcomes

Of the six major bakery chains in Tasmania at the time, four agreed to participate in bread fortification. Two did not agree because they were associated with national supermarket chains and baked their bread from premixes formulated interstate for stores throughout Australia. Most independent bakeries chose not to sign the memorandum of understanding, citing a lack of understanding of potential legal implications (Turnbull *et al.*, 2004). Nonetheless, a 2003 survey of independent bakeries concluded that the program had been widely adopted with minimal impact on cost or consumer satisfaction (Turnbull *et al.*, 2004). Approximately 70% of independent bakeries reported using iodized salt, none of which had reported reverting to regular salt. Among bakeries not using iodized salt, the use of salt-containing premixes or frozen dough formulated interstate was cited as the main barrier. Industry partners estimated the level of participation observed by major and independent bakeries accounted for approximately 80% of the bread available for consumption in Tasmania.

A 2004 survey of bread from the major bread manufacturers and 11 randomly selected independent bakeries reporting use of iodized salt found a median iodine concentration of 35 μg/100 g of bread, with the majority of samples falling between 20 and 70 μg/100 g (Seal, 2007). This may reflect batch-to-batch variation in salt iodine concentration and/or brand differences in salt addition to bread.

Comparisons of pre- and post-intervention surveys suggest a modest but significant increase in iodine population status consistent with predictions (Figure 26.1). The observed increase in MUIC of 30−38 μg/l in schoolchildren shifted the population into the lower end of the optimal range (Seal *et al.*, 2007). However, post-intervention surveys of pregnant women revealed that iodine status continued to be inadequate (Burgess *et al.*, 2007).

The voluntary approach to bread iodization adopted by Tasmania demonstrated that meaningful changes to population iodine status can be achieved through bakeries switching to iodized salt. However, not all bakeries adopted this approach, and the improvement in iodine status among pregnant women was not enough to achieve the desired outcome.

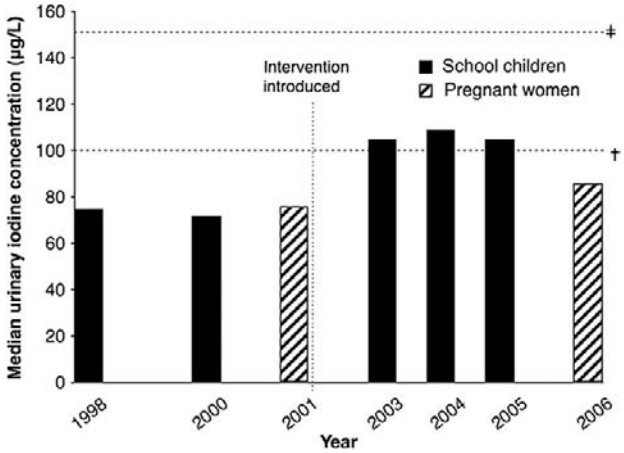

FIGURE 26.1
Median urinary iodine concentrations in children and pregnant women before and after the introduction of voluntary use of iodized salt in bread. †Median urinary iodine concentration (MUIC; 100 μg/l) cut point for adequate iodine status in the general population. ‡MUIC (150 μg/l) cut point for adequate iodine status in pregnant women. *Source: ICCIDD/UNICEF/WHO (2007).*

Development of binational mandatory fortification of bread with iodine

The binational food standards setting agency, FSANZ, was asked to explore fortification options to address iodine deficiency in both countries. The key processes employed are described here.

DIETARY INTAKE ASSESSMENT METHODS

Estimation of iodine intake in different segments of the population following fortification was essential for establishing safe and effective fortification levels and selecting appropriate food vehicles. The core objective was to optimize efficacy, by maximizing the proportion of different subpopulations with intakes above their EAR (known as the EAR cut-point method, this provides a close approximation of the proportion of a population with inadequate intakes of a nutrient provided certain criteria are met; Health Canada, 2006), and to mitigate risk by minimizing the proportion of subpopulations above their UL.

Multiple delivery vehicle combinations and iodine concentrations were modeled using the custom-made software DIAMOND (DIetAry Modelling Of Nutritional Data). This program merges regularly updated nutrient composition data with dietary intake data sets from two discrete national nutrition surveys that each collected one 24-h recall from all responders and a second recall from a subset of responders. The software uses analysis of variance to calculate the correction factors for various population subgroups to remove within-person variance and estimate a "usual" intake distribution of a nutrient for the population groups of interest.

There were challenges specific to the modeling of iodine fortification via iodized salt. The first was that food composition tables commonly just give the total sodium content of food. However, some sodium is present naturally, and not all added sodium is from sodium chloride (e.g., sodium bicarbonate for leavening and sodium cyclamate for sweetening). Failure to account for this would have overestimated the effect of fortification for some foods. Furthermore, salt reduction initiatives meant older food composition data were out-of-date. Iodine concentration data in existing databases were limited, and detailed data on iodized salt consumption were unavailable. Therefore, ingredient lists and direct analysis were used to develop new salt composition databases, analyses of the iodine content of core foods were commissioned, and sales data for iodized table/cooking salt were used to estimate intakes. Sales data probably overestimate mean intake because waste and non-food use for salt cannot be separated from consumption.

PROJECTED IMPACT OF BREAD FORTIFICATION

A range of options were modeled. Figure 26.2 shows the mean iodine intake prior to fortification in Australian children aged 2 to 3 years and Australian and New Zealand women aged 16–44 years (i.e., childbearing age). As the range of food vehicles increased from bread alone to all foods containing added salt, the concentration of iodine in salt needed to concomitantly decrease to avoid a substantial proportion of the population exceeding the UL for iodine. Specifically, the increase in mean intake using 45 mg/kg salt in bread alone was similar to the projected result if salt was iodized at 15 mg/kg in all salted foods.

Before fortification, mean iodine intake was similar across age groups (Table 26.4). Lower absolute iodine requirements in children meant that a smaller proportion of children had inadequate intakes relative to adults. Baseline iodine intakes were consistent with results from the surveys measuring urinary iodine in Australian children (see Table 26.3). Because only limited relevant dietary intake data in children were available for New Zealand, the results from the Australian data were extrapolated to New Zealand.

Bread fortification is projected to increase mean iodine intake by 54 and 84 μg/day in Australians aged 2 years or older and New Zealanders aged 15 years or older (see Table 26.4), respectively. The higher salt content of New Zealand bread at the time accounts for most of this

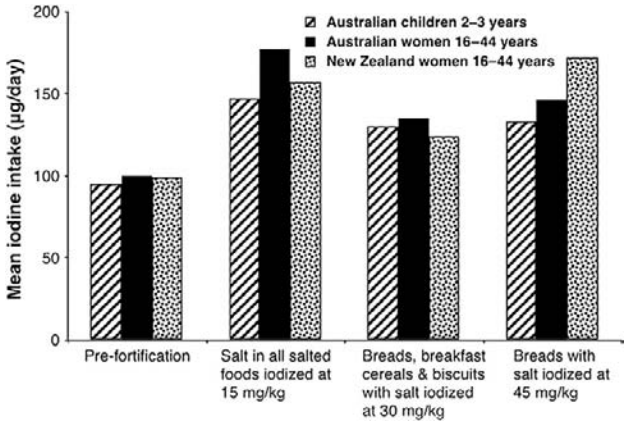

FIGURE 26.2
Estimated mean daily intake of iodine at baseline and under various fortification options. *Source: FSANZ (2008a,b).*

difference. The increase was similar across age groups in Australia—for example, 38 µg/day in 2- to 3-year-olds and 46 µg/day in women of childbearing age. The much lower UL for young children meant that the proportion of young children exceeding the UL was 6% at a fortification level, whereas 9% of women of reproductive age still had inadequate intakes.

Increasing the proportion of adults with adequate intakes by adding more iodine would further increase the proportion of young children with high intakes. Consequently, it was not feasible to meet the substantially higher iodine requirements of pregnant and lactating women, whose EAR is close to the UL for young children, without large numbers of young children exceeding their UL. Food intakes in these groups were examined in detail, but no foods suitable for fortification—and with high, widespread consumption by adult women but

TABLE 26.4 Mean Prefortification and Projected Postfortification Iodine Intakes, Percentage below Estimated Average Requirements, and Percentage above the Upper Level of Intake for Selected Groups

	Prefortification			Projected Postfortification		
Age (Years)/Group	**Mean (µg)**	**% <EAR**	**% >UL**	**Mean (µg)**	**% <EAR**	**% >UL**
Australia						
2–3	95	16	0	133	1	6
4–8	94	18	0	139	1	<1
14–18	121	35	0	179	4	0
19–29	119	41	0	177	6	0
30–49	110	47	0	166	5	0
Women 16–44	100	59	0	146	9	0
Pregnancy[a]	—	93	0	—	71	0
Lactation[a]	—	97	0	—	88	0
New Zealand						
15–18	106	27	0	193	0	0
19–29	106	49	0	190	0	0
30–49	109	46	0	195	0	0
Women 16–44	99	68	0	172	0	0
Pregnancy[a]	—	97	0	—	45	0
Lactation[a]	—	99	0	—	77	0

EAR, Estimated Average Requirement; UL, Upper Level of Intake.
Source: FSANZ (2008a,b).
[a]*The numbers of pregnant and lactating women in the nutrition surveys underpinning these predictions were too small to allow these groups to be modeled separately. Therefore, adequacy of intake in these groups was extrapolated by applying the dietary requirements for pregnant women and for lactating women to all sampled women aged 16–44 years. This may underestimate the total iodine intake because it does not allow for increased food intake during pregnancy or lactation.*

not young children—were found. A decision was made not to further increase the concentration of iodine in salt but, rather, to refer the need for an iodine supplementation initiative for pregnant and lactating women to relevant government bodies.

ADDITIONAL CONSIDERATIONS IN SELECTING BREAD AS THE DELIVERY VEHICLE FOR IODINE

As noted previously, the recommendation to use iodized salt in all foods containing added salt is problematic. As the number of foods covered by mandatory fortification increases, the costs to industry, trade barriers, the cost and complexity of enforcement, and the need to overcome technical difficulties increase while consumer choice decreases. Furthermore, government policy in both Australia and New Zealand requires that the least amount of regulation needed to achieve a purpose be adopted.

All changes to food regulations in Australia and New Zealand are subject to core objectives relating to public health and safety and the provision of adequate and non-misleading information (Australian Government, 1991). Furthermore, changes should be evidence based, and they should promote consistency between domestic and international food standards, an efficient and internationally competitive food industry, and fair trading in food. Guidelines specified that mandatory fortification should (Australian Government, 2009):

- be required only in response to demonstrated significant population health need, taking into account both the severity and the prevalence of the health problem to be addressed;
- be required only if it is assessed as the most effective public health strategy to address the health problem;
- be consistent as far as is possible with the national nutrition policies and guidelines of Australia and New Zealand;
- ensure that the added vitamins and minerals are present in the food at levels that will not result in detrimental excesses or imbalances of vitamins and minerals in the context of total intake across the general population; and
- ensure that the mandatory fortification delivers effective amounts of added vitamins and minerals with the specific effect to the target population to meet the health objective.

The selection of food vehicles for iodine fortification was therefore iterative. Fortification of all processed foods, a close approximation of Universal Salt Iodization, was considered, as was the option of fortifying bread, breakfast cereals, and biscuits (i.e., cookies and crackers). The efficacy of each option was similar (see Figure 26.2), but the burden of regulation decreased as foods were incrementally removed from the mandatory fortification model. Consultation identified a technical difficulty for one of the region's leading breakfast cereal manufacturers. Testing indicated that its salt addition method, involving a brine system, would result in highly variable and sometimes unsafe iodine concentrations in its products. Biscuits contributed least to increasing the population's iodine intake but posed the greatest impost on both import and export trade; there were also negative reactions from public health sectors to fortifying a "snack" food.

The final selection of bread as the food vehicle was consistent with the aforementioned guidelines and was supported by Tasmanian findings and extensive dietary modeling. Because bread is predominantly produced and consumed domestically, there were fewer trade issues than for other foods. Bread also contains a relatively narrow range of salt, making it easier to predict iodine intakes at the extremes of the distribution.

ECONOMIC ANALYSIS

Cost/benefit and cost-effectiveness analyses assessed the feasibility of using mandatory iodine fortification to address iodine deficiency in Australia and New Zealand (FSANZ, 2008b). Estimating the economic benefit of fortification proved difficult because the consequences of mild and moderate iodine deficiency, although clearly supported by research, were not well

quantified or easily costed even when quantified. Despite these limitations, both analyses concluded that the cost of moving individuals from deficiency to adequacy was small, especially compared with the potential benefits to health and productivity. The ongoing cost of fortification was estimated to be less than four cents per capita each year based on 2007 prices.

REACTIONS TO THE PROPOSAL OF MANDATORY FORTIFICATION

Changes to food regulation in Australia and New Zealand require formal public consultation. Four rounds of public consultation and targeted consultations with relevant parties were conducted for mandatory iodine fortification.

The majority of government, health professionals, and consumer organizations supported the proposed mandatory fortification. In contrast, the majority of industry representatives opposed mandatory fortification, citing the increased regulatory burden, increased costs, reduced consumer choice, and trade impacts as reasons for their opposition. Technical issues were also raised. These are discussed later.

Among health professionals, many noted the proposed fortification would not fully meet the needs of pregnant and lactating women and viewed it as an initial step in a broader approach. Some maintained that Universal Salt Iodization would provide higher intakes in these two groups. Some challenged the decision to minimize exceedance of UL in young children and urged review of the UL. Others viewed the initiative as promoting iodized salt and expressed concern that it might conflict with health messages to reduce sodium intake and/or prompt bakers to maintain or increase the amount of salt in bread. Health professionals generally agreed that mandatory fortification would provide greater certainty, sustainability, and equity than voluntary fortification.

Some consumers, especially in New Zealand, were concerned about reduced consumer choice and thought mandatory fortification of bread would alter the perception of bread as a "wholesome health product." Given the variable prevalence of iodine deficiency across Australia, some people considered nationwide mandatory fortification to be inappropriate. Individuals with a thyroid condition or an iodine sensitivity were concerned that they would be adversely affected by increased iodine in the food supply.

All groups agreed that comprehensive monitoring of the effects of mandatory fortification was needed. The majority considered that the greater public good outweighed the small and manageable risks associated with mandatory iodine fortification.

Implementation of mandatory iodine fortification

The mandatory iodine fortification regulation specified a transition period. This allowed salt manufacturers to increase their production of iodized salt and bakers to transition to using iodized salt and to alter packaging to declare "iodized salt" in the ingredients list. The period aligned with another major initiative, the introduction of mandatory fortification of bread-making wheat flour with folic acid, to reduce a key cost to industry by allowing one set of food label changes.

An early communication strategy was developed and modified throughout the mandatory fortification project. Its purpose changed from raising awareness of the consultations conducted prior to regulation to informing the public and industry of the new fortification requirement.

Consultation continued after the regulation was gazetted to assist industry compliance with fortification requirements. A guide outlining the responsibilities of the salt and bread industries was prepared and distributed. Information material for the public and health professionals was developed and distributed via newspapers, professional groups, websites, and in response to enquiries. Relevant health bodies have prepared recommendations for iodine supplementation during pregnancy and lactation.

Monitoring of mandatory fortification

Australian and New Zealand health authorities will monitor the effects of the initiative to ensure that it is safe and effective in addressing the iodine deficiency. Monitoring will focus on:

- ensuring industry compliance with the regulation;
- assessment of the iodine content of bread and other foods;
- collection of dietary intake and urinary iodine excretion data to assess status; and
- surveillance of the effect of iodine fortification on related health parameters (e.g., rates of thyroid disease) and assessment of consumer awareness, attitudes, and behavior with respect to fortified products.

TECHNOLOGICAL ISSUES

Ongoing consultations identified at least one bread manufacturer using brine to add salt to bread. Given the technical difficulty previously identified by a brine-using breakfast cereal manufacturer, a feasibility and safety assessment was undertaken for the addition of iodized salt to bread using a brine solution (FSANZ, 2008b). The key finding was that provided the iodized salt is completely dissolved, the addition of brine to dough is unproblematic, and iodine addition can be expected to be at least as effective as dry salt addition.

Although no technological issues associated with adding iodized brine solutions to bread were identified, the potential difficulty for those bakeries that do export bread to adjust their process lines to manufacture both export products without iodine and domestic bread with iodine was noted.

DISTRIBUTION RANGE OF IODINE IN SALT

Dietary intake modeling identified a mean iodine concentration of 45 mg/kg salt for use in bread to help address the re-emergence of iodine deficiency for most of the population. This was consistent with the existing permission to iodize salt voluntarily within a range of 25–65 mg/kg salt. A narrower range was initially considered to further improve consistency of the amount of iodine delivered via fortification. However, consultations with, and analyses provided by, the salt industry indicated that the existing range could be achieved consistently, whereas a narrower range could not. The existing permission of 25–65 mg iodine/kg salt was therefore retained.

SUMMARY POINTS

- Mandatory fortification of bread with iodine, using salt iodized at a concentration of 25–65 mg iodine/kg salt, was introduced in Australia and New Zealand in 2009 in response to re-emerging iodine deficiency.
- Bread was selected as the food vehicle because it is widely consumed by the population. Also, iodine fortification of bread is effective and safe, is technologically feasible, has minimal trade impacts, and is consistent with government polices and guidelines.
- Selecting an appropriate food vehicle required a combination of dietary intake and food composition data, biochemical surveys of population nutrient status, an assessment of technological issues, and consultation with affected parties.
- In Australia and New Zealand, use of iodized salt in bread was projected to result in similar iodine intake improvements as those predicted for use of iodized salt in all salted processed foods, provided iodine concentrations were adjusted accordingly.
- Mandatory iodine fortification could not deliver sufficient amounts of iodine to fully accommodate the elevated requirements of pregnant and breast-feeding women without causing large numbers of young children to exceed their Upper Level of Intake.
- To ensure the successful implementation of mandatory iodine fortification, sufficient time was allocated to allow the salt industry and bread manufacturers to change their processes and food labels.

- A monitoring system was established to ensure the ongoing safety and effectiveness of the mandatory iodine fortification.

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CHAPTER 27

Phytochemical Fortification of Flour and Bread

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

ALA α-Linolenic acid
DHA Docosahexaenoic acid
EPA Eicosapentaenoic acid
GSE Grape seed extract
GTE Green tea extract
LC Long chain
LDL Low-density lipoprotein
MDF Mango dietary fiber
MTO Microencapsulated tuna oil
PGBR Pregerminated brown rice
PUFA Polyunsaturated fatty acids
TC Total cholesterol
TG Triglyceride

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INTRODUCTION

Bread is the major product among baked foods and consumed worldwide in relatively large amounts. The demand for value-added cereal products has been growing steadily as consumers realize that food with health benefits is better than a supplement. Among different food systems, baked products provide an excellent opportunity to incorporate bioactive compounds. With appropriate formula and/or process optimization, breads with acceptable quality can be produced with the addition of phytochemical-based ingredients. This chapter reviews the use of health-beneficial phytochemicals in the bread making process.

FUNCTIONAL BREAD

In many countries, bread is a staple food product, and depending on the regional traditions, bread products and their production techniques vary widely. The basic ingredients are flour, water, yeast or other leavening agent, and salt (Sluimer, 2005). During bread making, the availability and levels of bioactive compounds in cereal grains can either decrease or increase. The interactions of ingredients are also important and affect the nutritional value of bread. Various ingredients can be used to improve processing ability of dough and the quality and nutritional value of the final product. Breads formulated with functional ingredients are becoming more important in the bakery industry, and functional breads are already available (Menrad, 2003). In August 2000, the Fazer Company in Finland made a request for plant sterol-enriched bakery products. The European Union Scientific Committee on Food confirmed with regard to the application of Fazer that the addition of phytosterols to a wide range of bakery products was safe. In January 2006, the request was granted for "rye bread $\geq$50% whole grain rye flour, $<$30% wheat flour, $<$4% added sugar to rye bread, and no added fat in the rye bread." Intake of plant sterols should not exceed 3 g per day. Later, the rye product was introduced into the marketplace (Anonymous, 2006).

In The Netherlands, independent experts appointed by The Netherlands Nutrition Centre approved claims for specific brands of bread with added OatWell oat bran, with Frutafit inulin, and with *n*-3 fatty acids (same as fish). The formulations for the first two claims are as follows (Schmitz and Marquart, 2009):

Product: Pró-FIT bread, containing 2.2 g β-glucan from OatWell oat bran per 100 g bread.
Health benefit: Daily consumption of 140 g of Pró-FIT bread (four slices, providing 3 g β-glucan from oat bran per day) has been shown to reduce the serum concentration of low-density lipoprotein (LDL) by 3% within 3 weeks in persons with elevated cholesterol levels.

Product: Vitaalbrood flora bread, containing at least 5 g Frutafit inulin per 100 g.
Health benefit: Consumption of three slices of Vitaalbrood flora per day supports a well-balanced gut flora composition and colonic function by selectively stimulating the growth of *Bifidobacterium*.

These health benefits have been approved by the European Food Safety Authority (2006).

PHYTOCHEMICAL FORTIFICATION OF BREAD

Herbs and spices

Bread samples flavored with garlic in proportion of 0.5, 1, and 1.5% and with sweet basil in proportion of 5, 10, and 15% have been prepared and analyzed for antioxidant activity. The antioxidant capacity was 0.053–0.197 mM Fe^{2+}/100 g for bread flavored with garlic and 0.059–0.368 mM Fe^{2+}/100 g for bread flavored with basil, whereas the antioxidant capacity was 0.036 mM Fe^{2+}/100 g for the control sample. The polyphenol content was 0.179–0.221 mM

TABLE 27.1 Selected Properties of the Breads Used for an Intervention Study[a]

	Control Bread	Prebiotic-Only Bread	Prebiotic and Antioxidative Bread
Composition (%)			
Apple fiber	2.0	2.0	2.0
Sourdough	3.0	3.0	3.0
Malt flour	0.8	0.8	0.8
Sunflower seeds	10.0	10.0	10.0
Wheat flour	66.9	50.9	48.1
Rye bran	15.0	15.0	15.0
Salt	2.3	2.3	2.3
Soya	—	6.0	6.0
Inulin	—	4.0	4.0
Linseed	—	4.0	4.0
Wheat gluten	—	2.0	4.0
Green tea	—	—	0.5
Spices	—	—	0.7
Tomato	—	—	0.5
Wheat (Se rich)	—	—	1.0
Antioxidant activity ((mmol l^{-1})/100 g)			
TEAC (hydrophile)	0.441 ± 0.015	0.758 ± 0.002	1.274 ± 0.033
TEAC (lipophile)	0.036 ± 0.002	0.068 ± 0.026	2.185 ± 0.041

TEAC, Trolox-equivalent antioxidant capacity.
Source: Glei, M., Habermann, N., Osswald, K., Seidel, C., Persın, C., Jahreis, G., and Pool-Zobel, B. L. (2005). Assessment of DNA damage and its modulation by dietary and genetic factors in smokers using the Comet assay: A biomarker model. *Biomarkers* 10, 203–217. Reprinted by permission of Taylor & Francis Group (http://www.informaworld.com).
[a]*The control bread consists of the basic mixture with wheat flour, coarsely ground rye grain, malt flour, sour dough, apple fiber, salt, and wheat gluten. Prebiotic bread contains inulin, linseed, and soy flours, and the antioxidant bread contains selenium-rich wheat, tomato extract, green tea, and spice extract.*

gallic acid/100 g for bread flavored with garlic and 0.194–0.278 mM gallic acid/100 g for bread flavored with basil. The polyphenol content for the standard sample was 0.177 mM gallic acid/100 g (Raba *et al.*, 2007).

In a study investigating DNA damage and its modulation by dietary and genetic factors in smokers, control bread consisted of the basic mixture with wheat flour, coarsely ground rye grain, malt flour, sourdough, apple fiber, salt, and wheat gluten (Table 27.1). Prebiotic breads were supplemented with inulin, linseed, and soy flours, whereas the antioxidant bread was additionally supplemented with the antioxidative ingredients selenium-rich wheat, tomato extract, as well as green tea and spice extract (Glei *et al.*, 2005).

Omega-3 fatty acids

Yazawa *et al.* (2001) studied docosahexaenoic acid (DHA)-supplemented breads, one of which contained 1 g DHA and 0.3 g of eicosapentaenoic acid (EPA) (Table 27.2). Serum total cholesterol (TC) and triglyceride (TG) were significantly decreased 4 weeks following consumption (TC before and after ingestion, 232 and 222 mg/dl, respectively; TG before and after ingestion, 204 and 147 mg/dl, respectively) with a concurrent increase in serum DHA and EPA, suggesting that DHA-supplemented bread can be consumed every day and is clinically effective for lipid reduction.

The effects of low doses of LC *n*-3 PUFA (<100 mg/day) on plasma LC *n*-3 PUFA levels using a novel delivery form, bread containing microencapsulated tuna oil (MTO), were investigated. MTO bread contained approximately 80 mg of LC *n*-3 PUFA/four slices. Plasma triacylglycerol fatty acid compositions were measured after an overnight fast and postprandially at 2 and 4 h. This study showed that a low dose of LC *n*-3 PUFA, consumed as MTO-enriched bread, was bioavailable, as measured by an increase in LC *n*-3 PUFA levels in the plasma of human subjects (Yep *et al.*, 2002).

TABLE 27.2 Effect of Ingestion of DHA-Supplemented Bread for 4 Weeks on the Serum Total Cholesterol, Tryglyceride, HDL-C, and LDL-C of Volunteers with Hyperlipidemia[a]

Lipid Class	Before (mg/dl)[b]	After (mg/dl)[b]
Total cholesterol	232.4 (28.6)	221.9 (29.9)**
Triglyceride	203.8 (119.6)	146.7 (75.7)*
HDL-C	51.2 (17.3)	53.3 (18.0)
LDL-C	142.2 (33.1)	136.2 (26.2)

DHA, docosahexaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
Source: Reprinted with permission from Yazawa, K., Terano, T., and Matsui, T. (2001). Serum lipid lowering effect of DHA supplemented bread. *J. Oleo Sci*. 50, 673–675.
[a]*Bread contains 1 g DHA and 0.3 g of eicosapentaenoic acid.*
[b]*Mean (SD)*, n = *19.*
*$p < 0.05$.
**$p < 0.01$.

In subjects with hyperlipidemia, intake of bread containing a small amount of fish oil (1.3 g) resulted in a significant increase in *n*-3 fatty acids, an increase in high-density lipoprotein cholesterol, and a decrease in triglycerides, which may reduce the risk of ischemic heart disease (Liu *et al.*, 2001).

COMMERCIAL EXAMPLES

Omega-3 bread, which is formulated to improve heart health, was launched in the United States by George Weston Bakeries. Grains & More Double Omega bread claimed to contain 25 mg of *n*-3 EPA/DHA per slice. Other examples include Cali-Wraps, which are *n*-3-enriched tortilla wraps, and whole grain flax bread in Canada. In Australia, Up Omega-3 bread, under George Weston's TipTop brand, contains encapsulated fish oil. Weston's Arnold brand (Horsham, PA) formulates its Smart & Healthy Omega-3 DHA/EPA bread with fish oil. Bread containing a concentrated hydroxytyrosol, Hytolive 2, is made by Spanish company Genosa R&D. The ingredient has been added to Puratos' Nostrum brand bread, which is made with a mixture of heart-healthy cereals such as oat, wheat, and barley. The firm claims that hydroxytyrosol is a valuable antioxidant extracted from olives that can help prevent aging. Kroger's Active Lifestyle Honey Oat and Whole Grain bread and 100% Whole Wheat Bread contain Cargill's CoroWise Naturally Sourced Cholesterol Reducer brand of plant sterols.

Phenolics

Phenolic glucosides—secoisolariciresinol diglucoside, *p*-coumaric acid glucoside, and ferulic acid glucoside—have been analyzed in commercial breads containing flaxseed (Table 27.3). The total phenolic glucoside content ranges from 15 to 157 mg/100 g dry bread (Strandâs *et al.*, 2008).

Common buckwheat has been used to substitute 15% of wheat flour to make buckwheat-enhanced wheat breads. Buckwheat-enhanced wheat bread has good antioxidant activity, reducing power, and 1,1-diphenyl-2-picrylhydrazyl radical scavenging ability, with unhusked buckwheat-enhanced wheat bread being the most effective. Overall, buckwheat-enhanced wheat bread has been suggested as a food with more effective antioxidant properties than unenhanced ones (Lin *et al.*, 2009).

A green tea extract (GTE) has been incorporated (50, 100, and 150 mg/100 g of flour) into bread as a source of tea catechins (Table 27.4). One piece of bread (53 g) containing 150 mg of GTE/100 g of flour provides 28 mg of tea catechins, which is 35% of those infused from one green tea bag (2 g) (Wang and Zhou, 2004).

TABLE 27.3 Flaxseed Content (mg/100 g Dry Bread) and the Relative Composition (%) of the Phenolic Glucosides SDG, *p*-Coumaric Acid Glucoside, and Ferulic Acid Glucoside in Breads[a]

		Phenolic Glucoside Content			
Bread	Flaxseed content (g/100 g Fresh Weight)[b]	*p*-Coumaric Acid Glucoside	Ferulic Acid Glucoside	SDG Soft Bread[c]	Total
1	6.5	33 (21%)	18 (12%)	105 (67%)	157
2	n.g.[d]	21 (18%)	13 (11%)	81 (70%)	114
3	9	27 (23%)	15 (13%)	74 (64%)	116
4	3	21 (22%)	17 (18%)	56 (60%)	93
5	3.9	11 (16%)	12 (18%)	44 (65%)	67
6	n.g.	12 (21%)	8.3 (15%)	35 (64%)	55
7	2.5	7.2 (16%)	3.7 (8%)	35 (76%)	46
8	3	11 (25%)	7.7 (18%)	24 (56%)	42
9	n.g.	4.7 (18%)	4.4 (16%)	17 (66%)	27
10	2	3.9 (15%)	4.6 (18%)	17 (67%)	26
11	1.5	3.5 (15%)	5.3 (22%)	15 (63%)	24
12	n.g.	3.8 (26%)	3.3 (22%)	7.6 (52%)	15
Crisp bread[e]					
13	2.5	8.2 (15%)	5.4 (10%)	42 (75%)	55
14	2.2	8.2 (21%)	5.2 (14%)	25 (65%)	38
15	3	4.1 (17%)	5.2 (22%)	15 (62%)	24
16	5	9.4 (29%)	7.7 (25%)	14 (46%)	31
17	4	3.3 (18%)	7.2 (39%)	7.9 (43%)	18

SDG, secoisolariciresinol diglucoside.
Source: Reprinted from *Food Chem.,* 110, Strandâs, C., Kamal-Eldin, A., Andersson, R., and Âman, P., Phenolic glucosides in bread containing flaxseed, pp. 997–999, Copyright 2008, with permission from Elsevier.
[a]*Phenolic compounds have been analyzed in commercial breads containing flaxseed.*
[b]*The flaxseed content in the bread was obtained from the product label.*
[c]*The soft breads had dry weight content ranging from 57 to 69%.*
[d]*Flaxseed content was not given (n.g.) on the product label.*
[e]*The crisp breads had dry weight content ranging from 90 to 94%.*

TABLE 27.4 Relative Retention Rate of Green Tea Catechins and Caffeine in Bread[a]

Component	Bread with 50 mg of GTE/100 g of Flour (%)	Bread with 100 mg of GTE/100 g of Flour (%)	Bread with 150 mg of GTE/100 g of Flour (%)
(-)-EGCG	80.6 ± 3.0	86.2 ± 4.9	82.6 ± 2.5
(-)-ECG	93.3 ± 2.9	90.9 ± 4.1	90.1 ± 4.4
(-)-EGC	66.8 ± 7.1	67.8 ± 5.2	62.8 ± 7.3
(-)-EC	93.6 ± 3.4	95.9 ± 2.4	97.1 ± 3.9
(-)-GCG	83.2 ± 5.4	84.8 ± 4.2	85.9 ± 4.0
(-)-CG	94.3 ± 2.8	94.9 ± 3.1	99.6 ± 4.3
Caffeine	95.0 ± 4.6	95.7 ± 5.1	96.5 ± 5.5
Total catechins	83.7 ± 3.8	85.8 ± 4.2	83.7 ± 3.2

(-)-CG, catechin gallate; (-)-EC, epicatechin; (-)-ECG, epicatechin gallate; (-)-EGC, epigallocatechin; (-)-EGCG, epigallocatechin gallate; (-)-GCG, gallocatechin gallate; GTE, green tea extract.
Source: Reprinted with permission from Wang, R., and Zhou, W. (2004). Stability of tea catechins in the breadmaking process. *J. Agric. Food Chem*. 52, 8224–8229. Copyright 2004 American Chemical Society.
[a]*Green tea extract was incorporated into bread as a source of tea catechins. Data are expressed as mean ± standard deviation of 12 samples.*

The supplementation of bread with green coffee has been shown to improve the chemoprotective property of normal bread under *in vitro* cell culture conditions. Supplementation also increases chlorogenic acid content and antioxidative capacity. The treatment of the cells with supplemented bread increases resistance of colon and liver cells to H_2O_2, a source of oxidative stress (Glei *et al.,* 2006).

Grape seed extract (GSE), a well-known nutraceutical product, is an abundant source of catechins and proanthocyanidins with a strong antioxidant and free radical scavenging activity. Moreover, it shows other biological effects, such as inhibition of platelet aggregation and anti-inflammation and anti-ulcer activity. The change in antioxidant activity of breads with added GSE has been investigated. Bread with added GSE had stronger antioxidant activity than bread without GSE, and increasing the level of GSE further enhanced the antioxidant capacity of the bread. However, thermal processing caused the antioxidant activity of the GSE added to bread to decrease by approximately 30–40%. The findings indicate that GSE-fortified bread is promising as a functional food with high antioxidant activity (Peng *et al.*, 2010).

Lemon flavonoid prepared from lemon peel contains 30% eriocitrin, a potent antioxidant and a functional food material. Sixty-five percent of eriocitrin was retained in the bread, and 78% of the antioxidative activity remained after baking bread with added lemon flavonoid. Sensory tests showed that a desirable taste could be retained with the addition of up to 0.50% lemon flavonoid, but larger amounts gave the bread a bitter taste. Lemon flavonoid thus seems to be a useful food material for enhancing the functions of bread (Kanae *et al.*, 2008).

Lignans

Flaxseed has been identified as a potential functional food because of its high content of the phytochemicals α-linolenic acid (ALA) and lignans. Lignan has been shown to have a chemoprotective effect against cancer. Flaxseed can be used in baked goods because it has a minimal loss of ALA. However, ALA is susceptible to oxidation and the development of off-aromas and off-flavors in food. Conforti *et al.* (2009) determined the effectiveness of both synthetic and natural antioxidants incorporated into yeast bread that contained 15% flaxseed as a partial replacement for bread flour.

Phytosterols

The relative effects of phytosterol ester-enriched low-fat foods such as bread on serum lipids, plasma phytosterols, and carotenoids have been investigated. Table 27.5 shows that plasma sitosterol increased by 23% and campesterol by 52% with phytosterol-enriched bread, indicating that such products still delivered and released phytosterols to the gut (Clifton *et al.*, 2004).

Fructooligosaccharides

Mujoo and Ng (2003) studied bread baked from flour blended with immature wheat meal rich in fructooligosaccharides and found that the overall quality of bread appeared to be acceptable, and the added fructooligosaccharides were retained after baking.

TABLE 27.5 Plasma Lathosterol, Campesterol, and Sitosterol after Ingestion of Control Foods and Sterol-Enriched Foods[a]

	Control (n = 58)	Bread (n = 36)	Milk (n = 40)
Campesterol (mg/ml)	3.72[a] (1.61)	5.36[b] (2.22)	5.68[b] (2.19)
Sitosterol (mg/ml)	3.54[a] (1.84)	4.66[b] (2.74)	4.51[b] (2.12)

Source: Reprinted by permission from Macmillan Publishers Ltd: Clifton, P. M., Noakes, M., Sullivan, D., Erichsen, N., Ross, D., Annison, G., Fassoulakis, A., Cehun, M., and Netsel, P. (2004). Cholesterol-lowering effects of plant sterol esters differ in milk, yoghurt, bread and cereal. *Eur. J. Clin. Nutr*. 58, 503–509, copyright 2004.

[a]*The relative effects of phytosterol ester-enriched low-fat foods such as bread and milk on plasma phytosterols have been investigated. Values (SD) with different superscripts are significantly different ($p < 0.05$) from each other.*

Dietary fibers

Contrary to whole bread, which is relatively high in fiber content (7 or 8%, dry matter basis), white bread contains only 2 or 3% fiber on a dry matter basis. Fibers, particularly soluble ones such as inulin and oligofructose, might help to prevent diseases such as intestinal infections, colorectal cancer, obesity, cardiovascular diseases, and type II diabetes. Therefore, to improve the nutritional quality of white bread, new formulae enriched with fibers such as inulin should be developed (Poinot *et al.*, 2010).

Mango dietary fiber (MDF) concentrate showed low lipid and high starch contents and balanced soluble DF/insoluble DF levels, which is important for the functionality of fiber in the human diet. *In vitro* starch digestibility tests of MDF bakery products indicated a low predicted glycemic index. MDF might be an alternative for use in the development of products with balanced DF components and low glycemic response for people with special carbohydrate/energy requirements (Vergara Valencia *et al.*, 2007).

Inulin

Development of high-fiber white bread containing added inulin (approximately 3–5%) has also been described, as well as bread containing inulin plus other functional ingredients (Ca (as calcium lactate) or linseeds + Ca). Results showed it was possible to produce fiber-enriched white bread with good sensory, nutritional, and physicochemical properties. Bread containing inulin, Ca, and linseeds had a Ca content of 250 μg/100 g and contents of dietary fiber and essential fatty acids of 2.5 and 3.9 g/100 g, respectively (Draganov *et al.*, 2004).

Germinated grains

Pregerminated brown rice (PGBR) contains phytic acid, which has excellent health benefits such as an antioxidative effect, protecting against cardiovascular disease and preventing platelet aggregation. However, it suppresses the absorption of metallic ions into the body. PGBR with germ length of 0.5–1.0 mm is produced as a healthy food by immersing the brown rice in water to provide PGBR bread with high functional properties (Morita *et al.*, 2007).

A wheat bread fortified with germinated wheat seedlings (30%, w/w) was reported to positively affect glucose-regulating factors compared to a control wheat bread in healthy volunteers (Andersen *et al.*, 2008).

SUMMARY POINTS

- An increased consumer desire for a healthy lifestyle has resulted in demands from the bakery industry for breads containing functional compounds.
- There is an immediate requirement for the food industry to prepare healthy bakery products to satisfy consumers' needs. New plant-derived natural ingredients or processing steps are needed to develop breads with similar qualities as those of white ones.
- As the number of available phytochemicals increases, the incorporation of these functional ingredients into bakery foods will become easier.
- Many phytochemicals exist for bakery applications, and producers have started formulating breads with soy isoflavones, β-glucans, conjugated linoleic acid, and *n*-3 fatty acids.
- Research is needed to evaluate the effects of the phytochemical ingredients on the functional and nutritional properties of bread.

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CHAPTER 28

Carotenoids of Sweet Potato, Cassava, and Maize and Their Use in Bread and Flour Fortification

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

OFSP Orange-fleshed sweet potato

INTRODUCTION

Carotenoids are among the most valuable food constituents in terms of food quality and human health effects. As natural pigments, they confer the pleasing yellow, orange, or red color of many fruits, vegetables, egg yolk, crustaceans, and some fish. Aside from the pro-vitamin A activity of some of these compounds, they have also been credited with other

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health-promoting effects, including immunoenhancement and reduction of the risk of developing degenerative diseases such as cancer, cardiovascular diseases, cataracts, and macular degeneration (Krinsky and Johnson, 2005).

The carotenoids' action against diseases has been widely attributed to their antioxidant activity, specifically to their ability to quench singlet oxygen and interact with free radicals. However, the following mechanisms are being increasingly reported: retinoid-dependent signaling, modulation of carcinogen metabolism, regulation of cell growth, inhibition of cell proliferation, enhancement of cell differentiation, stimulation of gap junctional communication, modulation of DNA repair mechanisms, induction of detoxifying enzymes, and filtering of blue light (Krinsky and Johnson, 2005).

The principal carotenoids found in foods are β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin (Figure 28.1). These carotenoids are also the most commonly found in human plasma and have been the most studied in terms of health benefits.

The highly unsaturated carotenoids are prone to geometric isomerization and oxidation (Rodriguez-Amaya, 1999). Losses during home preparation and industrial processing have been widely reported, and instability of carotenoids is a major concern in maintaining the food color and the beneficial effects on health. These losses are due to physical removal and enzymatic or nonenzymatic oxidation. Oxidative degradation depends on the availability of oxygen; is stimulated by light, heat, metals, enzymes, and peroxides; and is inhibited by antioxidants. It is known to increase with the destruction of the food cellular structure, surface area or porosity, duration and severity of the processing conditions, duration and temperature of storage, and with use of packaging permeable to oxygen and light. Enzymatic oxidation occurs prior to heat treatment, during peeling, slicing, pulping, or juicing.

Initial stages of oxidation involve epoxidation and cleavage to apocarotenals (Figure 28.2). Subsequent fragmentations result in compounds with low molecular masses. Now devoid of the color and biological activity attributed to carotenoids, these compounds can give rise to desirable flavor or off-flavor.

Isomerization of all-E(*trans*)-carotenoids, the usual configuration in nature, to the Z(*cis*)-isomers occurs along with oxidation (see Figure 28.2). It is promoted by acids, heat, and light, and it results in loss of provitamin A activity and alteration of bioavailability and metabolism. As with the all-E-isomers, the Z-carotenoids are subject to oxidation.

For a long time, the major concern about carotenoids in food processing was minimizing their degradation. In more recent years, processing (i.e., mechanical matrix disruption and/or heat treatment) has been shown to enhance bioavailability (i.e., the fraction of the carotenoid ingested that becomes available for utilization in normal physiological functions or for storage in the human body) (Rock *et al.*, 1998). It softens or breaks membranes and cell walls and denatures proteins complexed with carotenoids, facilitating the release of these compounds from the food matrix during digestion. Processing conditions should therefore be optimized to increase bioavailability without provoking significant degradation of the carotenoids.

In this chapter, the carotenoid compositions of three major staple foods consumed by millions of people in many countries, especially developing countries, are reviewed, along with their possible use in the fortification of flour and bakery products.

CAROTENOIDS OF SWEET POTATO (*IPOMOEA BATATAS* LAM.)

Carotenoids in roots and flour

All-E-β-carotene is the major carotenoid of sweet potatoes. The orange-fleshed varieties of sweet potato (OFSP) have this carotenoid almost exclusively and in considerable amounts.

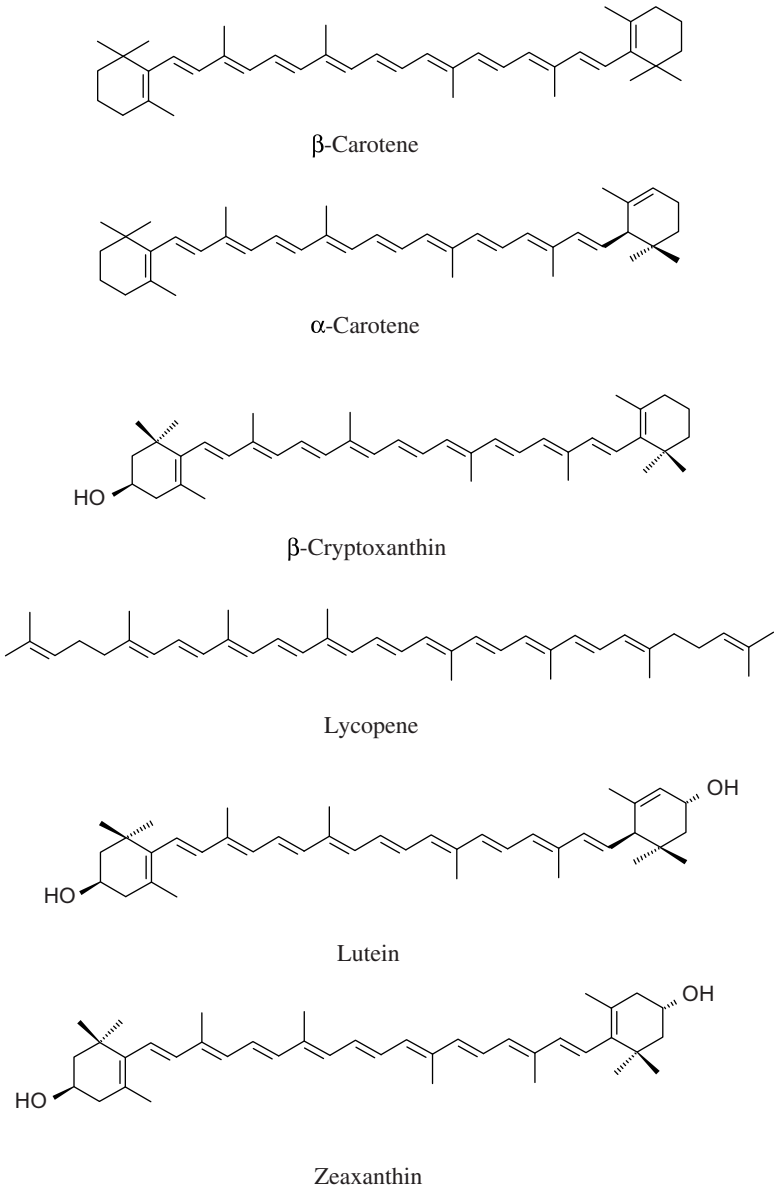

FIGURE 28.1
Structures of carotenoids demonstrated to be important to human health. Although there are many different carotenoids (approximately 100) in food, β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin are major food carotenoids. They are also the carotenoids commonly encountered in human plasma and have been the most studied and demonstrated to be important in terms of human health.

Other carotenoids (e.g., lutein and β-carotene epoxides) have comparatively higher levels in the lighter colored sweet potatoes, but β-carotene still predominates (Kimura *et al.*, 2007). The β-carotene concentration of sweet potato varies considerably (Table 28.1), from less than 1 μg/g in white-fleshed roots to more than 130 μg/g in OFSP. Unfortunately, the white-fleshed sweet potatoes are the most commonly consumed in Africa, Asia, and Latin America. Aside from the remarkable varietal differences, the carotenoid content is also influenced by such factors as root age and production site (K'osambu *et al.*, 1998).

Structurally, vitamin A (retinol) is essentially half of the molecule of β-carotene. This carotenoid is the most potent provitamin A; it is also the most widely distributed carotenoid

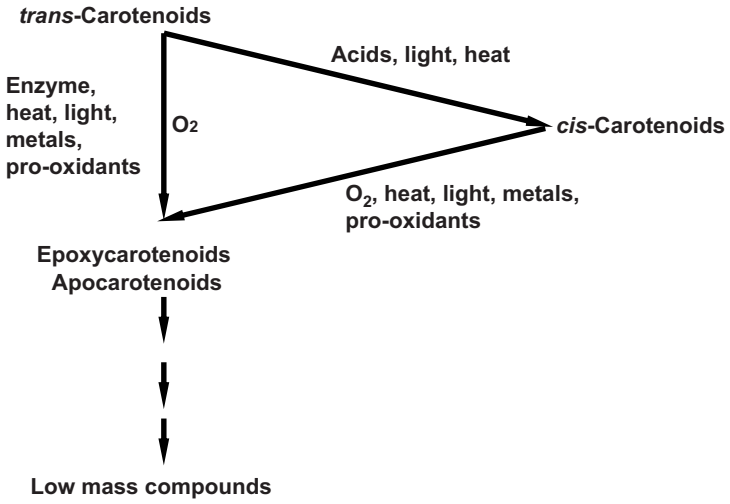

FIGURE 28.2
Possible scheme for the degradation of carotenoids. During processing and storage of food, carotenoids undergo isomerization from the *trans*-form to the *cis*-form, with both forms being oxidized to epoxycarotenoids and apocarotenoids. A series of fragmentations then occur forming small compounds, which no longer have the color and the biological activities of carotenoids. *Source: Reproduced with permission from Rodriguez-Amaya, D. B. (1999). Changes in carotenoids during processing and storage of foods.* Arch. Latinoam. Nutr. *49, 38S—47S.*

in foods. The minimum requirement for a carotenoid to have provitamin A activity is an unsubstituted β-ring with a polyene chain of 11 carbon atoms. Thus, α-carotene and β-cryptoxanthin have approximately 50% of the activity of β-carotene.

Sweet potatoes are commonly consumed as boiled roots, but in Africa they are traditionally sun-dried for consumption during the dry season when the fresh roots are not available (Bechoff *et al.*, 2009). The roots are crushed or chipped and then dried for several days. The dried product can be rehydrated or milled into flour to be used as porridge. In urban areas, the sweet potato flour can be used to partially replace wheat flour in a variety of baked products.

TABLE 28.1 β-Carotene Content of Sweet Potato Roots[a]

Origin of Samples	Description of Samples	β-Carotene Content (μg/g Fresh Root)[b]	Reference
Hawaii	4 purple-fleshed varieties	10—50	Huang *et al.* (1999)
	7 yellow/white-fleshed varieties	10—60	
	7 orange-fleshed varieties	67—131	
Kenya	6 white cultivars	<1	K'osambu *et al.* (1998)
	3 cream cultivars	1—10	
	7 pale orange to orange cultivars	10—80	
Kenya	9 white, white/yellow cultivars	<1	Hagenimana *et al.* (1999)
	7 cream, cream/yellow cultivars	20—110	
	7 light yellow, white/yellow cultivars	1—10	
	6 light to deep orange cultivars	21—63	
Kenya	6 yellow- and orange-fleshed cultivars	12—109	Kidmose *et al.* (2007)

[a]*The color of sweet potato varies from white to deep orange. The content of β-carotene, the provitamin A carotenoid responsible for the color, varies from <1 to 131 μg/g fresh root.*
[b]*All-E-β-carotene with traces of Z-β-carotenes.*

Several research groups have reported variable carotenoid losses in the production of sweet potato flour, which are affected by the variety utilized, the size and shape of the pieces submitted to drying, and the drying method and condition. Hagenimana *et al.* (1999) found that drying sweet potato slices in a forced-air oven at 60°C for 12 h reduced the total carotenoid content by 30%. Storing the dried chips in opaque paper bags at ambient conditions for 11 months resulted in a 10% loss. Van Jaarsveld *et al.* (2004) achieved better β-carotene retention with oven drying compared to sun drying, with the latter provoking greater degradation than drying in the shade. Drying thicker slices retained more β-carotene. Lower retention in open-air sun drying is apparently due to the destructive effect of direct sunlight and the uncontrolled environmental conditions, including greater and more prolonged exposure to oxygen. Kidmose *et al.* (2007) observed that shade drying of chips resulted in a significant reduction of all-E-β-carotene (approximately 21%), which was further reduced when flour was produced from the chips. Bechoff *et al.* (2009) reported that all-E-β-carotene losses in flour made from dried chips varied between 16 and 34% under different drying treatments. Hot air cross-flow drying retained significantly more provitamin A than open-air sun drying, but drying in a greenhouse solar dryer and sun drying did not result in significantly different provitamin A retention. The shape of the sweet potato pieces (chip or crimped slice) influenced retention during sun drying, with the crimped slices retaining more provitamin A. Bengtsson *et al.* (2008) found that drying slices of OFSP at 57°C in a forced-air oven for 10 h reduced the all-E-β-carotene content by 12%. Contrary to Bechoff *et al.*'s results, solar and sun drying yielded markedly different losses of 9 and 16%, respectively.

Application in the fortification of flour and bread

The nutritional quality of bakery products is low because of the inferior nutritional composition of the wheat grain, accentuated by the use of refined flours as the ingredient (Chavan and Kadam, 1993). Substitution of part of the wheat flour with non-wheat flours has been advocated for nutritional enrichment of these products and to reduce cost, utilizing local raw materials preferentially. Although most research has focused on increasing the protein content, the addition of carotenoid-rich flours is now being suggested.

Van Hal (2000) extensively reviewed the quality of sweet potato flour, including literature that is not easily accessible, such as reports, proceedings, and pamphlets. Some of the important findings are as follows:

- Processing sweet potato into flour improves shelf life and makes it easier to incorporate into food products.
- Because of its distinct properties, the use of sweet potato flour in the preparation of bread is restricted, with most researchers reporting substitution of 10–15% of wheat flour with sweet potato flour on a dry weight basis as most acceptable. For other baked products, especially sweet baked products, higher proportions (10–100%) of sweet potato flour can be used.
- Acceptability depends on the sensory evaluation of the flour, with color, odor, and a high degree of whiteness being the most important marketing quality factors.
- When made from OFSP, the flour has a high content of β-carotene and reasonable levels of vitamin C, calcium, phosphorus, iron, and potassium.

Utilization of OFSP as a means of alleviating vitamin A deficiency in developing countries has gained impetus with the launching of the VitaAfrica and HarvestPlus biofortification programs. Biofortification has been defined as the development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology (Nestel *et al.*, 2006). The potential to increase the micronutrient density of staple foods by conventional breeding is best exemplified in sweet potatoes, for which lines with high levels of β-carotene (>200 μg/g) have been identified.

Studies in South Africa (van Jaarsveld *et al.*, 2005) and Mozambique (Low *et al.*, 2007) demonstrated that the consumption of biofortified OFSP significantly increased the vitamin A status of children.

Rangel *et al.* (2008) reported that English cake formulations in which the wheat flour was mixed with OFSP flour (10 or 20%) produced cakes with smaller volume, a more round shape, darker color, and characteristic sweet potato flavor but that were as acceptable as the standard cakes. Cookies were also prepared with the substitution of 10, 30, and 50% wheat flour with OFSP flour or pumpkin flour (Siciliano *et al.*, 2009). Substitution of 30% of wheat flour with OFSP flour was significantly preferred, obtaining the highest score for intention to purchase.

Because an adequate gluten formation is necessary to keep the quality of bread, Rangel *et al.* (2008) evaluated the use of biofortified sweet potato flour (10, 20, and 30%) in the production of bread (sandwich loaves). It was observed that the bread loaves produced with OFSP flour had smaller final volumes and darker color with characteristic flavor, with the changes in the loaves' characteristics being accentuated by an increase in the OFSP flour level. The loaves with 10% substitution with OFSP better resembled the standard bread. Despite the differences, all the loaves produced with OFSP flour were considered as acceptable for consumption as the standard loaves.

The possibility of producing expanded snacks by extrusion using different proportions of OFSP and polished rice flours (15:85, 30:70, and 45:55) was investigated by Silva *et al.* (2008), with the objective of increasing the product's shelf life and offering low-cost processed biofortified products. The snacks with 30% OFSP flour presented high water solubility index and viscosity characteristics, indicating that they could be used in other products (e.g., soups). Although the expansion indexes were low, the snacks presented good sensory characteristics such as excellent flavor.

CAROTENOIDS OF CASSAVA (*MANIHOT ESCULENTA* CRANTZ)

Carotenoids in roots and flour

The roots of currently used varieties of cassava are of poor nutritional quality, having very low concentrations of carotenoids, iron, zinc, and protein, and they also contain toxic cyanogens. The major carotenoid is also all-E-β-carotene, but unlike sweet potato, in which the Z-isomers are present in only trace amounts, cassava roots have appreciable levels of Z-β-carotenes (Kimura *et al.*, 2007). The total β-carotene concentration varies from nondetectable in the white-fleshed roots to only approximately 5 μg/g in yellow-fleshed cassava (Table 28.2).

Using chips from the three cassava cultivars listed in Table 28.2, the average β-carotene retention was higher in oven drying (72%) than in shadow drying (59%), sun drying (38%), and gari production (34%) (Chávez *et al.*, 2007). The level of this carotenoid dropped further

TABLE 28.2 β-Carotene Content of Cassava Roots[a]

Origin of Samples	Description of Samples	β-Carotene Content (μg/g Fresh Root)[b]	Reference
Australia	5 cultivars	0.2–3.0	Adewusi and Bradbury (1993)
Colombia	3 cultivars	3.8–4.9	Chávez *et al*. (2007)
India	11 faint yellow to yellow exotic lines	0.4–3.1	Moorthy *et al*. (1990)
	10 faint yellow to yellow indigenous lines	0.4–3.1	

[a]*The color of cassava root varies from white to yellow. The β-carotene of the yellow cassava varies from about 0.2 to 4.9 μg/g fresh root.*
[b]*All-E-β-carotene with appreciable amounts of Z-β-carotenes.*

during storage in regular plastic bags at ambient temperature, with the retention after 4 weeks of storage being approximately 18% in sun-dried chips and in sun-dried chips stored as flour.

Application in the fortification of flour and bread

Cassava tubers have to undergo immediate processing after harvest because they deteriorate within 24 h. A versatile crop, different processing techniques have been developed and are shown in Figure 28.3.

The fortification of gari and flour with vitamin A, B vitamins, iron, iodine, zinc, and calcium was recommended by Asonye (2001). Gari is considered the most practical product for fortification because it is cheap, easy to prepare, palatable, and suitable for mechanical processing in an industrial setup. It is a fermented, roasted, granular meal that is regularly consumed by urban and rural populations. Fortification of gari and flour with β-carotene also appears feasible.

Rangel *et al.* (2008) used cassava flour in cake formulations. As for cakes with the OFSP flour, the cakes with cassava flour were acceptable but with smaller volume. Because it does not have strong characteristic flavor and color, a greater substitution (20%) of this flour resulted in cakes similar to the standard cake.

Evaluating the possibility of producing nonexpanded products by extrusion (pellets) using biofortified cassava flour, Silva *et al.* (2008) concluded that, according to the extrusion parameters studied (particle size, water absorption index, water solubility index, and viscosity), the production of pellets using a biofortified raw material is feasible.

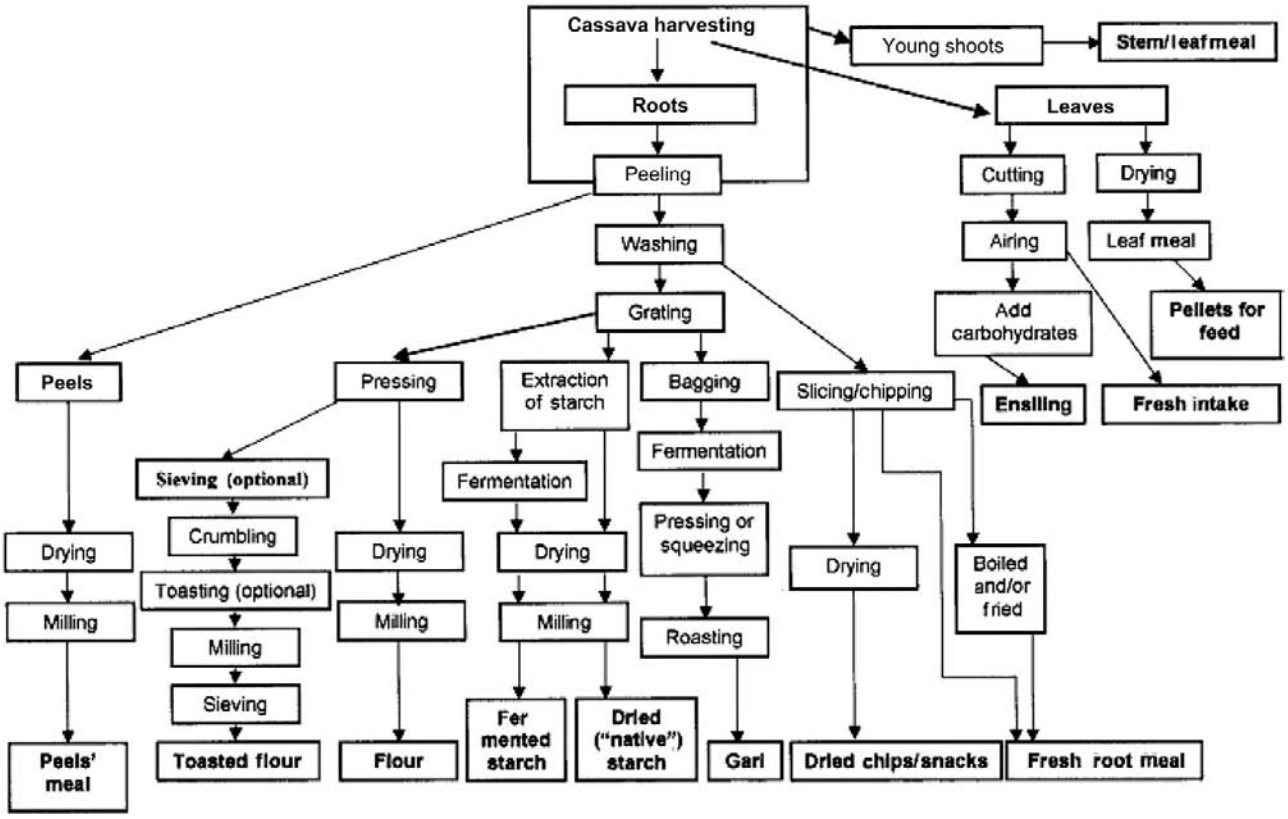

FIGURE 28.3
Schematic representation of cassava processing into different food and feed products. Cassava is a very versatile crop. The leaves can be eaten boiled or fried or transformed into meal or pellets for animal feed. The root can be processed into flour, chips, or starch. The peel can also be used as feed. *Source: Reproduced from the Organisation for Economic Co-operation and Development (2009).*

TABLE 28.3 Carotenoid Contents (μg/g Dry Kernel) of Maize Grains[a]

Origin of Samples	Description of Samples	Lutein	Zeaxanthin	β-Cryptoxanthin	β-Carotene	Reference
Brazil	3 cultivars	1.5–3.6	4.0–5.6	1.2–1.7	1.2–1.6	Kimura et al. (2007)
United States	44 genotypes	ND–28	0.01–7.7	0.08–2.45	0.09–7.6	Kurilich and Juvik (1999)
United States	41 genotypes	0.1–16	0.1–8.1	ND–1.8		Ibrahim and Juvik (2009)

The table shows the variation in the carotenoid contents of some maize cultivars/genotypes, with lutein and/or zeaxanthin as the major carotenoids. The provitamin A carotenoids β-carotene and β-cryptoxanthin are present at low levels.
ND, not detected.

CAROTENOIDS OF MAIZE (*ZEA MAYS* L.)

Carotenoids in kernels and flour

Yellow maize naturally contains the xanthophylls lutein and zeaxanthin as major carotenoids, with much lower levels of the provitamin A carotenoids β-carotene and β-cryptoxanthin. The concentrations of the different carotenoids vary markedly among maize genotypes: not detected to 28 μg/g lutein, 0.01–8.1 μg/g zeaxanthin, 0.08–2.45 μg/g β-cryptoxanthin, and 0.09–7.65 μg/g β-carotene (Table 28.3).

Lutein and zeaxanthin make up the yellow pigment in the macula of the human retina and are believed to be responsible for the protective effect of carotenoids against macular degeneration and cataract, acting as antioxidants and filters of high-energy blue light (Krinsky and Johnson, 2005). Lutein and especially zeaxanthin are not widely distributed in foods. Maize and maize products are among the few foods with high levels of these important xanthophylls.

An average of 36% loss of provitamin A was observed following nixtamalization and subsequent snack preparation by deep-frying (Lozano-Alejo *et al.*, 2007). The traditional nixtamalization in Mexico and Central America is the cooking and steeping of maize kernels in an aqueous suspension of calcium hydroxide. It is a central step in the conversion of maize grain to dough and ultimately to maize flour, snacks, and tortillas.

Application in the fortification of flour and bread

Because the focus is micronutrient deficiency, biofortification has been directed to increasing the provitamin A content of maize. Considering the important role of lutein and zeaxanthin in health and their infrequent occurrence in commonly consumed foods, maize and maize products being principal dietary sources, it is imperative that elevation of the provitamin A level be achieved not at the expense of these xanthophylls. This means increasing the levels of all carotenoids, not by blocking the formation of lutein and zeaxanthin so as to accumulate β-carotene and β-cryptoxanthin.

Aside from conventional breeding, generation of transgenic maize with enhanced provitamin A content in its kernels has been pursued in recent years. Overexpression of the bacterial genes for phytoene synthase and the enzymes (phytoene desaturase and ζ-carotene desaturase) that catalyze the four desaturation steps of the carotenoid pathway resulted in an increase in total carotenoids up to 34-fold with preferential accumulation of β-carotene in the maize endosperm (Aluru *et al.*, 2008). The high β-carotene trait was found to be reproducible over at least four generations. Gene expression analyses indicated that upregulation of the endogenous lycopene β-cyclase was responsible for the accumulation of β-carotene.

The retention of β-carotene in a high β-carotene (10 μg/g) maize during traditional African household cooking was investigated by Li *et al.* (2007). The cumulative losses in the final

cooked products were 24% for fermented porridge (ogi) and 25% for the unfermented porridge. The traditional preparation of ogi involves soaking of dried whole maize kernels, milling, addition of water to form a wet flour or dough, and spontaneous fermentation (24–72 h). The ogi is then cooked with added water to prepare the porridge.

Cookies were prepared from blends of soybean (10–90%) and maize flours (Akubor and Onimawo, 2003). The soybean flour had higher protein, fat, crude fiber, and ash contents than the maize flour. The soybean flour/maize flour blends possessed good water and oil absorption capacities, foaming capacity, and foam stability in relation to the maize flour. The emulsion activity of the blends was also dependent on soybean flour, whereas bulk density increased with the level of maize flour. Cookies prepared from the blends of 60% soybean flour and 40% maize flour were judged most acceptable. Above 60% soybean substitution levels, the sensory scores for overall acceptability and all sensory qualities evaluated (color, flavor, taste, and texture) except taste decreased steadily.

Silva *et al.* (2008) studied the production of snacks made with bean and maize flours at different proportions (15:85; 30:70, and 45:55) by extrusion. The products were characterized by particle size, water absorption index, water solubility index, viscosity, expansion index, and density. Snacks with 45% of bean flour presented the lowest water solubility index because of a reduction of the starch content and an increase of proteins; however, they showed high water absorption index and could therefore be recommended to prepare instant products using less water at room temperature. The high expansion index obtained with all formulations resulted in pleasant taste and color. In accordance with the extrusion parameters, the use of biofortified maize flour may be recommended for the production of these snacks.

TECHNOLOGICAL ISSUES

A major problem in fortification with carotenoids is their instability. Processing conditions should be optimized to ensure good retention of these compounds. This includes processing immediately after peeling, cutting, or maceration; minimum processing time and temperature; protection from light; and exclusion of oxygen. The heat treatment in blanching may provoke losses of carotenoids, but inactivation of oxidative enzymes will prevent further and greater losses during slow processing (as in drying) and storage.

SUMMARY POINTS

- Fortification with the three staple foods reviewed in this chapter is at different stages of development.
- For all three crops, nutrition education appears to be needed to promote acceptability, considering the current preference for varieties devoid of color in many countries.
- For OFSP, varieties with considerable amounts of all-E-β-carotene are available; however, for cassava and maize, enhancing the provitamin A content is still being pursued, especially by biofortification, although adding β-carotene to cassava flour may be an alternative.
- Carotenoid losses during processing and storage of the flours emphasize the need for using optimized conditions.
- The use of carotenoid-rich or biofortified flours in a variety of products has been shown to be technologically feasible; carotenoid retention during these processes needs to be demonstrated.

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CHAPTER 29

Production and Nutraceutical Properties of Breads Fortified with DHA- and Omega-3-Containing Oils

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

CVD Cardiovascular disease
DHA Docosahexaenoic acid
EPA Eicosapentaenoic acid
FA Fatty acid(s)
PG Prostaglandin
PPARα Peroxisome proliferator-activated receptor-α
PUFA Polyunsaturated fatty acid
SREBP Sterol regulatory element-binding protein
SSL Sodium stearoyl-2-lactylate

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INTRODUCTION

Bread is one of the oldest prepared foods, dating back to the Neolithic era. Its origins can be closely linked to the beginning of agriculture in the area known as the Fertile Crescent. Wheat was probably first eaten as a gruel of crushed grain and water. Then, the gruel or dough was baked on hot stones into primitive flat breads. Fermentation was the next development when doughs, exposed to natural yeasts and microflora, fermented before baking. The basic materials for bread making have changed little; however, bread has diverged into a wide array of types (Serna-Saldivar, 2010). Bread continues to be the most widely consumed food and, with rice, the largest supplier of calories, protein, and certain essential vitamins and minerals for the 6.8 billion people that currently inhabit the earth. An average human obtains approximately 22 and 24% of the daily calories and protein from wheat (Food and Agriculture Organization, 2010). Flour and bread are commonly fortified with selected essential micronutrients; however, the recent trend is to use these to provide fiber, other proteins, and nutraceuticals that can prevent chronic diseases currently responsible for approximately 60% of deaths.

It has been suggested that the typical Western diet, which is relatively high in omega (ω)-6 and low in ω-3 fatty acids (FA), may not provide the appropriate balance for proper biological function. The imbalance is related to cardiovascular disease (CVD), hypertension, hyperlipidemia, hypercholesterolemia, inflammatory disorders, and certain disrupted neurological functions (Newton and Snyder, 1997). The nutritional benefits of incorporating docosahexaenoic acid (DHA) and other ω-3 FA into bakery products have gained interest in recent years. The beneficial role of algal and fish oils, rich in ω-3 polyunsaturated fatty acids (PUFAs) such as DHA and eicosapentaenoic acid (EPA), has been well documented in more than 2000 clinical studies pointing to health improvements of various human disorders, such as CVD, breast and prostate cancer, rheumatoid arthritis, and inflammatory diseases (Kumar Rudra *et al.*, 2001; Leitzmann *et al.*, 2004). There is also evidence indicating the benefit of slowing the progression of Alzheimer's disease with the consumption of DHA/EPA-containing fish (Morris *et al.*, 2003). DHA is considered the most important ω-3 FA because it plays an important role in brain chemistry and development. It constitutes approximately half of the lipids found in neuron membranes and in the photoreceptors of the retina (Connor *et al.*, 1992). DHA supplementation is critical in infants because they are very limited in the conversion of linolenic acid into DHA. A cross-sectional study conducted with a population of 1613 individuals ranging from 45 to 70 years of age concluded that consumption of marine ω-3 PUFA and fatty fish was associated with a reduced risk of impaired cognitive function, whereas an increased intake of cholesterol and saturated FA was associated with an increased risk (Kalmijn *et al.*, 2004).

TECHNOLOGICAL ISSUES AND PRODUCTION OF BREADS FORTIFIED WITH DHA AND ω-3 OILS

Bread is a convenient vehicle for introducing DHA/EPA and other nutraceuticals because it is a common staple in most cultures throughout the world. Newton and Snyder (1997) concluded that bread is an ideal medium for ω-3 PUFA because the CO_2 produced during dough fermentation protects the oil from oxidation, especially while it is exposed to high temperatures during baking. Serna-Saldivar *et al.* (2006) researched the production of different breads containing ω-3 FA in oils and emulsions. The aim was to substitute part of the vegetable shortening so as to produce slices of bread (32 g) with 25 or 50 mg DHA or ω-3 FA. DHA/EPA-rich oils were obtained from algae or fish oil, whereas the high-linolenic oil was obtained from flax.

Fortified bread production and evaluation

Breads were produced following the straight or sponge dough procedures. The first procedure (method 10-10B; American Association of Cereal Chemists (AACC), 2000) was used in order to detect possible deleterious effects of the ω-3 oil sources and estimate optimum water absorption and mix times. The formulation included 100 g commercial bread flour, 5.5 g sugar, 3 g vegetable shortening, 1.5 g salt, and 2 g dry yeast (*Saccharomyces cerevisiae*). Bake absorption, mixing time, proof height, loaf height, oven spring, loaf weight, loaf volume, loaf apparent density, and crumb grain texture were determined (Serna Saldivar *et al.*, 2006).

Sponge dough breads were manufactured to obtain commercial loaves for sensory and texture analyses throughout 14 days of storage at room temperature. Sponges were produced from 604 g flour (14% mb), 356 ml water, and 14 g dry yeast. Sponges were fermented for 4.5 h in a proof cabinet set at 29°C and 85% relative humidity. Resulting sponges were mixed with the dough-stage ingredients (326 g flour, 56 g sugar, 28 g shortening, 28 g nonfat dry milk, 18.6 g salt, 9.3 g vital gluten, 1.86 g diastatic malt, 1.86 g lecithin, 0.94 g sodium stearoyl-2-lactylate (SSL), 55.8 mg sodium ascorbate, and 18.6 mg potassium bromate) and 258 ml of water until optimum dough development was obtained. Resulting doughs were weighed and cut into two identical parts before punching, final proofing, baking (28 min at 225°C), cooling, and slicing. One slice per loaf from 16 different loaves was submitted for determination of its full FA profile using a gas chromatograph flame ionization detector.

Bread was cut into 1-inch-thick slices for determination of texture, color, and sensory properties. Bread firmness throughout 14 days of storage at room temperature was objectively evaluated according to AACC (2000) method 74-09 with a texture analyzer equipped with a cylindrical probe. Crumb color was determined with a Minolta color meter. L, a, and b were obtained and color index E was determined by the following equation: $E = (L^2 + a^2 + b^2)^{1/2}$. Between 25 and 30 untrained panelists evaluated the color, flavor, texture, and overall acceptability of the control bread and breads enriched with 25 or 50 mg DHA/slice throughout 14 days of storage. Breads were evaluated using a 9-point hedonic scale (Serna-Saldivar *et al.*, 2006).

Baking performance

According to the FA composition of all experimental oil sources, the vegetable shortening was partially substituted to yield 32-g slices of bread containing either 25 or 50 mg DHA, 25–50 mg total ω-3 in the case of fish oil, or 120 mg linolenic acid from flax oil. The source of the oil did not significantly affect optimum water absorption but reduced mix time by 10–15% (Table 29.1). A similar performance was observed in commercial breads produced by the sponge dough procedure. The utilization of dough conditioners and improvers such as vital gluten, oxidizing agents, lecithin, and SSL counteracted the deleterious effects of the ω-3-rich oils (Table 29.2).

Fatty acid composition

According to Newton and Snyder (1997), the prehistoric human diet provided a relatively equal balance of ω-6 and ω-3 FA. The modern diet in Western societies has dramatically changed this ratio to approximately 10–25:1. This unbalance is believed to exacerbate chronic diseases.

Breads fortified with algae and fish oils required the least weight addition of ω-3 oils because they contained relatively high levels of DHA/ω-3 FA. The algae oil breads contained approximately 25 or 50 mg DHA/serving, whereas the fish oil supplemented bread contained approximately half of the amount but still maintained the 25–50 mg of total long-chain ω-3 FA, namely contributed by EPA and DHA. As expected, the flax oil bread did not contain DHA but contained the highest level of linolenic acid (Table 29.3). Experimental loaves supplemented with the highest amount of algal DHA or DHA/EPA fish oils contained at least four times more ω-3 FA than did the control or flax supplemented bread. Flax oil enriched breads

TABLE 29.1 Effect of Different Types and Concentrations of DHA/ω-3-Rich Oils on Baking Performance Estimated with the Straight Dough Procedure[a]

Sample	Water Absorption (%)	Mixing Time (min:sec)	Proof Height (cm)	Bread Height (cm)	Oven Spring (cm)[b]	Bread Weight (g)	Bread Volume (cm^3)	Apparent Density (g/cm^3)	Crumb Texture[c]
25 mg DHA									
Control	64	3:43[a]	7.625[a]	10.875[a]	3.250[a]	143.2[a,b]	888.3[a]	0.162[c]	5.3[a,b]
Algae oil	64	3:35[a,b]	7.500[a]	10.575[a]	2.975[a]	144.2[a,b]	826.3[b]	0.175[b]	5.0[a–c]
Emulsion-P	64	3:23[b,c]	7.525[a]	10.575[a]	3.050[a]	143.4[a,b]	830.8[a,b]	0.174[b,c]	6.0[a]
Emulsion-L	64	3:28[a–c]	7.700[a]	10.350[a,b]	2.725[a,b]	142.2[b]	828.3[a,b]	0.172[b,c]	5.8[a]
Flax oil	64	3:20[b,c]	7.475[a]	10.325[a,b]	2.850[a,b]	142.5[b]	823.3[b,c]	0.174[b,c]	5.8[a]
Fish oil	64	3:15[c]	7.550[a]	10.300[a,b]	2.750[a,b]	144.1[a,b]	823.8[b,c]	0.175[b,c]	4.3[b,c]
50 mg DHA									
Control	64	3:33[a,b]	7.700[a]	10.175[a,b]	2.475[b]	140.5[b–d]	841.3[a]	0.167[a,b]	4.8[b–d]
Algae oil	64	3:23[b,c]	7.625[a,b]	10.475[a,b]	2.850[a,b]	140.4[c,d]	842.5[a]	0.167[a,b]	5.0[a–c]
Emulsion-P	64	3:28[a–c]	7.475[b,c]	10.500[a]	3.025[a,b]	139.8[d]	853.8[a]	0.164[b]	5.8[a,b]
Emulsion-L	64	3:23[b,c]	7.525[a,b]	10.425[a,b]	2.900[a,b]	141.6[a–d]	841.3[a]	0.168[a,b]	6.3[a]
Flax oil	64	3:18[c]	7.475[b,c]	10.350[a,b]	2.875[a,b]	142.0[a–c]	840.0[a]	0.169[a,b]	3.8[c,d]
Fish oil	64	3:28[a–c]	7.550[a,b]	10.575[a]	3.025[a,b]	141.4[a–d]	841.3[a]	0.168[a,b]	5.8[a,b]

Source: From Serna-Saldivar *et al*. (2006)
[a]*Means with a different letter(s) within column and enrichment level are statistically different* ($p < 0.05$).
[b]*Oven spring = bread height - proof height.*
[c]*1, poor; 3, regular; 5, good; 7, excellent.*

TABLE 29.2 Effect of Different Types and Concentrations of DHA/ω-3-Rich Oils on Baking Performance Estimated with the Sponge Dough Procedure[a]

Sample	Water Absorption (%)	Mixing Time (min:sec)	Proof Height (cm)	Bread Height (cm)	Oven Spring (cm)[b]	Dough Weight (g)	Bread Weight (g)	Bread Weight/ Dough Weight (%)	Crumb Texture[c]
25 mg DHA									
Control	65.5	4:40[a]	10.53[a]	12.33[a,b]	1.80[b,c]	830.7[a]	748.1[c,d]	90.1[b,c]	7.0[a]
Algae oil	65.5	4:35[a]	10.40[a]	12.23[a,b]	1.83[b,c]	836.0[a]	750.9[b–d]	89.8[b,c]	6.5[a,b]
Emulsion-P	65.5	4:32[a,b]	10.28[a]	12.80[a]	2.52[a,b]	834.0[a]	752.1[b,c]	90.2[b,c]	7.0[a]
Emulsion-L	65.5	4:40[a]	10.18[a]	12.33[a,b]	2.15[a–c]	832.8[a]	744.2[d,e]	89.4[c]	6.5[a,b]
Flax oil	65.5	4:40[a]	10.15[a]	12.53[a,b]	2.38[a,b]	831.5[a]	756.0[b]	90.9[a,b]	6.5[a,b]
Fish oil	65.5	4:42[a]	10.60[a]	11.80[b]	1.25[c]	835.5[a]	768.2[a]	92.0[a]	6.0[b]
50 mg DHA									
Control	65.5	4:35[a]	11.05[a]	12.60[a,b]	1.55[a,b]	830.8[b,c]	742.8[b,c]	89.4[b–d]	7.0[a]
Algae oil	65.5	4:33[a]	11.23[a]	12.90[a,b]	1.67[a,b]	834.2[b]	739.4[c]	88.6[d]	7.0[a]
Emulsion-P	65.5	4:35[a]	10.83[a,b]	12.48[a,b]	1.65[a,b]	832.7[b,c]	740.6[c]	88.9[c,d]	6.5[a,b]
Emulsion-L	65.5	4:35[a]	10.38[b]	12.38[b]	2.00[a,b]	829.9[c]	738.9[c]	89.0[c,d]	6.0[b]
Flax oil	65.5	4:30[a]	10.83[a,b]	12.83[a,b]	2.00[a,b]	834.7[b]	754.0[a]	90.3[a,b]	6.0[b]
Fish oil	65.5	4:10[b]	10.80[a,b]	13.10[a]	2.30[a]	832.9[b,c]	754.6[a]	90.6[a]	6.3[a,b]

Source: From Serna-Saldivar *et al*. (2006).
[a]*Means with a different letter(s) within column and enrichment level are statistically different* ($p < 0.05$).
[b]*Oven spring = bread height - proof height.*
[c]*1, poor; 3, regular; 5, good; 7, excellent.*

contained at least 2.5 times more linolenic acid than did the other experimental counterparts and were practically free of long-chain FA. The ω-6:ω-3 FA ratio of the control bread was approximately 9 (see Table 29.3). Addition of the different oil sources improved the ratio to less than 3.5 and 2.2 for the 25- and 50-mg fortified breads, respectively. The most balanced ratio was observed in the flax oil enriched bread because it was the richest source of linolenic acid.

TABLE 29.3 Effect of Different Types and Concentrations of DHA/Omega-3-Rich Oils on Fatty Acid Composition of Breads (Expressed Per 32-g Serving)[a]

Fatty Acid (mg/Serving)	Control	Algae Oil	Emulsion-L	Emulsion-P	Flax Oil	Fish Oil
	Low Enrichment Level					
Dodecanoate	20	20	23	19	21	21
Myristate	24	27	24	22	18	32
Myristoleate	6	5	5	4	4	6
Palmitate	149	153	129	129	147	154
Palmitoleate	22	20	17	17	18	29
Stearate	95	91	70	69	90	93
Oleate	156	150	118	117	146	154
Linoleate	138	135	128	129	149	135
Arachidate	2	2	2	2	3	3
Linolenate	14	14	12	13	93	18
Eicosenoate-11	4	3	3	3	3	6
Eicosapentaenoate	1	2	2	2	0	11
Lignocerate	0	2	2	2	0	1
Docosapentaenoate *n*-6	0	11	12	12	0	0
Docosahexaenoate	0	26	29	29	0	16
Omega-6	138	146	140	141	149	138
Omega-3	15	42	43	44	93	46
Omega-6:omega-3	9.20	3.47	3.25	3.20	1.60	3.00
Saturated	290	295	250	243	279	305
Monounsaturated	178	178	143	141	171	196
Polyunsaturated	153	188	183	185	242	184
Saturated: monounsaturated: polyunsaturated	47:29:25	45:27:28	43:25:22	43:25:33	40:25:35	45:29:27
	High Enrichment Level					
Dodecanoate	19	21	21	20	21	20
Myristate	25	30	21	19	11	29
Myristoleate	6	5	2	0	3	5
Palmitate	162	153	100	103	108	148
Palmitoleate	24	20	12	11	13	27
Stearate	104	82	34	36	52	82
Oleate	167	138	70	69	112	141
Linoleate	138	136	119	124	153	132
Arachidate	3	2	0	0	3	2
Linolenate	16	14	12	3	172	18
Eicosenoate-11	4	3	3	0	0	6
Eicosapentaenoate	0	4	4	4	0	18
Lignocerate	1	2	2	3	2	0
Docosapentaenoate *n*-6	0	20	24	23	0	2
Docosahexaenoate	0	51	59	58	0	27
Omega-6	138	156	143	147	153	136
Omega-3	16	71	77	67	172	65
Omega-6:omega-3	8.63	2.20	1.86	2.19	0.89	2.09
Saturated	313	290	178	181	197	281
Monounsaturated	201	166	87	80	128	179
Polyunsaturated	154	227	220	214	325	201
Saturated: monounsaturated: polyunsaturated	47:30:23	42:24:33	36:18:45	38:17:45	30:20:50	42:27:30

Source: From Serna-Saldivar *et al*. (2006).

[a]*Values are means of three observations. The standard deviations of the fatty acid determinations were less than 0.05 mg/g.*

Bread color, texture, and sensory evaluations

Within enrichment concentration, addition of different sources of algae oils did not significantly affect lightness (*L*), *a*, *b*, and color indexes (*E*) determined at the center portion of the crumb. The color remained the same throughout 14 days of storage at room temperature (Serna-Saldivar *et al.*, 2006). Sensory evaluations of 25- and 50-mg enriched breads indicated that all breads were equally preferred compared to the control bread. Objective color values were similar in terms of lightness and color scores. As expected, panelists graded with lower scores bread texture stored for longer periods of time. However, they considered that experimental breads had similar texture or firmness throughout storage compared with the control bread. The flavor and overall acceptability of all breads were rated similarly during the first 6 days storage. However, the 14-day-old bread enriched with the highest level of fish oil had a significantly lower flavor score and overall acceptability compared to the rest of the treatments. Presumably, the high PUFA oil became rancid and/or its flavor reverted, negatively affecting organoleptic properties. This bread was the only one rated as "neither like or dislike" or worse after 14 days of storage at ambient temperature (Serna-Saldivar *et al.*, 2006).

SYNTHESIS OF DHA AND EPA

Humans are only capable of synthesizing EPA and DHA from α-linolenic acid, although the conversion has been reported to be extremely limited (John-Bjarne *et al.*, 1998). Therefore, the diet should either provide the precursor or these PUFA or these two FA. However, several plants, fungi, and fish have the capacity to produce EPA and DHA from other FA. The biosynthetic pathway starts when stearic acid (18:0) is elongated to a 20-carbon chain and desaturated in the 5, 8, 11, 14, and 17 positions to yield EPA. DHA is produced similarly, but the chain is elongated to 22 carbons with six insaturations in positions 4, 7, 10, 13, 16, and 19. These reactions occur in the endoplasmic reticulum. Key enzymes for the production of these FA are the 2-carbon elongases and Δ4, -6, -9, -12, and -15 desaturases (Figure 29.1).

METABOLIC IMPLICATIONS AND HEALTH BENEFITS OF DHA, EPA, AND ω-3 FATTY ACIDS

Dietary EPA and DHA are packaged and transported through chylomicrons produced in the intestinal epithelial cells. The most relevant tissues that contain ω-3 PUFA are hepatic, lung, kidney, spleen, plasma, heart, retina, and the vascular endothelium. EPA concentrates in the hepatic, renal, and blood cells, whereas DHA concentrates in the heart, retina, and brain. These PUFAs have three major roles: cell membrane structure, precursors of eicosanoids, and effectors of protein activity (enzymes, receptors, and ionic channels). EPA is preferably incorporated associated with phosphatidyl choline (67%) and ethanolamine (13%) in platelets, whereas DHA accumulates with phosphatidyl ethanolamine (45%) and choline (37%). DHA is also incorporated into cardiolipin, a phospholipid only found associated with the cardiac muscle. Eicosanoides are hormones synthesized from 20-carbon FA, mainly araquidonic and EPA, and the enzymes cyclooxygenases and lipooxygenases (Figure 29.2). EPA is precursor of series 3 prostaglandins (PGs) and thromboxanes and series 5 leukotrienes. These are known to affect inflammatory response, vasodilatation, platelet aggregation, nerve stimulation, atherosclerosis, asthma, arthritis, diabetes, psoriasis, and possibly cancer tumors (Table 29.4). In addition, their presence in semen favors the contraction of the uterus and the movement of the spermatozoids through the fallopian tubes. In females, PGs are liberated during menstruation, favoring the detachment and elimination of the endometrial epithelial cells.

Platelet aggregation requires fibrinogen and is stimulated by thromboxane A2, whereas prostacycline (PG-I) decreases aggregation and stimulates vasodilatation. Both thromboxane A2 and prostacycline are produced by cyclooxygenase of endothelial cells. EPA reduces the synthesis of thromboxane A2; produces thromboxane A3 (weak platelet aggregator);

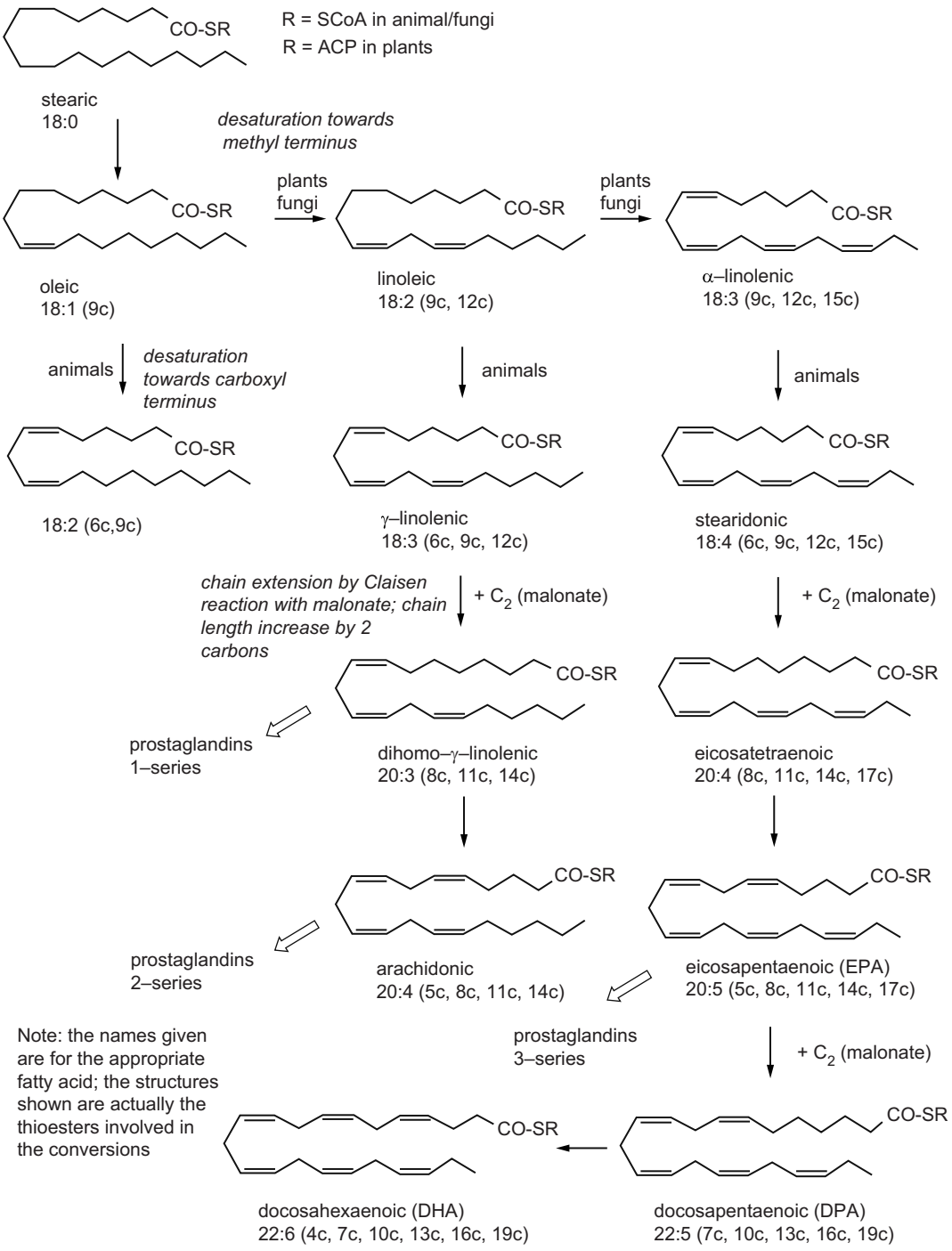

FIGURE 29.1
Synthesis of EPA and DHA from stearic acid in plants, fungi, and animals. *Source: Reproduced with permission from Dewick, P. M. (2001).* Medicinal Natural Products. A Biosynthetic Approach, *2nd edn. Wiley, West Sussex, UK.*

maintains the production of prostacycline; and yields PG-I3, which prevents platelet aggregation (Nettleton, 1995).

EPA and DHA lower serum triglycerides and cholesterol because they bind to peroxisome proliferator-activated receptors (PPARs), which act as transcription factors of specific genes. These receptors control cell differentiation and development related to carbohydrate, lipid, and protein metabolism (Torrejon *et al.*, 2007). The other two receptors (alpha and gamma) have

TABLE 29.4 Metabolic Effects and Health Implications of DHA and EPA

Health Implication	Metabolic and Health Effects
Hyperlipidemia and cholesterolemia	Both EPA and DHA reduce serum TG levels. However, only DHA increases HDL and improves the LDL:HDL ratio (Egert *et al*., 2009). Jump *et al*. (2008) found that the key transcription factors PPARα and SREBP-1 are regulated by ω-3 PUFA. DHA activates PPARα (lipid oxidation gene) and suppresses the nucleus expression of SREBP-1 (lipid synthesis gene). As a result, it promotes FA oxidation and suppresses lipogenesis; however, EPA is a more potent activator of PPARα compared to DHA
CVD	DHA and EPA lower total cholesterol, VLDL, HDL, and TG by inhibiting the synthesis of VLDL and apoprotein B-100. The ω-3 FAs also affect atherogenesis because they are precursors of PG that interfere with blood coagulation. Both FA inhibit sodium channels that decrease intracellular calcium transport affecting the electric behavior of the cardiac muscle and heart rate. An epidemiological study indicated that ω-3 PUFA reduced levels of C-reactive protein (29%), interleukin-6 (23%), E-selectin (10%), the intracellular adhesion molecule-1 or sICAM-1 (7%), and the vascular adhesion molecule or sVCAM-1 (8%). As a result, these reduced endothelial inflammation and CVD (Lopez-Garcia *et al*., 2004). Studies indicate that the daily supplementation of 250−500 mg of EPA + DHA reduces the risk of CVD, cardiac arrhythmia, and mortality in approximately 35% (Harris *et al*., 2009). Dewailly *et al*. (2003) compared the effect of fish consumption of three ethnic groups in Québec, Canada. Interestingly, the Inuit (Indigenous Canadians) people who regularly consumed fish had a lower risk of CVD, despite their high obesity rate and the habit of smoking
Immune response	DHA and EPA stimulate the immune response due to the increment of these FA in cell membranes (Kew *et al*., 2003). EPA inhibits cytokines and the enzymes involved in the degradation of connective tissue, which are the main enzymes responsible for rheumatoid arthritis (Simopoulos, 2002). Weldon *et al*. (2007) indicated that DHA is more effective than EPA in reducing pro-inflammatory cytokine production by macrophages
Diabetes	Hu *et al*. (2003) prospectively examined the association between intake of fish and ω-3 PUFA and risk of CVD and total mortality among 5103 female nurses with diagnosed type-2 diabetes but who were free of CVD or cancer. Compared with women who seldom consumed fish (<1 serving/month), the relative risks of CVD adjusted for age, smoking, and other established coronary risk factors were 0.70 for fish consumption one to three times per month, 0.60 for consumption once per week, 0.64 for consumption two to four times per week, and 0.36 for consumption five or more times per week. Higher consumption of fish was also associated with a significantly lower total mortality
Cancer	Consumption of ω-3 PUFA is related to lower cancer risk. The mechanism is unknown, but it may be related to the synthesis of PG, activation of the immune system, peroxy lipid radicals, membrane fluidity, hormone secretion, activity of growth factor, and intracellular signaling. Animal studies concluded that the consumption of fish oil rich in EPA and DHA reduces the synthesis of PG and thromboxanes-2 in tumor tissues. The changes in eicosanoids also affect the immune system, PG-2 of macrophages increases tumor dissemination and induces the production of interleukin-1 by monocytes and the tumor necrotic factor. EPA and DHA inhibit mammary cancer cell growth, whereas ω-6 FAs induce *in vivo* and *in vitro* growth (Hammamieh *et al.*, 2007)
Neurological and retina	DHA is the most abundant lipid in the nervous system, especially the brain. It is incorporated into the phospholipidic membrane of neuron cells and retina. DHA is mainly associated with the synaptosomes, and myelin. It accumulates in brain, retina, liver, and adipose tissue during the third trimester of pregnancy. DHA and fish oil supplementation helps patients with neurological disorders such as attention deficit disorder syndrome, schizophrenia, and Alzheimer's disease (Ross *et al*., 2007; Sorgi *et al*., 2007)
Fetus and child development	DHA/EPA have an important and critical role in fetus and newborn development because they help enhance proper neuron synaptic connections and cognitive and visual development (Jacobson *et al*., 2008; Sorgi *et al*., 2007). DHA supplementation during lactation increases plasma levels and promotes a higher Bailey psychomotor development in 30-month-old infants (Jensen *et al*., 2005)

CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid(s); HDL, high-density lipoprotein; LDL, low-density lipoprotein; PG, prostaglandins; PPARα, peroxisome proliferator-activated receptors-α; PUFA, polyunsaturated fatty acid; SREBP-1, sterol regulatory element binding protein-1; TG, triglyceride; VLDL, very low-density lipoprotein.

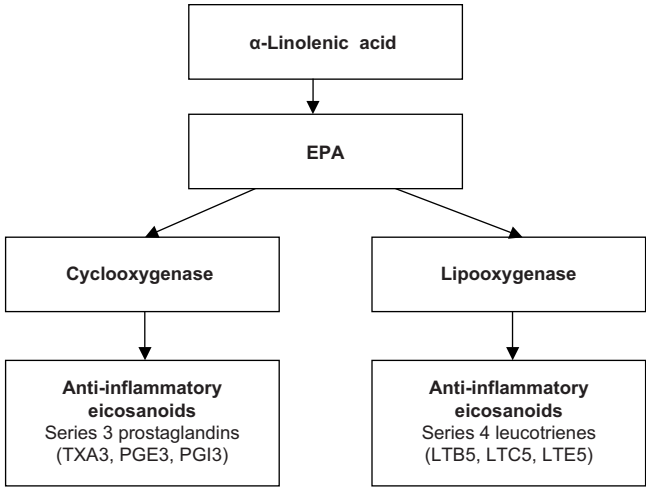

FIGURE 29.2
Synthesis of eicosanoids from α-linolenic acid and EPA.

affinity for EPA and DHA, enhancing β-oxidation and adipogenesis. These FA also inhibit other important transcription factors known as sterol regulatory element-binding proteins (SREBPs), which enhances gene expression responsible for sterol and cholesterol synthesis (Larsson *et al.*, 2004). EPA and DHA affect the activity of acyl-CoA:cholesterol acyltransferase, which esterifies cholesterol and avoids its accumulation in the cytosol (Deckelbaum *et al.*, 2006).

The consumption of EPA and/or DHA contributes more significantly than the consumption of α-linolenic acid to the prevention of CVD, thrombosis, hypertension, and death (Kumar Rudra *et al.*, 2001; Torrejon *et al.*, 2007). Their positive effects are related to the alteration of the eicosonoids' metabolism and the inhibition of proteins involved in cardiomyocytes sodium transport, which reduces heart rate. In addition, they help to maintain calcium "L" channels, avoiding the cytosolic saturation, especially during ischemic stress. The beneficial effect against thrombosis is associated with the liberation of nitric oxide, which improves vascular health via the inhibition of vasoactive protanoids.

SUMMARY POINTS

- Baking tests demonstrated that it was feasible to produce DHA/ω-3 fortified breads containing 25 or 50 mg DHA/slice (32 g). Enriched breads had similar properties as those of the control. The flax oil bread contained the highest amount of linolenic acid but lacked the important long-chain EPA and DHA. The fish oil bread had adequate baking properties but lost flavor and overall acceptability during the last stages of storage.
- Consumption of three slices of bread enriched with algae oil can provide almost all of the recommended daily amount of DHA (160 mg). The supplementation of 32 mg DHA/serving of a particular food (20% of the recommendation) allows the nutritional claim that the food is enriched with DHA.
- EPA and DHA play an important role in cell membrane structure and integrity, are precursors of eicosanoids, and are involved in gene regulation affecting the activity of important enzymes, receptors, and ionic channels. Both FA reduce the risk of CVD (arrhythmias, thrombosis, atherosclerosis, and hypertension) and diabetes, and they modulate the synthesis of eicosanoids involved in inflammatory/immune (psoriasis, asthma, cancer cell proliferation, and inflammatory intestinal disease) mechanisms and in the regulation of transcription factors that affect lipid metabolism (PPARα and SREBP-1). In addition, these FA affect neuron development in fetuses and neonates and photoreceptors present in retina.

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CHAPTER 30

Fortification with Free Amino Acids Affects Acrylamide Content in Yeast Leavened Bread

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CHAPTER OUTLINE

INTRODUCTION

Acrylamide, especially formed in heated carbohydrate-rich foods, is a known neurotoxin, a carcinogen in animals, and a probable carcinogen in humans (International Agency for Research on Cancer, 1994). Research on acrylamide is of particular importance because the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives considers its presence in food a human health concern. Acrylamide is formed via Maillard reactions involving free asparagine and reducing sugars under high temperatures (Zyzak *et al.*, 2003). Among cereal products, breads, rolls, pastries, biscuits, and breakfast cereals are the main foods contributing to acrylamide intake. The contribution of these and other major foods to acrylamide intake (i.e., fried potato and roasted coffee products) depends on the country and food habits (Joint FAO/WHO Expert Committee on Food Additives, 2005). It has been shown that free asparagine is the limiting precursor for

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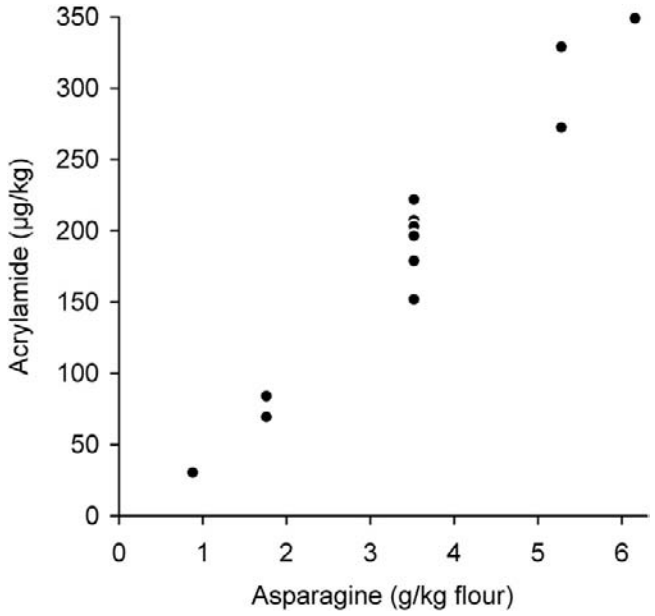

FIGURE 30.1
Relationship between added asparagine and acrylamide content in rye crispbread. The content of acrylamide increases as a result of asparagine addition for rye crispbread baked for 8 min at 250°C. *Source: Adapted from Mustafa, A., Andersson, R., Rosen, J., Kamal-Eldin, A., and Åman, P. (2005). Factors influencing acrylamide content and color in rye crisp bread.* Journal of Agricultural and Food Chemistry, *53, 5985–5989.*

acrylamide formation in bread and that reducing sugars are available in excess in most cases (Mustafa *et al.*, 2005; Surdyk *et al.*, 2004) (Figure 30.1).

Since the discovery of the occurrence of acrylamide in foods, many studies have investigated the factors affecting its formation and measures to control them (Claus *et al.*, 2008; Konings *et al.*, 2007). The acrylamide content in bread can be limited by adopting measures affecting the level of asparagine in the dough and/or hindering the Maillard reactions between asparagine and reducing sugars. In this chapter, we review the different measures that can be controlled to minimize the acrylamide content in breads. The suggested strategies for controlling the formation of acrylamide in bread include selection of raw materials, processing and fortification measures (e.g., the addition of amino acids, yeast, enzymes, certain metal ions, antioxidants, and organic acids), and controlling the time and temperature of baking.

CEREAL GRAINS AND MILLING FRACTIONS

The content of the acrylamide precursors (i.e., free asparagine and reducing sugars) varies with plant species/cultivar and growing conditions, but data on cereal raw materials are rather limited. The content of asparagine is higher in whole grain rye flour than in whole grain wheat and oat flours (Mustafa *et al.*, 2007) (Figure 30.2). In wheat flour, the content has been reported to vary between 0.1 and 0.7 mg/kg (Claus *et al.*, 2006). Selection of flour with low content may therefore offer a possibility to lower the acrylamide content in cereal products. Nitrogen fertilization has a strong impact on crude protein and free asparagine content in wheat flours, with up to a fourfold increase when 0 kg N/ha was compared to 220 kg N/ha (Claus *et al.*, 2006). In the study by Claus *et al.* (2006), sulfur fertilization did not increase the content of free asparagine in wheat flour. Sulfur deficiency seems to lead to the greatest accumulation of free asparagine in the grain, and under very severe deficiency an up to 30-fold increase in grain content of free asparagine has been noted (Muttucumaru

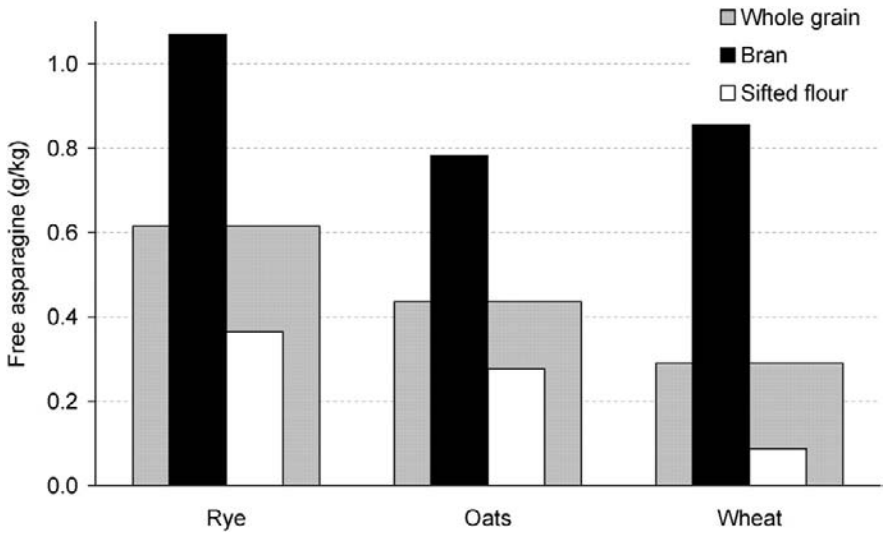

FIGURE 30.2
Content of free asparagine in whole grain and milling fractions of rye, oats, and wheat. The content of free asparagine is higher in rye whole grain compared to oats and wheat whole grain. Free asparagine is enriched in the bran fraction. *Source: Adapted from Mustafa, A., Åman, P., Andersson, R., and Kamal-Eldin, A. (2007). Analysis of free amino acids in cereal products.* Food Chemistry, *105, 317–324.*

et al., 2006). The sulfur content in the soil is therefore recommended to be at least 25 kg/ha. Sulfur deficiency has also been shown to affect the distribution of free asparagine in wheat kernels, resulting in a disproportional increase of the free asparagine in the inner parts of the wheat kernels (Shewry *et al.*, 2009). Genetic approaches to reducing acrylamide risk in cereals include identification of cultivars and germplasm in which free asparagine is low and the manipulation of genes involved in amino acid metabolism and signaling (Muttucumaru *et al.*, 2006).

Cereals are frequently processed by milling, resulting in fractions such as bran and sifted flour. Free amino acids have been determined in selected cereal fractions, revealing a wide range of contents (Mustafa *et al.*, 2007). Asparagine, aspartic acid, and glutamic acid were the most abundant free amino acids, and asparagine comprised 30–50% of the total. The acrylamide precursor asparagine is generally enriched in the outer parts of the kernels and is thus found at higher concentrations in the bran milling fraction. Sifted flours have lower contents of free asparagine (approximately 0.2 g/kg for wheat and 0.6 g/kg for rye), whereas the shorts and bran fractions contain significantly more (approximately 2 g/kg for wheat and 3 g/kg for rye) and the germ has the highest content of free asparagine (5 g/kg in wheat). The degree of enrichment of free asparagine in the bran fraction depends not only on the distribution of the amino acid in the tissue but also on the fractionation efficiency of the milling process. The separation of bran from the starchy endosperm is generally most efficient in wheat, giving a relatively low level of free amino acids in sifted wheat flour and a high content in wheat bran. Based on these results, it is evident that selection of grains and milling fractions is of great importance for the minimization of acrylamide formation during baking of bread.

CONTROLLING THE TIME AND TEMPERATURE OF BAKING

The Maillard reactions between amino acids and reducing sugars are accelerated by high temperatures, and their products are increased by long heat exposure. The effect of time and temperature of baking on acrylamide content in cereal-based products has been investigated in

numerous studies (Mustafa *et al.*, 2005; Surdyk *et al.*, 2004). In soft wheat bread, more than 99% of the acrylamide content is found in the crust; the content of acrylamide increased with baking time and temperature—an effect that can be attributed to the high rate of water loss from the surface of the bread (Surdyk *et al.*, 2004). Time and temperature of baking also significantly affected the acrylamide content in rye crispbread baked using a standard recipe, where acrylamide increased from 8 to 31 μg/kg bread (Mustafa *et al.*, 2005). These results show that the acrylamide content in bread can be reduced by optimizing the time and temperature of baking.

YEAST FERMENTATION AND ENZYME ADDITION

The content of the acrylamide precursor asparagine in dough can be reduced by yeast fermentation or the addition of the enzyme asparaginase. Yeast fermentation lowers asparagine levels as the yeast consumes it and other free amino acids as nitrogen sources for its metabolic activities (Benedito De Barber *et al.*, 1989). It was shown in a model system of yeast leavened wheat bread that highly reduced levels of formed acrylamide at long fermentation times correspond to the reduction of asparagine (Fredriksson *et al.*, 2004). Approximately 85% of free asparagine was used up during fermentation and baking (Figure 30.3), with most of the depletion occurring during fermentation (Mustafa *et al.*, 2007). A similar phenomenon was observed in rye crispbread, with up to an approximately 90% decrease in the content of asparagine during fermentation and baking. However, lengthy yeast fermentation should be avoided because of adverse effects including the degradation of the protein network and flattening of bread rolls and/or the production of monochloropropanediol isomers, defined as potential genotoxic carcinogens (Claus *et al.*, 2008).

The formation of acrylamide could be limited by applying more than one of the controlling measures. When testing the effect of two parameters, a significant interaction effect indicates that their effect occurs simultaneously and always the effect of one variable depends on the value of the other. In a model study using soft wheat bread, the effect of fermentation time and added asparagine at two levels (high and low) on the content of asparagine in the fermented dough and the acrylamide content in bread was investigated (Mustafa *et al.*, 2009). The fermentation time was found to have a significant decreasing effect on asparagine content in the dough, and an interaction effect was observed between the fermentation time and the level

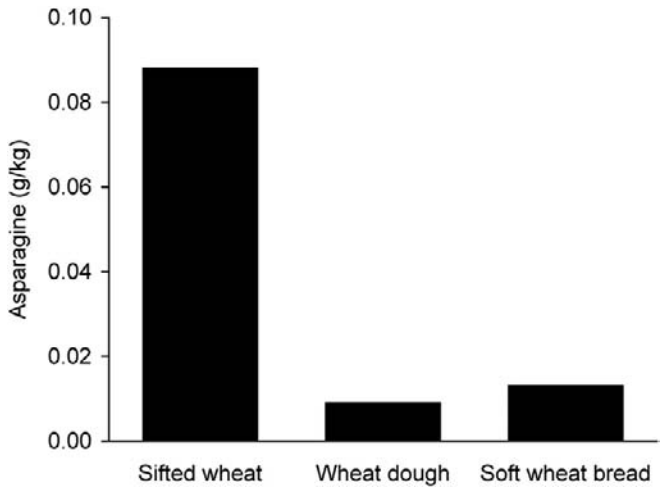

FIGURE 30.3
Content of asparagine in sifted flour, dough, and bread of wheat. The content of free asparagine is reduced during yeast fermentation (contents are given on a dry weight basis). *Source: Adapted from Mustafa, A., Åman, P., Andersson, R., and Kamal-Eldin, A. (2007). Analysis of free amino acids in cereal products.* Food Chemistry, *105, 317–324.*

of added asparagine in the dough, indicating that the reducing effect of fermentation time depends on the level of asparagine present in the system. The reduction of asparagine content in the dough containing low levels of the amino acid was translated into a significant reduction of acrylamide content in bread, but this effect was not observed in bread made from the dough with high concentrations of asparagine. When there is a large amount of asparagine, a long fermentation time may not reduce its content in dough to a large extent. A strong correlation between asparagine content in dough and acrylamide content in bread was obtained at all levels of added asparagine, which further supports the previously discussed finding that asparagine is the critical precursor for acrylamide content in bread. Consequently, it could be deduced that the efficacy of yeast fermentation time is controlled by the levels of asparagine present in the system. Therefore, ingredients that might enhance asparagine content should be avoided in raw materials for bread and similar products.

Addition of the enzyme asparaginase to the dough was suggested as a measure to control the formation of acrylamide. Asparaginase hydrolyzes asparagine to aspartic acid and ammonia and presents a potentially effective means for reducing asparagine formation in bread (Zyzak *et al.*, 2003). Asparaginase was effective in reducing asparagine content in bakery products, giving a decrease of up to 75%, which translated into a 55% reduction in acrylamide content in bread (Amrein *et al.*, 2004). The incompleteness of the hydrolysis of asparagine was related to the limited mobility of the enzyme and substrate within the dough. The reported effective dose for enzyme addition to reduce acrylamide formation was 200–1000 U/kg dough (Amrein *et al.*, 2007). Hendriksen *et al.* (2009) tested the potential of asparaginase in dough-based applications and obtained a 34–92% reduction of acrylamide contents in the final products. The critical parameters in these trials were enzyme dose, dough resting time, and water content.

FORTIFICATION WITH AMINO ACIDS OTHER THAN ASPARAGINE

Addition of amino acids, other than asparagine, to the dough reduces the acrylamide level in bread because they compete with asparagine in the reactions with reducing sugars. In a food model system, the reaction rates of all amino acids in the Maillard reactions were found to be similar so that the competition, and the reduction of acrylamide, depends on the number of molecular species of each amino acid (Wedzicha *et al.*, 2005). Addition of cysteine to the dough resulted in significantly lower acrylamide content in an asparagine–glucose model system (Claeys *et al.*, 2005). Addition of cysteine favors acrylamide reduction in bakery products, but care should be taken with this measure because it can affect dough rheological and sensorial properties at elevated dosages (Claus *et al.*, 2008).

The effect of added glycine and asparagine at different fermentation times on acrylamide content was studied in yeast leavened wheat bread by Mustafa *et al.* (2009). This study showed that there is an interaction effect between added glycine and asparagine in the reduction of acrylamide, indicating that the reducing effect of added glycine depends on the level of asparagine in the system (Figure 30.4). This effect was attributed to the competition between asparagine and glycine for reducing sugars and/or to reaction(s) with the formed acrylamide (Rydberg *et al.*, 2003). Furthermore, the addition of glycine and glutamine caused a reduction of acrylamide content in wheat bread depending on the levels of addition (Bråthen *et al.*, 2005). In another study, no reducing effect of added glycine on acrylamide formation was observed at low levels of asparagine in the dough (Fink *et al.*, 2006). Lysine and cysteine were also shown to reduce the formation of acrylamide, whereas alanine had a neutral effect and glutamine increased the yields of acrylamide in sealed aqueous model systems (Claeys *et al.*, 2005).

ADDITION OF IONS

An alternative measure based on the addition of metal ions to control the formation of acrylamide was proposed by Gökmen and Senyuva (2007). They found that divalent cations

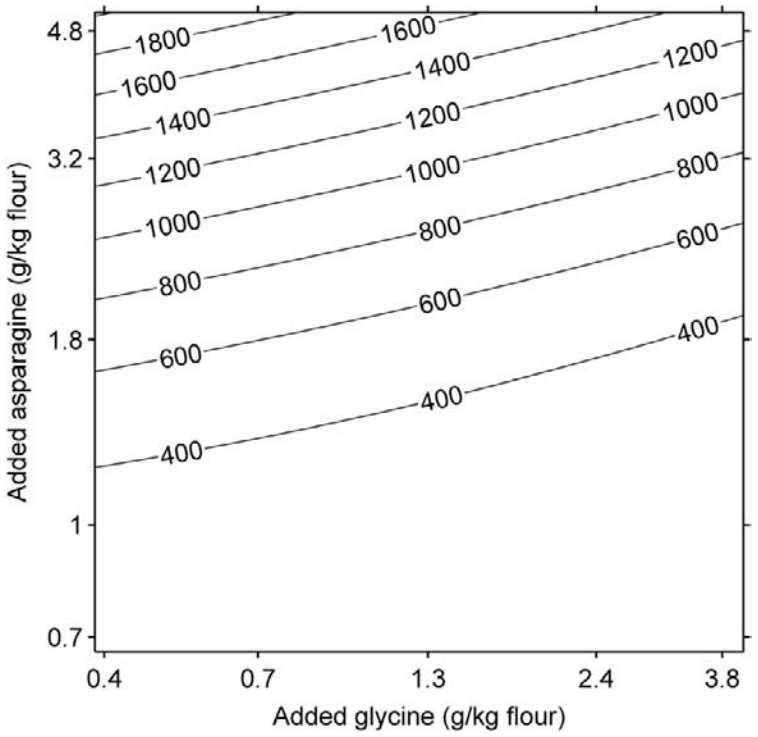

FIGURE 30.4
The response surface describing the effect of added glycine and asparagine on acrylamide content in wheat bread (g/kg). Acrylamide content is increased by the addition of free asparagine but reduced by addition of free glycine. The addition of free glycine can reduce the levels of acrylamide, especially at high levels of asparagine. *Source: Adapted from Mustafa, A., Åman, P., Andersson, R., and Kamal-Eldin, A. (2007). Analysis of free amino acids in cereal products.* Food Chemistry, *105, 317–324.*

(e.g., Ca^{2+} and Mg^{2+}) completely hindered the formation of acrylamide in a model system, whereas the monovalents (e.g., Na^{+} and K^{+}) resulted in a 50% reduction in acrylamide content. Adding up to 1% NaCl salt in a model food matrix caused a decrease in acrylamide content of 23% (Kolek *et al.*, 2006). The addition of Ca^{2+} into the reaction mixture increased the rate of glucose decomposition, whereas most asparagine remained unreacted. The reducing effect was related to the ability of these ions to stop the formation of Schiff base, which is an important intermediate in the Maillard reaction leading to acrylamide. However, the addition of cations has an adverse effect because they increase the formation of other harmful substances, such as hydroxymethylfurfural (HMF) and furfural, during heating (Gökmen and Senyuva, 2007). HMF and its derivative are known to have cytotoxic, genotoxic, and carcinogenic effects. The effect of added Na^{+} on acrylamide content in wheat bread depends on its level of addition, with a lowering effect at doses of up to 2% due to the inhibition of enzyme activities and a considerable increase in acrylamide content at higher levels of addition because of inhibition of the yeast growth (Claus *et al.*, 2008).

ADDITION OF ORGANIC ACIDS

Addition of organic acids and change in pH were proposed as a further measure to control the formation of acrylamide in bread (Amrein *et al.*, 2004). In a study using gingerbread, 1% citric acid in the dough reduced acrylamide content by a factor of 40 (Amrein *et al.*, 2004). In addition to citric acid, lactic, tartaric, and hydrochloric acids caused a substantial decrease in acrylamide content when tested in semifinished biscuit and biscuit models (Taeymans *et al.*, 2004). The reduction in acrylamide was attributed to the formation of aspartic acid and ammonia from asparagine as well as to moderation of the Maillard reactions at the low pH.

Nevertheless, this mitigation measure was not considered satisfactory because the resulting gingerbread had an acidic taste and insufficient browning and leavening related to the forced protonation of NH_3, which reduced the gas volume during baking. Thus, it would be best to evaluate the possibility of acid additions for each product individually.

ADDITION OF ANTIOXIDANTS

Addition of antioxidants has also been addressed as a measure to control the formation of acrylamide in foods. A study of the effect of prooxidants and antioxidants on the formation of acrylamide in bread showed that addition of 1% rosemary extract resulted in a 57–67% reduction in acrylamide levels depending on the nature of the extract (Hedegaard *et al.*, 2008). The flavonoids epicatechin and epigallocatechin gallate were also reported to reduce acrylamide formation in a model system of glucose and asparagine. Because of this interception by antioxidants, an unknown free radical pathway in the formation of acrylamide has been suggested (Hedegaard *et al.*, 2008).

INGREDIENTS AND PRODUCT FORMULATION

It has been reported that using ammonium bicarbonate (NH_4HCO_3) substantially increased acrylamide content in gingerbread and biscuits (Amrein *et al.*, 2004). However, when it was replaced with sodium bicarbonate ($NaHCO_3$), a decrease on the order of 70% was observed in all the tested products. In further studies, it was shown that when sodium hydrogen carbonate was used in combination with citric acid, gingerbread was almost devoid from acrylamide; this was explained by the reduction in pH. Amrein *et al*. reported a correlation between the content of asparagine, baking additives, and acrylamide. Products that were found to enhance acrylamide formation include almonds, hazelnuts, and sesame and poppy seeds.

Effect of storage

In two studies, we observed a reduction in acrylamide levels upon storage of rye crispbread (Mustafa, Andersson, *et al*., 2008; Mustafa, Kamal-Eldin, *et al*., 2008). The reduction was 13% when the bread powder samples were stored for 56 days at 20°C, and it was 17% when the samples were stored for 14 days at 40°C (Mustafa, Andersson, *et al*., 2008) (Figure 30.5). In addition to temperature, we have also found that the decrease in acrylamide content in the rye crispbread powder is enhanced by moisture (Mustafa, Andersson, *et al*., 2008). There was no effect of the type of container, oxygen, or surface area. In our studies, a strong correlation was found between the level of acrylamide in the fresh bread and that in the stored bread, suggesting that the decrease is proportional to the content in the starting material (Mustafa, Andersson, *et al*., 2008).

TECHNOLOGICAL ISSUES

Some of the mitigation measures described previously may compromise product quality, especially those measures that affect other Maillard reactions contributing to desirable bread color and flavor. This fact gave rise to the question of whether a correlation exists between acrylamide content and color and flavor intensity and would the measures used to control the formation of acrylamide jeopardize the organoleptic qualities of the final product. In a laboratory baking trial of rye crispbread with different heating protocols, we showed that the color intensity of the bread as well as the acrylamide content increased simultaneously (Mustafa *et al*., 2005). When correlating the color intensity and formed acrylamide, it was found that the addition of asparagine resulted in more formed acrylamide with limited variation in color formation. This indicated that asparagine contributes to the pathway(s) leading to acrylamide with a limited effect on color formation. Thus, measures leading to reduction of asparagine content—that is, choice of grains and milling fraction and optimization of yeast/enzyme addition—should be highlighted because limiting the major precursor asparagine would not

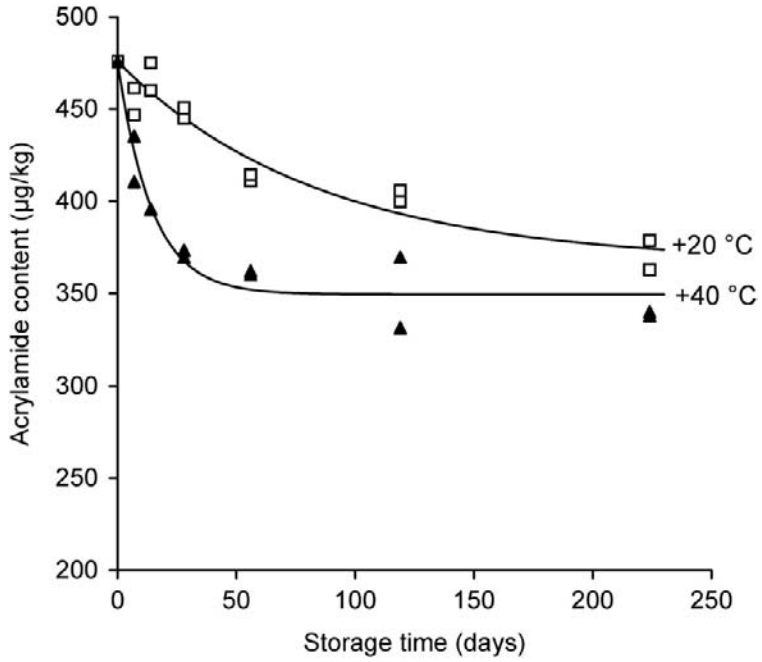

FIGURE 30.5
Levels of acrylamide content in rye crispbread stored at different temperatures. Storage of the bread can lead to reduction in the content of acrylamide. The reduction was faster and more pronounced at 40°C. *Source: Adapted from Mustafa, A., Kamal-Eldin, A., Petersson, E. V., Andersson, R., and Åman, P. (2008). Effect of extraction pH on acrylamide content in fresh and stored rye crisp bread.* Journal of Food Composition and Analysis, *21, 351–355.*

influence color formation. On the other hand, the quality of color may be affected if time and temperature of baking are changed in a suboptimal way.

Addition of glycine is used as an effective measure to control the formation of acrylamide. Furthermore, glycine is known to enhance color formation in bakery products when added before dough fermentation or on the surface of the dough before baking (Bråthen *et al.*, 2005; Fink *et al.*, 2006). This finding has been tested in a model study of soft wheat bread in which color formation was correlated with added glycine, asparagine, and fermentation time (Mustafa *et al.*, 2009). A correlation was only obtained between glycine and the a^* value (degree of redness) (Figure 30.6); the observed deviation from the regression line could be explained by variation in the fermentation time. The correlation between glycine and color indicates that unlike asparagine, glycine takes part in the Maillard reaction(s) leading to color formation. We observed an initial increase in the intensity of color with glycine addition and fermentation time, where the highest color intensity was achieved at intermediate fermentation times (Mustafa *et al.*, 2009). Longer fermentation times resulted in lesser hue of the final product, which might be partially attributed to the consumption of other amino acids and sugars (i.e., other main precursors contributing to color). Therefore, glycine could be used as an additive together with acrylamide reducing measures that tend to produce insufficiently colored products, for example, when replacing reducing sugars with sucrose or when using lower temperatures for baking.

Novel baking technologies have been proposed to limit the formation of acrylamide without substantial effect in the product quality. Extrusion is regarded as a mild technology that suits products with low water content, and because it involves mild toasting, less Maillard products will be formed (Claus *et al.*, 2008). Other technologies include air jet impingement, infrared radiation baking, and steaming baking. The infrared radiation gave bread with approximately 60% less acrylamide yet sustained product qualities similar to the standards.

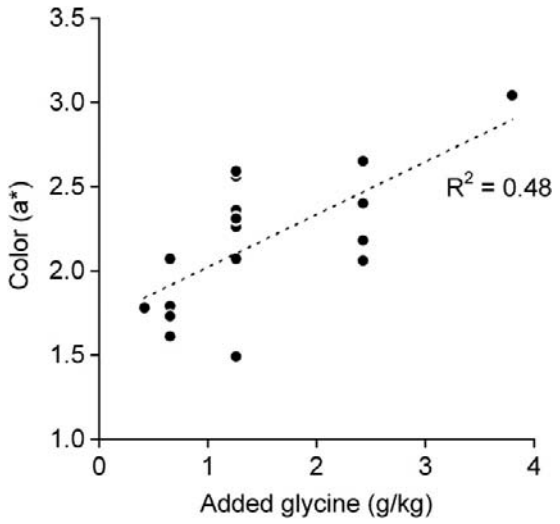

FIGURE 30.6
Effect of added glycine on the color of yeast leavened bread. Different fermentation times were used at each level of glycine. The degree of redness (a^*) was increased with increased levels of free glycine addition. *Source: Adapted from Mustafa, A., Fink, M., Kamal-Eldin, A., Rosén, J., Andersson, R., and Aman, P. (2009). Interaction effects of fermentation time and added asparagine and glycine on acrylamide content in yeast-leavened bread.* Food Chemistry, *112, 767–774.*

Steaming baking resulted in a satisfactory decrease of 40% with bread qualities similar to the standards (Heatox, 2007).

CONCLUSION

This chapter reviewed different measures that can be used to reduce the level of acrylamide in bread. These include choice of ingredients (flour and milling fractions, added amino acids, salts, organic acids, antioxidants, and enzymes), yeast fermentation, and heating protocols during baking. Some of these mitigation measures lead to compromise in product quality (color, flavor, and leavening), but these can be optimized or different measures can be combined to obtain the desired quality. Addition of metal ions might lead to the formation of toxic compounds such as hydroxymethylfurfural and should be used with care. The addition of the amino acid glycine, and possibly other amino acids, is particularly interesting because it improves bread color and might compete with asparagine and hinder acrylamide formation.

SUMMARY POINTS

- Free asparagine in dough is the limiting precursor for acrylamide formation during baking.
- The content of free asparagine in dough can be controlled by the choice of ingredients with low asparagine content; yeast fermentation, which consumes the free asparagine; and the use of asparaginase during dough making.
- Optimizing the time and temperature of baking could be used as a means to minimize acrylamide formation during baking.
- Addition of free amino acids other than asparagine, metal ions, organic acids, and antioxidants and selection of baking powder can in certain cases be used to reduce the formation of acrylamide during baking.
- Because free asparagine does not contribute to color formation during baking, it can be reduced in dough without affecting color formation. Addition of other amino acids, such as glycine, can reduce acrylamide by competition with asparagine in the Maillard reaction and enhance color formation during baking, leading to desirable color at reduced heating.

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CHAPTER 31

Barley β-Glucans and Fiber-Rich Fractions as Functional Ingredients in Flat and Pan Breads

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

CSP Canadian short process
FRF Fiber-rich fractions
GI Glycemic index

INTRODUCTION

In recent years, the role of diet has expanded from the past emphasis on survival and hunger satisfaction to the use of food to promote better health and to manage and reduce the risk of certain chronic diseases. Consumers' interest in purchasing health-promoting foods and food ingredients continues to increase as the link between diet and health benefits becomes substantiated by positive scientific evidence and clinical trials. The mixed linkage (1→3)(1→4)-β-D-glucans, commonly known as β-glucans, are the major constituents of dietary fiber in cereal grains such as barley and oats. β-Glucans have been implicated in controlling appetite, attenuating postprandial blood glucose and insulin levels, and lowering serum cholesterol levels (Wood, 2007). The effect of β-glucans on the postprandial glucose metabolism is related to their ability to increase viscosity in the gut

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causing a delayed and/or decreased absorption of glucose into the bloodstream. The role of β-glucans in lowering cholesterol and serum lipids has been a subject of numerous studies that showed that by increasing the viscosity of the contents of the gastrointestinal tract due to the presence of soluble fibers, a decrease in absorption and readsorption of cholesterol, bile acids, and their metabolites occurs, resulting in a reduced rate of diffusion of nutrients (Wood, 2007).

Enough positive evidence of the efficacy of barley β-glucans to reduce the risk of coronary heart disease has been found, and in the United States, foods containing barley are allowed to carry a health claim. This claim stipulates that 3 g per day of β-glucan soluble fiber is needed for an effect on cholesterol levels (Food and Drug Administration (FDA), 2005). FDA allows barley products to claim reduction in risk of coronary heart disease (FDA News, December 23, 2005. Online at: http://www.fda.gov/bbs/topics/news/2005/NEW01287.html). Similar claims linking β-glucans with bowel regularity and heart health have been approved in the European Union (Bryngelsson and Asp, 2007).

Given the evidence supporting the physiological benefits of β-glucans in the human diet, a considerable amount of technological research has gone into developing ways of incorporating these polysaccharides into food products. Typically, white bread is a poor source of dietary fiber and is considered to be a high glycemic food; therefore, incorporation of β-glucans into bread is desirable and at the same time an effective way to increase the intake of these polysaccharides because of the high consumption of bread products worldwide. This chapter discusses various technological and functional aspects of incorporating different forms of β-glucan preparations into pan and flat bread products.

BARLEY β-GLUCAN ISOLATES IN BREAD PRODUCTS

In an attempt to specifically assess the role of β-glucans during various stages of the bread making process, several baking studies using isolated and purified polymers from barley grain were conducted. Extraction of β-glucans from barley usually involves several steps, including inactivation of endogenous enzymes in the grain, extractions of grain with water or alkali solutions, removal of contaminating protein and starch from extracts, precipitation of β-glucans from the purified solutions with ethanol, and drying. Most of the resulting preparations contain between 70 and 85% of β-glucans. A partial replacement of wheat flour with purified water-soluble β-glucans significantly increases the farinograph and baking water absorption of dough during mixing (Table 31.1). The effect is attributed to a high water-absorbing capacity of these polymers, and its magnitude is dependent on the amount of β-glucans in dough and their molecular weight. Noticeable changes to the dough rheology were reported by Cleary *et al.* (2007), who observed higher resistance to extension and lower extensibility in doughs containing 5% of high- (510 kDa) and low-molecular-weight (160 kDa) β-glucan preparations. The effects of β-glucans on the rheological properties of wheat doughs are additionally influenced by the quality of the base wheat flour. Skendi *et al.* (2010) reported that β-glucan addition (0.2−1.4%) improved somewhat the extensibility of the poor bread making quality dough, thus strengthening the gluten matrix and increasing the gas retention capacity of doughs (see Table 31.1). Wang *et al.* (1998) pointed to a possible role of β-glucans in improvement of bread crumb grain by stabilizing air cells in the bread dough and preventing their coalescence. Inclusion of β-glucans in bread generally results in a significant decrease in loaf volume and height, and the reduction in height and volume worsens with increasing amounts and molecular weights of β-glucans (see Table 31.1). However, Skendi *et al.* observed that small improvements of the specific volume of bread are achievable through optimization trials that take into consideration the quality of wheat flour as well as the level and molecular features of β-glucans used for fortification. It is postulated that the negative effect of β-glucans on loaf volume is due to

TABLE 31.1 Properties of Bread Supplemented with Isolated β-Glucans

	Farinograph		Dough Rheology			
Isolate	Water Absorption (%, 14% mb)	Dough Development Time (min)	Resistance to Extension	Extensibility (mm)	Loaf Volume	Reference
Control	58.4	5.7	—	—	768 ml	Cavallero *et al*. (2002)
+20% WF	67.0	8.2	—	—	385 ml	
Control			33.34 g[a]	−29.83[a]	212 ml	Cleary *et al*. (2007)
+5% HMW	—	—	74.28 g	−22.30	100 ml	
+5% LMW	—	—	49.18 g	−23.75	118 ml	
Control	62	—	—	—	905 ml	Jacobs *et al*. (2008)
+0.5% BG	69	—	—	—	855 ml	
+1.0% BG	75	—	—	—	765 ml	
Control-Dion	50.3	1.8	103 BU[b]	241[b]	2.46 ml/g	Skendi *et al*. (2010)
+0.2% BG-100	52.6	4.5	148 BU	202	2.69 ml/g	
+0.6% BG-100	52.8	4.8	118 BU	215	2.88 ml/g	
+1.0% BG-100	52.8	4.7	95 BU	222	2.29 ml/g	
+1.4% BG-100	53.0	5.3	188 BU	195	2.26 ml/g	

BG, water extracted β-glucan (~85% β-glucan); BG-100, commercial concentrate (84.5% β-glucans, 100 kDa); Dion, poor bread making quality wheat flour; HMW, high-molecular-weight β-glucans (~95%, 510 kDa); LMW, low-molecular-weight β-glucans (~95%, 160 kDa); WF, water extracted fraction (33.2% β-glucan).

[a]*Resistance to extension (mean max force, g) and extensibility (mean distance at max force, mm) of doughs were measured using a TA-XT2 texture analyzer.*

[b]*After 135 min resting time in a fermenting cabinet, each dough piece was stretched in the Brabender Extensograph by a hook until rupture. The stretching force was recorded as a function of time, and the resistance to constant deformation after 50-mm stretching (R_{50}) and the extensibility were obtained.*

the combined effects of gluten dilution and disruption of gluten networks. The high water-binding properties of β-glucans interfere with proper hydration of gluten, resulting in underdeveloped gluten networks. Cleary *et al*. proposed that a reduction of steam production during baking as a result of strong water binding of β-glucans also contributes to the decreased loaf volume.

The increase in hardness of the bread crumb fortified with β-glucans at a higher level of addition (>1%) has been observed and various interpretations have been put forward. Rosell *et al*. (2001) proposed that the increase in crumb firmness may be a consequence of the thickening of walls surrounding the gas cell that occurs upon addition of hydrocolloids into bread formulas. Furthermore, an increase in bread firmness may be a consequence of a decrease of the total area of the gas cell in bread containing β-glucans; indeed, the greatest crumb firmness is usually observed in breads with the lowest loaf volume. Also, water promotes starch recrystallization, and the water content of β-glucan-enriched breads is generally higher than that of control breads. However, Skendi *et al*. (2010) did not report an increase in starch retrogradation with the addition of β-glucans, and Gill *et al*. (2002) proposed that β-glucans added to wheat flour reduce swelling and solubilization of starch during baking and thereby reduce bread firmness.

The effectiveness of barley β-glucan inclusion on the *in vitro* digestibility of breads was shown by Symons and Brennan (2004) and Cleary *et al*. (2007). Both studies reported significant reduction of sugars during *in vitro* digestion of breads prepared by replacing 5% of wheat flour with purified β-glucans. The effects were partly attributed to the inhibition of enzyme accessibility to starch polymers due to the increased digesta viscosity and/or altered rheological properties of breads containing β-glucans. Scanning electron micrographs of *in vitro* digests of bread containing barley β-glucan illustrated the more compact structure of bread and the retention of undigested starch granules compared to control breads. Cavallero *et al*. (2002) demonstrated a potential to regulate, *in vivo*, the sugar release from breads containing β-glucans; a significant reduction in the area under the blood glucose curve and delay in the

mean peak of blood glucose was observed in human subjects who consumed bread supplemented with 20% of barley β-glucans.

BARLEY FIBER-RICH FRACTIONS

Pure β-glucan isolates obtained via traditional extraction procedures described previously are not suitable for commercial applications because the procedures are time-consuming and costly. The alternative sources of β-glucans are fractions obtained by dry grain fractionation such as milling, pearling, sieving, and/or air classification. The nonuniform distribution of components within the barley kernel allows fractionation by physical means into products enriched in various constituents, including β-glucans and arabinoxylans. Such naturally obtained products may be more desirable food ingredients for health-conscious consumers than products obtained through chemical processes.

Izydorczyk *et al.* (2003) developed and optimized roller milling flow conditions for the production of barley flour and a coarse fiber fraction ("shorts") that originates mainly from the endosperm cell walls and contains large amounts of β-glucans, other bioactives, and dietary fiber constituents. This fraction, designated as a "fiber-rich fraction" in barley milling, is potentially of more value as a functional food ingredient than barley flour that is enriched mainly in starch. The milling flow, as shown in Figure 31.1, is composed of break passages through four sets of corrugated rolls. Following the fourth break, the ground product is sieved through two sieves with different apertures, and the coarse material retained on the sieves is directed to a shorts duster. The impact action occurring in the shorts duster effectively cleans the fiber by releasing starch granules that are encapsulated in the endosperm cell walls. The next stages of fiber refinement include a single passage through sizing rolls, sieving, and another shorts duster passage. The original FRF can be further enriched by additional pin milling, sieving, and another shorts duster passage. Depending

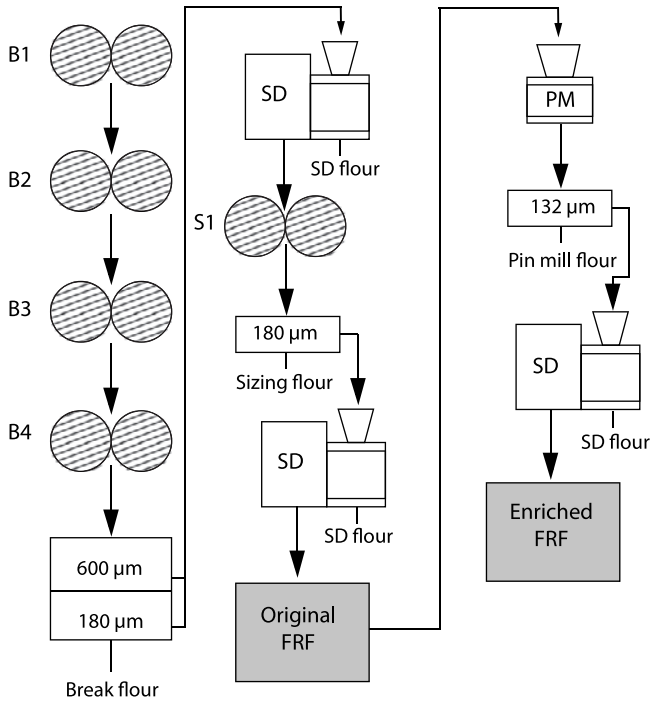

FIGURE 31.1
Roller milling flow for the original and enriched fiber-rich fractions (FRF) from whole barley. B, break passage; PM, pin mill; S, sizing passage; SD, shorts duster.

TABLE 31.2 Composition and Properties of Fiber-Rich Fractions (FRF) Obtained from Hull-less Barley Genotypes

	CDC Fibar			HB08302		
Properties and Composition	**Whole Barley**	**Original FRF**	**Enriched FRF**	**Whole Barley**	**Original FRF**	**Enriched FRF**
Yield (%)		38	19		36	16
Brightness, L^*		82.8	85.7		81.9	84.3
Swelling (ml/g)		13.1	16.9		10.0	13.9
Particle size, $d_{0.5}$ (μm)		300.6	171.8		272.0	175.3
Total β-glucan (%)	10.8	17.4	27.0	7.7	13.0	21.0
Soluble β-glucan (%)	5.4	8.3	12.1	3.4	5.5	8.3
Total arabinoxylans (%)	4.2	8.2	10.0	4.7	10.3	13.0
Starch (%)	52.3	34.4	24.3	56.2	36.4	23.9
Protein (%)	15.8	17.8	15.7	12.7	14.4	13.3
Ash (g/kg)	22.4	32.2	36.6	20.7	31.9	37.6
Mn (mg/kg)	18.3	22.1	18.7	18.5	19.4	17.1
Zn (mg/kg)	29.5	37.7	39.6	23.6	33.2	39.6
Fe (mg/kg)	55.6	79.6	91.8	44.1	66.6	83.4
Ca (mg/kg)	221	227	224	228	300	279
Mg (mg/kg)	1500	2330	2700	1390	2330	2830
P (mg/kg)	4070	6600	7670	4300	6400	7900
Total ferulics (μg/mg)	0.82	1.45	1.50	0.79	1.57	2.08
Vitamin B_3 (mg/100 g)	8.2	13.1	15.7	9.3	14.5	18.9
Vitamin E (mg/100 g)	0.96	1.32	0.98	0.43	0.77	0.51

CDC Fibar, hull-less barley with waxy starch; HB08302, an experimental hull-less barley line with high amylose starch.

on barley genotype, the β-glucan content of the enriched FRF obtained using this expanded roller milling flow design ranged from 21 to 27% (Table 31.2). A major benefit of the dry separation technologies is that the cell wall structures remain intact so there is very little chance of altering the physicochemical properties of β-glucans. Also, in contrast to β-glucan isolates or commercial β-glucan concentrates, barley FRF have the added benefit of containing other dietary fiber and bioactive components as well as being obtained by a chemical-free process.

If barley is not pearled before milling, the FRF contain not only the endosperm cell walls but also portions of the outer grain layers, specifically pericarp, aleurone, and subaleurone tissues (Figures 31.2A and 31.2B). Compared to the original FRF (150−500μm) (Figure 31.2A), the enriched FRF consist of smaller particles (100−300 μm) with fewer starch granules (Figure 31.2B); the particles generally have irregular shape and porous structure (Figure 31.3).

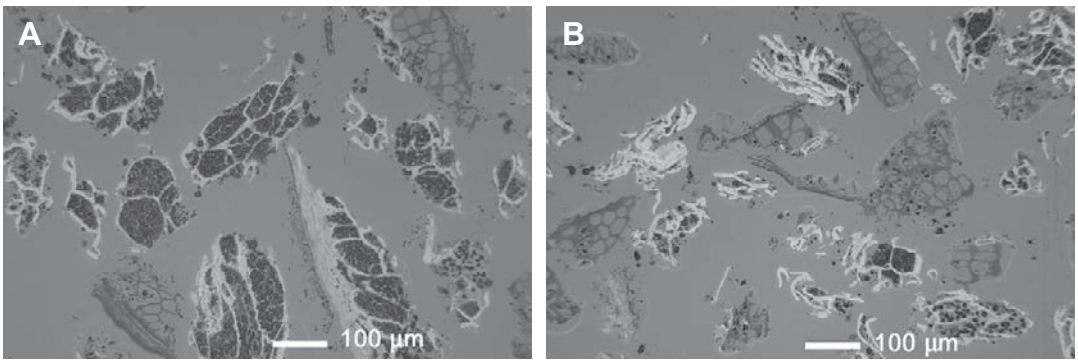

FIGURE 31.2
Light photomicrographs of sections of the (A) original and (B) enriched fiber-rich fractions.

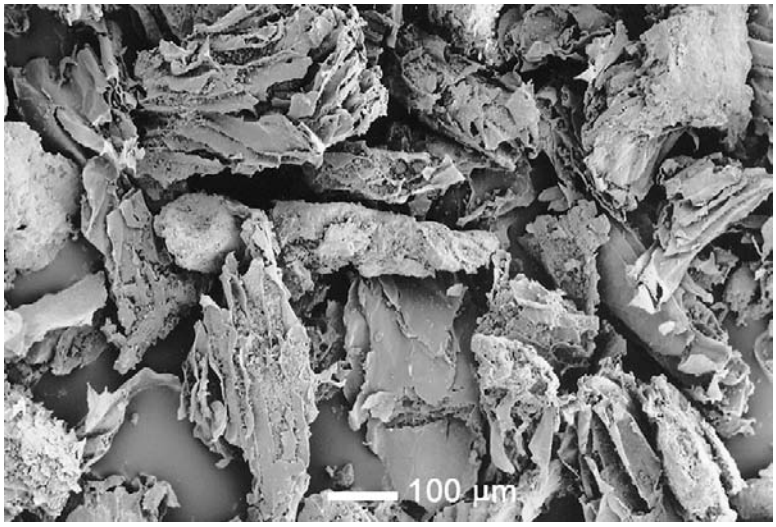

FIGURE 31.3
Scanning electron photomicrograph of barley original fiber-rich fractions.

Both the original and the enriched FRF from two barley cultivars (CDC Fibar and HB08302) contained higher amounts of β-glucan, arabinoxylans, protein, Mn, Zn, Fe, Ca, Mg, P, total ferulic acids, and vitamins B_3 and E than the respective whole grain samples (Table 31.2). Although the yield for the enriched FRF decreased, the refinement steps increased the content of β-glucan, arabinoxylans, total ferulics, vitamin B_3, as well as Zn, Fe, Mg, and P. The enriched FRF were also slightly brighter and contained fewer dark specks than their original counterparts.

BARLEY FIBER-RICH FRACTIONS IN PAN BREADS

Although the addition of β-glucans to bread has the potential to improve its nutritional benefits, its incorporation at the level recommended by the FDA—that is, 0.75 g of β-glucan per serving—has proven to be challenging, resulting in lower product quality and reduced consumer acceptability. Satisfactory bread products supplemented with barley flour have been produced, but often the level of supplementation is too small to achieve any significant health benefits. Compared to white or whole barley flour, the FRF have the advantage of being enriched in β-glucan and other bioactive components, and as a result, lower supplementation levels can be used to achieve the desired health benefits.

We assessed the potential of the original and enriched FRF from waxy and high-amylose hull-less barley (CDC Fibar and HB08302) as functional ingredients in bread prepared by the Canadian short process (CSP). The formula included wheat flour (100 g), whey (4 g), shortening (3 g), yeast (3 g), sugar (4 g), salt (2.4 g), and ascorbic acid (150 µg). To obtain the FDA-recommended dosage of 0.75 g of β-glucan per two-slice serving, 10 and 13% of white wheat flour was replaced with the original FRF from CDC Fibar and HB08302, respectively. Because of the higher concentration of β-glucans in the enriched FRF, the replacement level was reduced to 6 and 8% when using the enriched FRF from CDC Fibar and HB08302, respectively. In each case, the water absorption of the FRF-supplemented doughs was higher than that of 100% white flour but comparable to that of the 100% whole wheat flour (Table 31.3). As a consequence of higher baking absorption, the barley FRF-supplemented loaves and the 100% whole wheat loaves were heavier than the white flour control. The loaf volume of breads supplemented with original FRF was reduced by approximately 20–22% compared to the white flour bread and by 7–10% compared to the 100% whole wheat flour bread (Table 31.3). The crumb structure of breads containing the original FRF scored higher than that of the 100%

TABLE 31.3 Quality Characteristics of Wheat and Barley Fiber-Rich Fraction (FRF)-Supplemented Breads

				Visual Assessment			C-Cell Parameters			
	Water Absorption (%)	Loaf Volume (cm^3)	Loaf Weight (g)	Appearance	Crumb Texture	Brightness, L^*	No. of Cells	Cell Diameter (mm)	Cell Wall Thickness (mm)	Nonuniformity
Control loaves										
100% White flour	65	2210	286	7.5	6.5	85.0	5777	2.498	0.476	2.944
100% Whole wheat flour	73	1910	304	6.0	5.5	72.9	4712	2.790	0.494	1.818
FRF-supplemented loaves										
CDC Fibar										
+10% Original FRF	74	1770	300	5.5	5.8	77.5	4551	2.427	0.490	3.143
+6% Enriched FRF	74	2060	302	7.5	5.8	81.7	5308	2.396	0.482	1.567
HB08302										
+13% Original FRF	73	1720	303	6.5	6.0	78.5	4682	2.375	0.484	3.200
+8% Enriched FRF	74	2020	308	7.5	6.2	82.1	5247	2.404	0.483	1.567

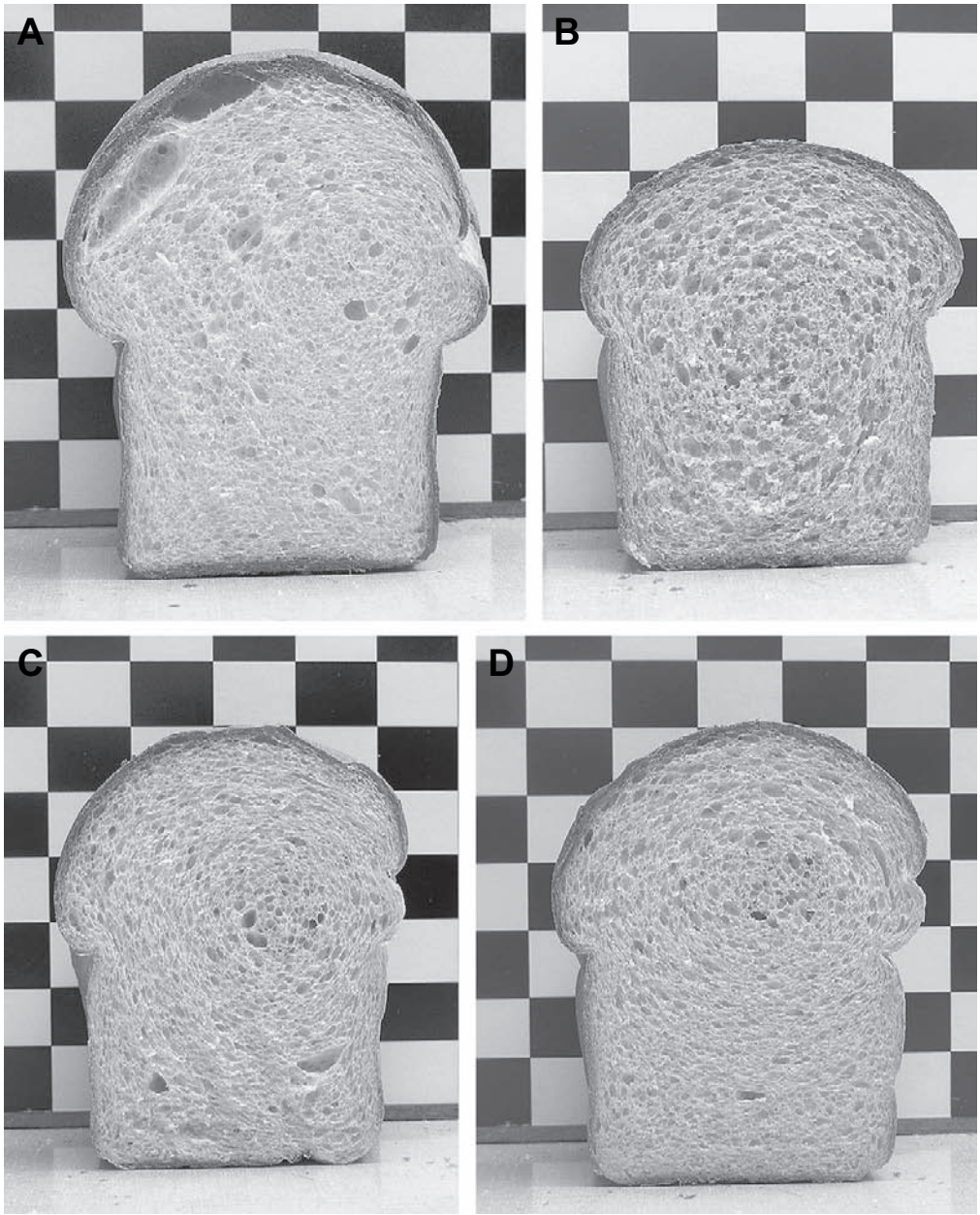

FIGURE 31.4
Control loaves and barley fiber-rich fraction (FRF)-supplemented breads. (A) 100% white flour, (B) 100% whole wheat flour, (C) +6% CDC Fibar-enriched FRF, and (D) +8% HB08302-enriched FRF.

whole wheat bread but slightly lower than that of the white bread (Table 31.3). Replacement of wheat flour with the enriched FRF preparations significantly improved the overall quality of the supplemented bread compared to the bread supplemented with the original FRF. The most striking improvements were in the loaf volume, appearance, crumb structure, and color of the bread (Table 31.3). The loaf volume of bread supplemented with both enriched FRF was superior to that of 100% whole wheat bread, and when compared to white flour bread, the volume was reduced by only 6–9% (Figure 31.4). The loaf volume of breads supplemented with the enriched FRF was superior to that of breads containing the original FRF. The number of cells in bread slices increased substantially when the enriched rather than the original FRF were used. Overall, the FRF-supplemented breads exhibited slightly finer crumb structure than wheat breads, as indicated by the lower diameter of the cells in the former. The cell wall

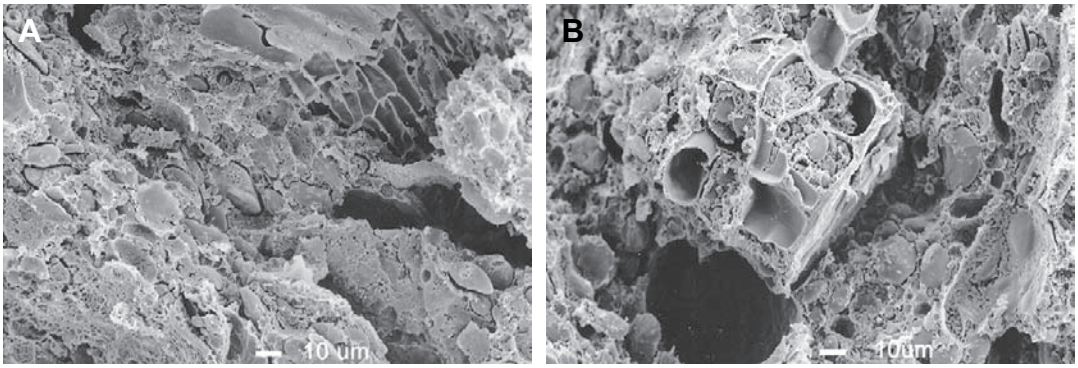

FIGURE 31.5
SEM micrographs of barley fiber-rich fraction (FRF)-supplemented bread dough. Discrete cell wall fragments are visible in the FRF-supplemented dough, giving it an appearance of a composite with particles of texture different than the matrix.

thickness in the FRF-supplemented breads was higher than that in white flour bread but lower than that in the whole wheat flour bread. The enriched FRF only slightly decreased the cell diameter or the wall thickness of bread but substantially improved the texture uniformity of bread slices compared to bread supplemented with the original FRF. A significant improvement in brightness of the bread supplemented with the enriched FRF (Table 31.3) was a definite advantage because often breads containing barley ingredients are characterized as having a grayish tint.

The mechanisms by which the FRF affect the dough rheology and bread textural attributes are complex, and the overall effects depend on the interactions among water, protein, starch, and fiber polysaccharides at the microscopic and molecular levels. Although the barley FRF can be considered mostly as particles containing insoluble dietary fiber, a portion of fiber may become partially solubilized during the bread making processes. As shown in Figure 31.5, discrete cell wall fragments are visible in the FRF-fortified dough, giving it the appearance of a composite with particles of texture different than the matrix. In bread, both the β-glucan-containing endosperm cell wall fragments (Figure 31.6A) and the arabinoxylan-rich aleurone cell walls (Figure 31.6B) can still be detected, although the fiber particles appear smaller and distorted, indicating their partial solubilization. The potential mechanisms by which insoluble fiber ingredients, such as bran, exert negative effects include gluten dilution, water entrapment, and mechanical disruption of gluten films during mixing, proofing, or the early stages of baking. Decreasing the level of fiber addition lessens the aforementioned effects, as clearly shown in breads containing the enriched barley FRF. Solubilization of the major FRF constituents, β-glucans and

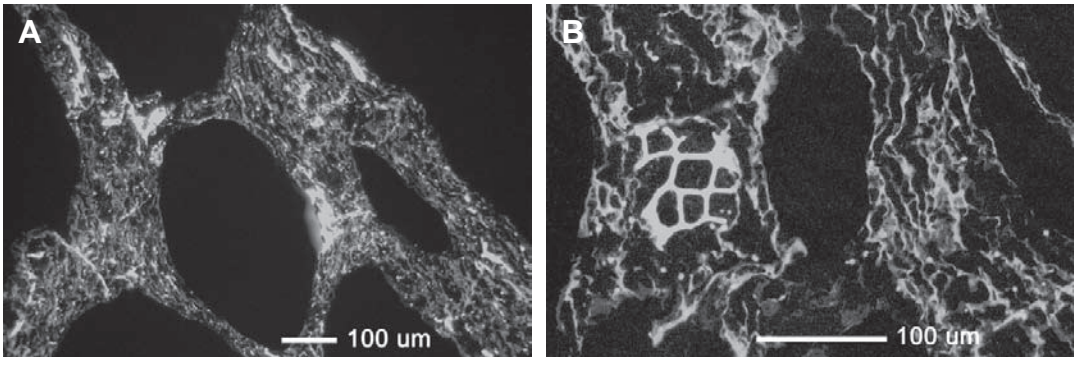

FIGURE 31.6
Light micrographs of barley fiber-rich fraction supplemented breads. (A) Bread stained with Calcofluor indicating the presence of β-glucans (lighter spots); (B) bread showing autofluorescence due to the presence of arabinoxylans in the aleurone cell walls.

TABLE 31.4 Extractability and Molecular Weight of β-Glucans Obtained by *in Vitro* Digestion of Fiber-Rich Fractions (FRF) and FRF-Supplemented Breads

	Extractability of β-Glucans (% of Total)	Molecular Weight (g/mol)
CDC Fibar		
Enriched FRF	55.6	279,000
Bread + enriched FRF	33.0 ± 3.9	247,200
HB08302		
Enriched FRF	39.7	287,300
Bread + enriched FRF	40.4 ± 4.3	495,500

arabinoxylans, during the bread making processes could lead to increased viscosity and/or formation of separate viscoelastic polysaccharide networks, resulting in changes to rheological properties of dough, reduction of dough expansion during proofing, and decreased loaf volume.

From a physiological standpoint, increased solubilization of β-glucan in a food product is highly beneficial. The amount of β-glucan ingested accounts for only part of the physiological effects; water solubility and a high molecular weight of β-glucans contribute to increased viscosity of digesta in the gastrointestinal tract, and these properties of β-glucans are critical for their health benefits (Keogh *et al.*, 2003; Yokoyama *et al.*, 1997). Whereas the increased solubility of β-glucans that might occur during bread preparations and baking is beneficial, a decrease in the molecular weight of these polymers is detrimental. The degradation of β-glucans appears to occur mainly during dough mixing, proofing, and fermentation—partly due to activation of endogenous β-glucanases—and to a lesser extent during baking (Tiwari and Cummins, 2009). Thus, to retain the high molecular weight of β-glucans, the mixing and fermentation time should be as short as possible, and the β-glucanase activity in any ingredients used in baking should be eliminated by appropriate methods. In our studies, the FRF used for baking were obtained from sound barley grain with no detectable β-glucanase activity, and wheat flour was also derived from unsprouted wheat. The CSP is a no-time mechanical dough development baking procedure involving fast mixing followed by short rest (15 min) and relatively short (70 min) proof at 37.5° C. The rich formula of the CSP dough was altered in our study by omitting the malt extract to avoid the addition of β-glucan degrading enzymes. As a consequence, the molecular weight of β-glucans extracted from the FRF-enriched breads after *in vitro* digestions with α-amylase, pepsin, and pancreatin was preserved and comparable or even higher than that of β-glucans extracted from the FRF under similar conditions (Table 31.4).

BARLEY FIBER-RICH FRACTIONS IN FLAT BREAD

Compared to pan or hearth breads, flat breads have modest flour quality requirements and their appeal is not derived from an aerated structure. Also, flat breads have been made from various grains, and generally consumers have greater tolerance for different color or taste of these products. From a technological standpoint, flat breads are better vehicles for delivering high dietary fiber ingredients into the diet, and they appear suitable for inclusion of β-glucans and/or barley fiber fractions. Barley flour has been successfully incorporated into single-layer flat breads, including chapatis and Turkish bazlama bread, but only a moderate increase of β-glucan in these products was achieved. Thondre and Henry (2009) showed that supplementation of whole wheat flour with a commercial barley β-glucan fiber preparation created a palatable chapatis for diabetic patients, and the glycemic index (GI) of chapatis with 4 g of β-glucan per serving was significantly reduced.

TABLE 31.5 Composition and Physicochemical Properties of Barley Fiber-Rich Fractions (FRF) before and after Pin Milling (PM)[a]

Barley Type/ FRF	Total β-Glucans (%)	Water-Soluble β-Glucans (%)[b]	Total Arabinoxylans (%)	Water-Soluble Arabinoxylans (%)[b]	Proteins (%)	Starch (%)	Swelling Capacity (ml/g)	Brightness, L^*	Particle Size, $d_{0.5}$ (μm)
Normal									
N-FRF	8.93 ± 0.05	4.55 (50.9)[c]	10.11 ± 0.04	1.41 (13.9)[b]	19.1 ± 0.1	36.2 ± 0.3	9.09[c]	79.02[e]	204
N-FRF-PM		4.79 (53.6)[c]		1.47 (14.5)[a]			8.16[d]	80.59[d]	139
Waxy									
W-FRF	13.84 ± 0.30	6.88 (49.7)[a]	8.87 ± 0.00	0.87 (9.8)[e]	20.0 ± 0.1	38.1 ± 0.2	10.95[b]	84.00[c]	220
W-FRF-PM		7.21 (52.1)[a]		0.91 (10.3)[e]			9.28[c]	84.95[b,c]	155
High amylose									
HA-FRF	14.06 ± 1.10	6.25 (44.5)[b]	9.98 ± 0.28	0.99 (9.9)[d]	19.5 ± 0.1	36.4 ± 0.2	12.04[a]	85.46[b]	220
HA-FRF-PM		6.90 (49.1)[a]		1.06 (10.6)[c]			11.89[a]	86.83[a]	164

HA, high amylose; N, normal; PM, pin mill; W, waxy.

[a] *Means in columns followed by a different letter(s) are significantly different ($p \leq 0.05$) as determined by Duncan's multiple range test.*

[b] *The values in parentheses indicate solubility expressed as a percentage of either total β-glucans or arabinoxylans, respectively.*

We have tested the potential of barley FRF as functional ingredients in two-layer flat bread (Izydorczyk *et al.*, 2008). The study was designed to investigate the effect of particle size of the FRF preparations on the technological and nutritional characteristics of flat breads. It was postulated that apart from chemical composition of fiber preparations, the properties that are technologically and nutritionally relevant include the particle size, bulk volume, the surface characteristics, hydration, and rheological properties. The original FRF were obtained according to the milling flow shown in Figure 31.1. A portion of the FRF was subsequently pin milled but not subjected to any further processing or sieving. As a consequence, the composition of the original and pin milled FRF remained the same (Table 31.5). In addition to particle size reduction, pin milling slightly increased the water solubility of β-glucans and arabinoxylans, increased the viscosity of FRF water slurries, but decreased their swelling capacity. The particle size reduction was expected to improve the solubility of fiber components by increasing the surface area of particles. It appears, however, that the reduction of particle size of FRF preparations caused a collapse of the porous fiber structure, making it unable to imbibe as much free water as the rough fiber.

Flat breads were prepared by mechanically mixing the ingredients, which included wheat flour (100 g; a blend of 80% white flour and 20% whole wheat flour), salt (1 g), sugar (1 g), and fresh compressed yeast (1.5 g), followed by fermentation (45 min at 30°C), division, rest (10 min), manually pinning the dough into circular sheets, proofing (25 min at 30°C), and baking (540°C for 70 s) in an electric traveling flat bread oven. The FRF-fortified flat breads were prepared by replacing 20% of white flour with the original and pin milled FRF. All FRF-containing doughs exhibited higher water absorption but good handling characteristics at division and sheeting (Table 31.6). The layer separation during baking was very good, and flat breads with FRF did not show any tendency for burning (Figure 31.7). Characteristics of FRF-fortified breads such as appearance, diameter, crumb structure, and aroma were comparable to those of the control breads (Table 31.6). However, the addition of FRF increased hardness and chewiness and decreased the brightness (L^*). The greatest hardness was observed for FRF derived from high amylose barley, and it was noticed that properties of starches associated with the FRF (especially at a higher level of FRF addition) could also contribute to the technological properties of breads. Flat breads supplemented with FRF from high amylose barley displayed slightly denser crumb structure than control or bread fortified with FRF from waxy barley (Figure 31.8). Some differences in the quality characteristics of flat breads supplemented with FRF from different barley types were also observed (Table 31.6). However, the quality and texture of supplemented breads were not significantly influenced by pin milling of the FRF preparations.

The addition of 20% of FRF significantly increased the amount of total and soluble β-glucans in flat breads. Assuming that single flat bread constitutes one serving, the FRF-supplemented breads would provide between 1.84 and 2.97 g of total β-glucans and between 0.58 and 1.42 g of water-soluble β-glucans depending on the origin of the FRF preparations (Table 31.7). With the exception of the waxy barley FRF preparations that showed a slight decrease, the water solubility of β-glucans was higher in flat breads containing pin milled FRF compared to the original FRF preparations. The addition of FRF also significantly increased the content of total arabinoxylans in the flat breads. The amount of water-soluble arabinoxylans, on the other hand, changed little with the addition of barley FRF, indicating the insoluble nature of these polysaccharides in barley. The results of the *in vitro* digestibility of starch showed that the inclusion of barley FRF into flat bread significantly decreased the solubilization and digestibility of starch at two different time intervals (Table 31.7). The pin milled FRF exerted a slightly greater effect than the original FRF in most cases. The reduction in starch digestibility of FRF-supplemented flat breads might be associated with the ability of fiber constituents to restrict the enzyme accessibility to the food either by changing (compacting and/or hardening) the food structure or by physically entrapping starch granules in their mucilaginous matrix. Brennan and Samyue (2004) postulated that the digestion of starch and sugar release from

TABLE 31.6 Quality Characteristics of Barley Fiber-Rich Fraction (FRF)-Supplemented Flat Breads[a]

		Dough Handling Scores[b]		Bread Scores[b]					Texture	
Flat Bread	Water Absorption (%)	Division[c]	Sheeting[d]	Appearance	Diameter	Crumb	Aroma	Brightness, L^*	Hardness (kg)	Chewiness
Control[e]	57[c]	4.25	4.25	4.25	5.0	3.75	4.75	74.09	0.47[c]	0.36[d]
+N-FRF	68[a]	4.25	4.25	4.25	5.0	3.5	4.5	68.96	0.54[a,b,c]	0.47[a,b,c,d]
+N-FRF-PM	68[a]	4.0	4.25	4.25	5.0	3.5	4.25	67.45	0.54[a,b,c]	0.48[a,b,c]
+W-FRF	64[b]	4.0	4.0	4.25	5.0	4.0	4.25	69.01	0.52[b,c]	0.45[b,c,d]
+W-FRF-PM	64[b]	4.25	4.5	4.25	5.0	4.0	4.5	71.84	0.49[b,c]	0.42[c,d]
+HA-FRF	67[a]	4.0	4.0	4.25	5.0	4.0	5.0	74.31	0.62[a,b]	0.54[a,b]
+HA-FRF-PM	67[a]	4.25	4.25	4.5	5.0	4.0	4.75	73.22	0.66[a]	0.58[a]

HA, high amylose; N, normal; PM, pin mill; W, waxy.

[a]*Means in columns followed by a different letter(s) are significantly different (*$p \leq 0.05$*) as determined by Duncan's multiple range test.*

[b]*Each quality parameter was given a score from 0 to 5.*

[c]*Division is the step in flat bread processing in which the dough was cut into five 125-g pieces after fermentation and before sheeting.*

[d]*Sheeting is the step in flat bread processing in which the dough is pinned into circular sheets followed by proofing and then baking.*

[e]*Control flat bread consisted of 80% straight grade Canadian Western Red Spring wheat flour and 20% whole wheat flour.*

FIGURE 31.7
Barley fiber-rich fraction supplemented flat breads exiting the traveling oven.

foods might be delayed due to dietary fiber constituents adhering to starch granules and possibly increasing the digesta viscosity. The decrease in *in vitro* starch digestibility due to the presence of β-glucans may indicate reduction of the GI of breads. Jenkins *et al.* (2002) showed that in a 50-g carbohydrate food portion (a prototype β-glucan-enriched breakfast cereal and bar), each gram of β-glucan could reduce the GI by 4 units, making it a useful functional food component for reducing postprandial glycemia.

TECHNOLOGICAL ISSUES

Consumers' interest in healthy and low-calorie foods has created demand and opportunity for innovative dietary fiber preparations and their incorporation into new and traditional food products. However, producing functional food products that fulfill expectations in terms of sensory appeal and indeed deliver the expected health benefits is challenging and requires innovative approaches and careful attention throughout the manufacturing process. The source of dietary fiber and type and degree of processing involved in fiber preparation are often key factors influencing its physiological functionality. The physiological benefits of β-glucans

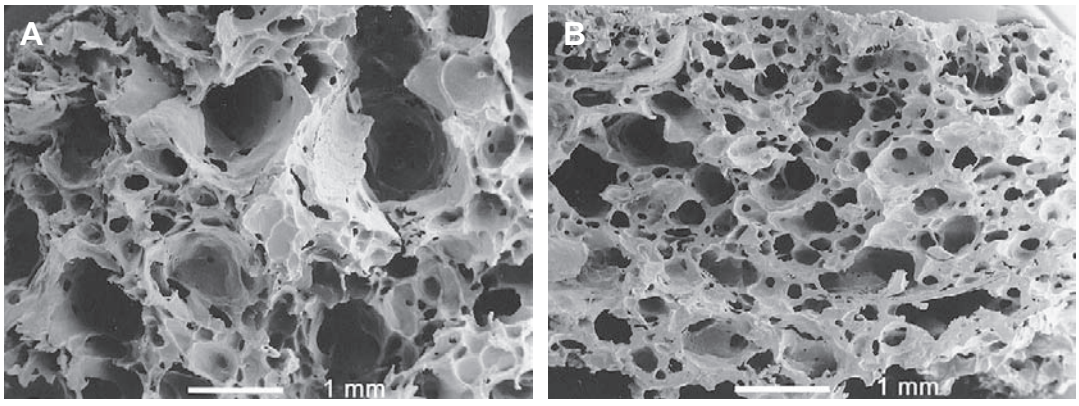

FIGURE 31.8
SEM micrographs of barley fiber-rich fraction (FRF)-supplemented flat breads. (A) Control flat bread (80% white flour/20% whole wheat flour; (B) flat bread with 20% of barley FRF.

TABLE 31.7 Nutritional Characteristics of Barley Fiber-Rich Fraction (FRF)-Supplemented Flat Breads[a]

	β-Glucans (%)		Arabinoxylans (%)		Digestible Starch (g/g)	
Flat Bread	Total	Water-Soluble[b]	Total	Water-Soluble[b]	15 Min	60 Min
Control	0.23^{d}	0.09^{g}	2.40^{b}	0.71^{b}	0.203^{a}	0.436^{a}
+N-FRF	1.85^{c}	0.58^{f}	4.90^{a}	0.91^{a}	0.184^{b}	0.374^{c}
+N-FRF-PM	1.84^{c}	0.62^{e}	4.50^{a}	0.91^{a}	0.172^{c}	0.368^{c}
+W-FRF	2.70^{b}	1.22^{c}	3.70^{a}	0.68^{c}	0.177^{b,c}	0.384^{b}
+W-FRF-PM	2.74^{b}	1.10^{d}	3.90^{a}	0.65^{c}	0.161^{d}	0.352^{d}
+HA-FRF	2.91^{a}	1.31^{b}	4.47^{a}	0.75^{b}	0.180^{b}	0.352^{d}
+HA-FRF-PM	2.97^{a}	1.43^{a}	4.20^{a}	0.71^{b}	0.164^{d}	0.348^{d}

Results are expressed as gram of starch released upon digestion with α-amylase per gram of available starch in flat bread samples.
HA, high amylose; N, normal; PM, pin mill; W, waxy.
[a]*Means in columns followed by a different letter(s) are significantly different ($p \leq 0.05$) as determined by Duncan's multiple range test.*
[b]*Water-soluble β-glucans and arabinoxylans were measured after extracting cubes of flat bread in water at 40°C for 120 min.*

are associated not only with their amount but also with their viscosity-building properties linked to the high molecular weight of these polymers. Because β-glucans are easily degraded by β-glucanases, care has to be taken during the preparation of β-glucan fiber to either choose sound barley or to inactivate the endogenous enzymes in grain by appropriate treatments. Changes to the molecular weight of β-glucans can also occur during bread making processes such as mixing and fermentation; keeping these steps as short as possible and avoiding introduction of β-glucanase activity from other ingredients used in the bread formulae are also necessary.

In bread making, the incorporation of a sufficient amount of β-glucans to ensure beneficial physiological impact is often associated with replacing large amounts of wheat flour, and the resulting detrimental technological effects are caused by a dilution of functional gluten proteins. The use of fiber preparations with a high concentration of β-glucans eliminates the necessity of high supplementation levels to achieve the desired β-glucan content in the final product. Several hull-less barley genotypes with high β-glucan contents have been bred specifically for food uses and are suitable for production of β-glucan-enriched fiber fractions. The adverse technological effects in bread manufacture associated with high contents of β-glucan fiber can be counteracted to some degree with the use of strong gluten flours, the addition of vital gluten and surfactants, and the incorporation of hemicellulases. Jacobs *et al.* (2008) reported that the addition of xylanase to the sponge-and-dough formulae improved the loaf volume, appearance, and crumb structure of bread fortified with barley FRF and potentially increased the health benefits by increasing the amount of soluble fiber content in the bread. These authors also reported that the method of bread production strongly influenced bread quality; the best quality FRF-enriched bread was obtained by the sponge-and-dough process because the gluten network was allowed to fully develop during the 4-h sponge stage and the barley FRF were incorporated only at the dough stage. The shorter exposure of FRF to fermentation was also a potential advantage because extended fermentation times might reduce the cholesterol-lowering capacity of β-glucans due to their degradation by β-glucanases.

SUMMARY POINTS

- Fiber-rich fractions, obtained by dry fractionation of barley grain, are excellent sources of β-glucans; the yield of FRF and concentration of β-glucans can be tailored to specific needs by adjusting processing steps using standard milling equipment. FRF consist of untreated fragments of pericarp, aleurone, and endosperm cell walls, with their natural constituents including other fiber constituents (arabinoxylans and cellulose), vitamins, minerals, and phenolic compounds in addition to β-glucans.

- Fortification of bread with barley FRF significantly improves the nutritional quality of bread due to increased levels of dietary fiber and β-glucans and qualifies the product (in some countries) to carry a health claim relating the presence of β-glucans with reducing the risk of cardiovascular diseases, heart diseases, and constipation.
- Because of the high concentration of β-glucans in enriched FRF, a relatively small amount of wheat flour (6−8%) has to be replaced with FRF, resulting in a high-quality bread product with a sufficient amount of β-glucans to exert physiological impact.
- Improving the composition and physical properties of FRF, either by choosing barley with specific characteristics (e.g., hull-less barley varieties with a high β-glucan content or altered starch composition) as a source of FRF or by additional reduction of particles in the FRF, will ensure efficacy and might even expand the physiological functionality of FRF-fortified food products.

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CHAPTER 32

Antioxidant Activity and Phenolics in Breads with Added Barley Flour

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

FRAP Ferric reducing antioxidant power
FRAP-S Antioxidant activities in the acetone soluble extracts
FRAP-IS Antioxidant activities in the alkali extracted acetone insoluble residue
Σ-FRAP Total antioxidant activity (FRAP-S + FRAP-IS)
P-S Amounts of aqueous acetone soluble phenolics
P-IS Amounts of the bound phenolics solubilized by alkali from the residue of the P-S extraction
Σ-P Summarized content of phenolics (P-S + P-IS)

INTRODUCTION

Breads and cereals are a major part of Western-style diets and are important sources of nutrients such as fiber and antioxidants. Different cereals have different dietary fiber and phenolic profiles proving variation in nutritional value depending on the flours used. Traditionally, wheat has been the first choice for bread production worldwide, whereas rye is a popular raw material in northern Europe and Russia. Barley has traditionally been of little use, and it is generally more a constituent of or source for animal fodder.

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For disease prevention and health maintenance, food products can play an essential role. The increasing focus on health-promoting components in foods has resulted in an increased desire to utilize more healthy raw materials for food products. As such, barley has received increased attention as a food or ingredient. As a beneficial food choice, barley has an advantage due to its high content of health-promoting components. Not only the high amount of dietary fiber in this cereal but also the high content of phenolic acids and other phenolic compounds place barley as an excellent dietary source of natural antioxidants (Holtekjølen *et al.*, 2006; Madhujith *et al.*, 2006; Peterson, 1994).

Barley is incorporated in already established as well as in new food products either as a whole grain or as a food ingredient. Examples of relatively new uses include pasta and bread. The latter has gained increasing importance in Norway. Barley has a wide diversity of genotypes with large variations in polysaccharide content and compositions (dietary fiber) as well as amounts and solubility of phenolics with possible antioxidant properties (Holtekjølen *et al.*, 2010; Izydorczyk and Dexter, 2008; Madhujith and Shahidi, 2009). These differences provide opportunities for different end uses, and it is obvious that barley can make a contribution to a nutritious and balanced diet. However, there are challenges regarding the increased use of barley for human consumption. There are limited quality criteria for evaluating barley for food. Because of the wide diversity of barley varieties, barley provides an inconsistent flour quality to the industry. Determination of natural variations within barley with respect to bioactive and health-related compounds and their resulting effects on different products is therefore essential to ensure a predictable product quality. Detection of unwanted effects of different processes on the health-promoting components is also important and necessary to ensure a healthy food. For example, the common physical processing of barley, referred to as pearling, is well-known to decrease the amount of health-related compounds. The amounts of both dietary fiber and phenolics will decrease because many of these health-related components in barley are located in the outer part of the kernels. Unfortunately, many of the grains currently consumed are highly processed, which reduces the levels of health-beneficial compounds. Thus, whole grain products are recommended for healthy diets because they contain high levels of dietary fiber and antioxidant substances. Furthermore, the addition of barley or barley fractions in foods will influence product quality in terms of color, taste, and texture. The polysaccharides in barley affect the quality of baking products (Holtekjølen, Olsen, *et al.*, 2008; Izydorczyk and Dexter, 2008), and the baking process itself may alter the fiber constituents (depolymerization) (Andersson *et al.*, 2004). Thus, the naturally occurring phenolics in barley will not only play an important role in disease prevention and health maintenance but also relate to the sensory and technical qualities of food products. Few studies have examined the possible effects on bread quality by a natural variation in phenolic contents of well-characterized barley flours. There is also limited literature on the effect of processing on the antioxidant properties of breads with a fairly high amount of barley incorporated.

This chapter discusses how a natural variation in phenolic profile (content and composition) among different barley varieties can impact bread quality parameters. It also discusses the effect of the baking process itself on the phenolic contents and antioxidant activities of breads with added barley flours. Particular attention is paid to work reported on Norwegian barley varieties.

BARLEY FLOUR CHARACTERISTICS WITH EMPHASIS ON PHENOLICS

Barley contains a variety of phenolics, such as cinnamic acids, benzoic acids, flavonoids, and tannins. For barley as for other cereals in general, the phenolics exist as free (extractable/soluble in certain solvents) or chemically bound to other constituents, and the latter are insoluble in most aqueous or organic solvents. The majority of the free components in barley

are flavanols and tocols (tocopherols and tocotrienols), and there is a small amount of free phenolic acids, whereas the bound phenolics are mainly phenolic acids (Cavallero *et al.*, 2004; Holtekjølen *et al.*, 2006; Mattila *et al.*, 2005). The flavanols exist as monomers and polymers (proanthocyanidins), and the most abundant bound phenolic acid is the cinnamic acid-derivative ferulic acid (FA). The second most abundant phenolic acid is *p*-coumaric acid. These acids are often covalently bound to other macromolecules, such as arabinoxylans and lignin. FA can dimerize to form diferulic acid bridges, cross-linking arabinoxylan chain and thus fortifying the cell wall, and reduce the solubility of such fiber (Renger and Steinhart, 2000). Both free and bound phenolics are known to possess antioxidant activity and thus possible health benefits (Beecher, 2004; Chesson *et al.*, 1999; Rechner *et al.*, 2002; Rice-Evans *et al.*, 1996). However, the antioxidant activity of phenolic acids is dependent on the structure.

The phenolics are not evenly distributed in the cereal grains and are mostly found in the outer part of the kernels. The proanthocyanidins (flavanols) in barley are found in the seed coat (testa), just outside the aluerone layer (Aastrup *et al.*, 1984), whereas the barley endosperm, germ, and the hulls have a substantial tocol content (Peterson, 1994). The phenolic acids are covalently linked to arabinoxylan, and the extent of feruloylation is tissue dependent (Izydorczyk and Dexter, 2008). A higher concentration of phenolic acids is found in the aluerone layer and pericarp tissues than in the starchy endosperm. Due to the nonuniform distribution of phenolics in barley kernels, the degree of pearling has a major impact on the total amount of phenolics as well as the antioxidant activity in the remaining flour (Abdel-Aal and Gamel, 2008; Bellido and Beta, 2009; Holtekjølen *et al.*, 2010; Madhujith *et al.*, 2006; Verardo *et al.*, 2008a,b). This also provides a possibility for physical fractionation, giving products enriched in antioxidants because the abraded barley material is a rich source of phenolics.

IMPACT OF NATURALLY OCCURRING PHENOLICS IN COVERED WHOLE GRAIN BARLEY FLOURS ON HEARTH BREAD QUALITY PARAMETERS

It is well-known that polysaccharides in barley affect the baking quality of breads (Holtekjølen, Olsen, *et al.*, 2008; Izydorczyk and Dexter, 2008). However, there are few studies dealing with possible technological properties of the naturally occurring phenolics within the raw material (barley flours) and their impacts on bread quality. Phenolics have been studied for changes occurring during bread making (Hansen *et al.*, 2002). Certain phenolic compounds, such as FA, have also been added in pure form to study their possible technological impact. Adding FA to the dough has been shown to increase the rate of dough breakdown and simultaneously make it more sticky (Okada *et al.*, 1987). Ferulic acids are also important because they cross-link pentosans, thereby affecting viscosity, or directly link to the gluten, affecting the gluten network. Such interactions are assumed to contribute to a decrease in gluten and hence dough extensibility (Wang *et al.*, 2004a,b).

The naturally occurring phenolics in barley varieties have been shown to impact bread quality parameters in wheat-based hearth breads containing 40% covered whole grain barley flours (Holtekjølen, 2005). Variations in proanthocyanidins and phenolic acid contents (predominately FA and *p*-coumaric acid) within the different barleys were shown to contribute significantly to the prediction of some bread quality parameters according to partial least square regression (PLSR) (Figure 32.1). In the PLSR model, the variability in the bread parameters could be explained by the variation in phenolic profiles in the different barley varieties. The proanthocyanidins were shown to correlate positively with dough consistency (used as a measurement of the water absorption capacity (WAC)), bread weight, and form ratio. Thus, higher contents of proanthocyanidins in the barley flours, especially the trimeric oligomers, increase the WAC of the dough and then the weight of the breads. As seen in Figure 32.1, this is especially valid for hull-less (H-L) and atypical starch barley varieties (A H-L). These samples

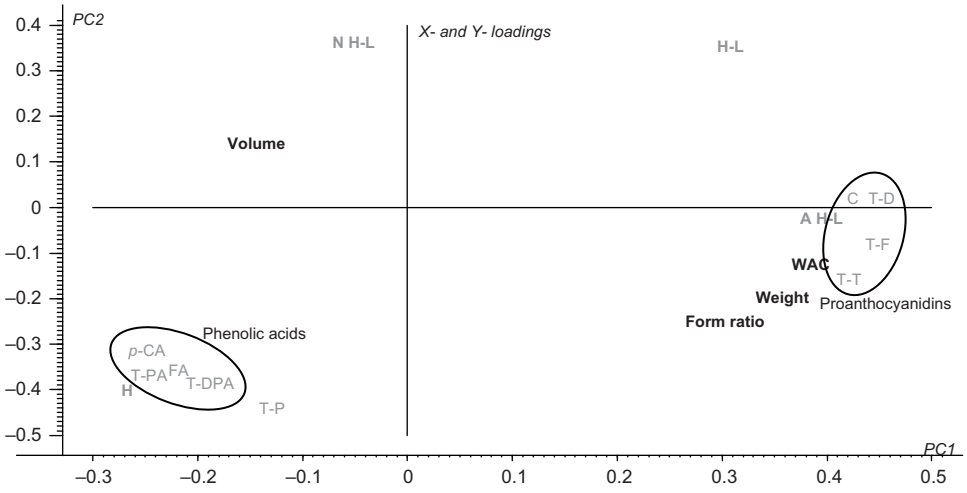

FIGURE 32.1
Partial least square regression loading plot with the different barley bread quality characteristics (black and bold) as response variables (y variables) and the different amounts of phenolics and barley variety characteristics (gray) as regressor variables (x variables). The proanthocyanidins (C, catechin; T-D, total dimeric flavanols; T-F, total flavanols; T-T, total trimeric flavanols) are related to the bread weight, the form ratio of the bread, and the water absorption capacity (WAC) of the dough. The phenolic acids (FA, ferulic acid; *p*-CA, *p*-coumaric acid; T-DFA, total dimeric ferulic acids; T-PA, total phenolic acids) and the total phenolics (T-P) as sum of all individual quantified phenolics are related to the bread volume. *Source: Recalculated from original data from Holtekjølen (2005).*

cluster closer to the proanthocyanidins showing higher levels of these phenolics in these varieties compared to hulled (H) or normal starch hull-less varieties (H-L N). The phenolic acids, however, cluster more to the left toward bread volume in the PLSR plot. A correlation between bread volume and phenolic acids in the barley is seen; however, it is strongly dependent on the type of barley. No clear relation is seen between the bread volume and phenolic acids including barley varieties of all different types. However, grouping the barley varieties by their characteristics (hulled, hull-less, and starch type) provides strong negative correlations within the normal starch barley varieties (Figure 32.2). Such a correlation is not seen among the atypical starch varieties tested (circles in Figure 32.2).

A natural variation in phenolics content and composition among barley flours does impact some bread quality parameters and will provide an additional contribution to the possible variability found in these parameters compared to polysaccharides.

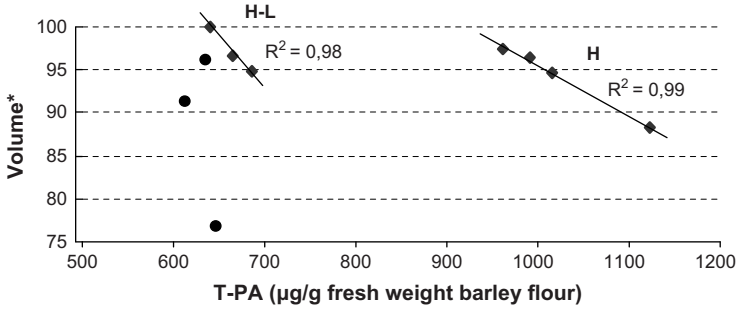

FIGURE 32.2
Bread volumes are reduced by an increased content of total phenolic acids depending on the barley type. A higher content of total phenolic acids (T-PA) results in lower bread volumes within normal starch varieties independent of hull content. Atypical barley types differed and are shown as solid circles. Volume is expressed as relative differences. H, hulled; H-L, hull-less. *Source: Recalculated from original data from Holtekjølen (2005).*

IMPACT OF BARLEY VARIETY, BAKING PROCEDURE, AND STORAGE ON THE ANTIOXIDANT PROPERTIES OF BREADS WITH ADDED BARLEY FLOURS

The phenolics occurring in barley impact bread quality, but the baking process itself will also affect the total amount of phenolics found in the finished product as well as the overall antioxidant properties of the breads. Barley and wheat have different phenolic profiles. This is also valid among barley varieties. Thus, the antioxidant properties of different flour mixtures and the resulting barley breads vary according to the different barley flours incorporated in the bread formula (Figure 32.3) (Holtekjølen, Bævre, *et al.*, 2008). As seen in the figure, the barley–wheat flour mixtures contain a higher total amount of phenolics (Σ-P; determined by the Folin–Ciocalteu assay) than the whole grain wheat mixture, except for barley variety 3. Pearling affects the amount of phenolics found in the resulting flours, and a higher extraction rate increases the amount of phenolics. However, despite the lower extraction rate of the barley flours tested, compared to the whole wheat (100% extraction rate) added in the control wheat bread, the barley breads contain a higher ratio of free phenolics (P-S) than the whole wheat bread. This demonstrates that barley has a significantly higher amount of P-S than wheat (determined by the Folin–Ciocalteu assay). This has been confirmed in other studies. The incorporation of whole wheat in the wheat bread increases the content of bound phenolic acids. This provides comparable levels of bound or insoluble phenolics (P-IS) in the wheat bread and the barley breads. Substitution of an amount of wheat flour with barley flour will therefore increase the overall phenolic content compared to that of 100% baking wheat flour. This will further be a healthier food choice because barley also provides a significant amount of dietary fiber.

As mentioned previously, the baking process increases Σ-P (see Figure 32.3). This is shown to be due to a significant increase in the amount of P-IS. The content of P-S decreased during the baking process in all the barley varieties tested (Holtekjølen, Bævre, *et al.*, 2008). The increase in phenolic content during baking is often addressed as a side effect of the baking process. Maillard-type reaction products may possibly contribute to this increase

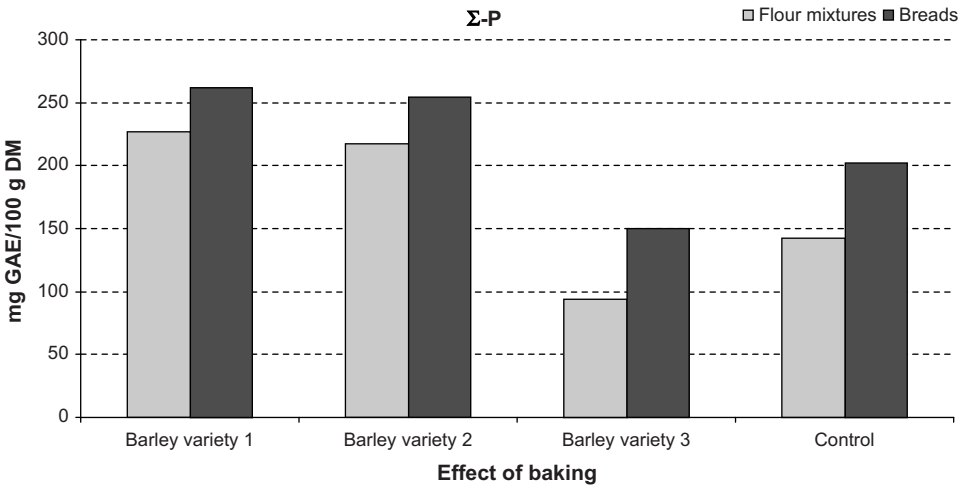

FIGURE 32.3
The total amount of phenolics increased during baking. The baking process increased the total amount of phenolics (Σ-P), measured as gallic acid equivalents (GAE) in the Folin–Ciocalteu's assay. Barley flour mixtures were 40% barley and 60% baking wheat, and the control was 40% whole wheat flour with 60% baking wheat. Gray and black bars refer to flours and breads, respectively. *Source: Recalculated from original data from Holtekjølen* et al. *(2008a).*

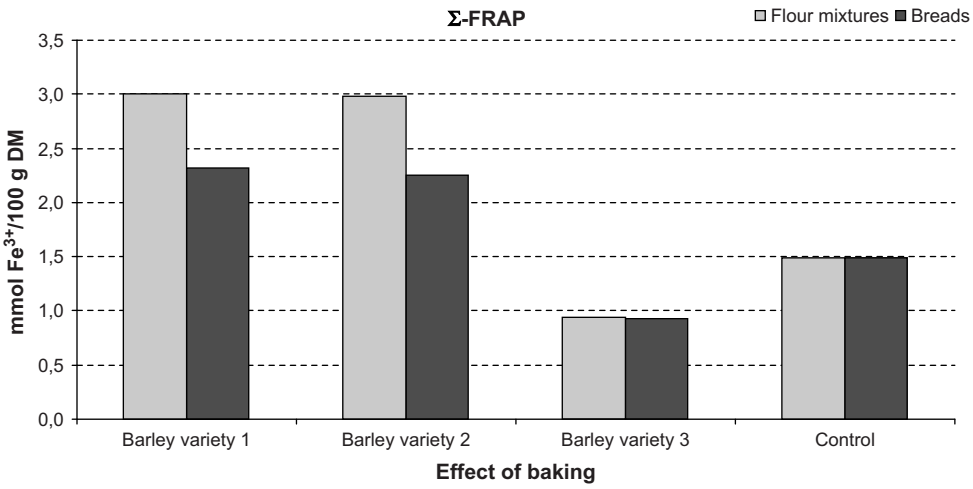

FIGURE 32.4

The total antioxidant activity decreased or was more or less constant during the baking process. The total antioxidant activity (Σ-FRAP), measured as millimole Fe^{3+} equivalents in the assay, decreased or was more or less constant during the baking process. Barley flour mixtures were 40% barley and 60% baking wheat, and the control was 40% whole wheat flour with 60% baking wheat. Gray and black bars refer to flours and breads, respectively. *Source: Recalculated from original data from Holtekjølen et al. (2008a).*

(Samaras *et al.*, 2005). The heat-induced products from the Maillard reaction, polyphenolic oxidation products, and caramelization products may affect the estimation of phenolics with the Folin assay by contributing false positives. It is important to note that the FRAP methodology is nonspecific, giving similar inaccurate estimations. Although the amounts of bound phenolics significantly increase upon baking, the corresponding measured antioxidant activities of these extracts are relatively unchanged. A decrease in the summarized antioxidant activities (Σ-FRAP) in barley varieties 1 and 2 (Figure 32.4) is due to a decrease in the measured antioxidant activity of the extracts with the free phenolics after baking.

The significance of barley variety, baking procedure (pan or hearth baking), and storage on the antioxidant properties of the breads is demonstrated in the PLSR biplot (Figure 32.5). The different varieties span out the first principal component (PC1), whereas baking procedure and storage show less influence on the antioxidant properties. The differences between pan and hearth breads are not consistent, and the changes during storage are not significant compared to those of fresh breads. Thus, the antioxidant properties of breads with added barley flours depend mostly on the different barley varieties used and their respective phenolic profiles (Holtekjølen, Bævre, *et al.*, 2008). Because the phenolics are also concentrated in the outer part of the grain, the extraction rate of the barley flour will influence the total amount as well as the ratio of free to bound phenolics in the breads.

The amounts of phenolics in the barley varieties correspond well to sensory evaluation of barley breads (Holtekjølen, Bævre, *et al.*, 2008). The barley variety containing the highest Σ-P (variety 1; Figure 32.3) gives rise to a barley bread with a more intense flavor as well as odor, and it also has the highest score for bitterness, off-odor, and off-flavor (Figure 32.6). A variety with low Σ-P shows low intensity in the sensory evaluation and is less bitter than the rest (variety 3; Figure 32.6) (Holtekjølen, Bævre, *et al.*, 2008). This relates to both proanthocyanidins and phenolic acids. Both are reported to have a bitter and astringent taste (Dimberg *et al.*, 1996; Lesschaeve and Noble, 2005). Storage does not significantly alter the antioxidant properties, but does affect the odor, flavor, and texture of the barley breads (Holtekjølen, Bævre, *et al.*, 2008).

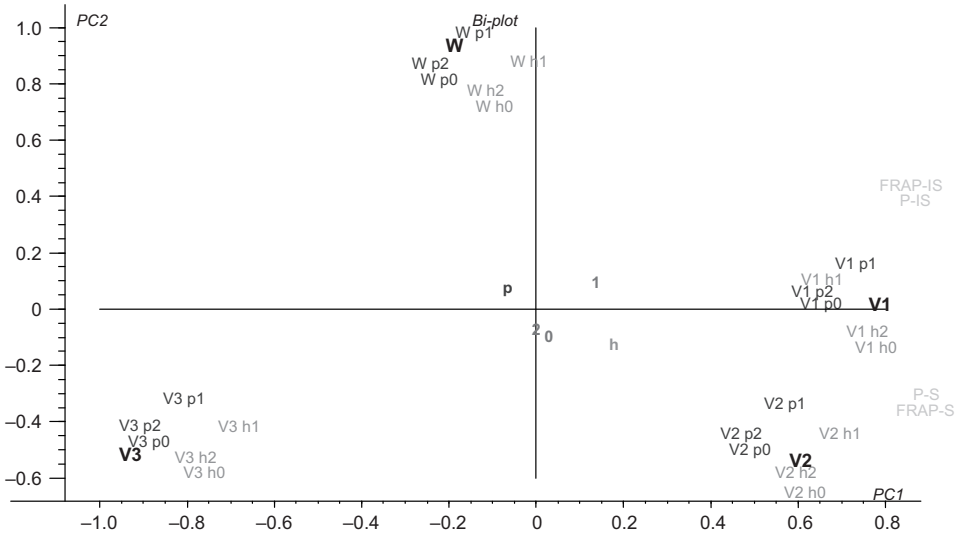

FIGURE 32.5
Partial least square regression biplot with the content of free and bound phenolics (P-S and P-IS) and their antioxidant activity (FRAP-S and FRAP-IS) as response variables (y variables) and the different varieties, baking processes (pan and hearth baking), and storage (0, 1, 2) as regressor variables (x variables). The biplot shows the influence of variety, different baking processes (pan and hearth baking), and storage on the content of free and bound phenolics (P-S and P-IS) and their antioxidant activity (FRAP-S and FRAP-IS) of breads with added barley flours (40%). h, hearth baked; p, pan baked; V1, bread with barley variety 1; V2, bread with barley variety 2; V3, bread with barley variety 3; W, whole grain wheat bread; 0, fresh; 1, 1 day of storage; 2, 2 days of storage. *Source: Modified from Holtekjølen, Bævre,* et al. *(2008).*

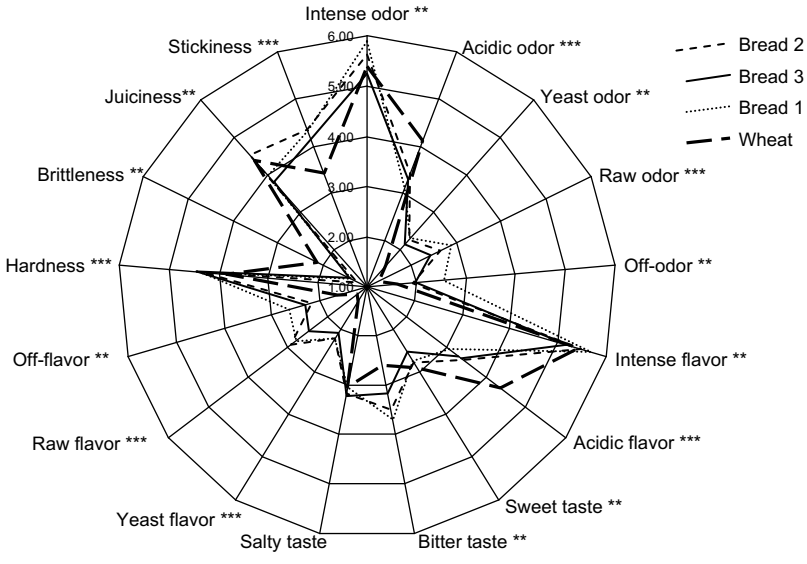

FIGURE 32.6
Spider web diagram of the sensory evaluation of different breads with added barley flours (40%). The different breads with added barley flours (40%) of different barley varieties differed in sensory evaluation compared to the whole grain wheat bread. The different barley breads also differed according to the barley variety. Asterisks indicate significant levels: $** p < 0.05$; $*** p < 0.01$. *Source: Reprinted from Holtekjølen, Bævre, et al. (2008).*

SUMMARY POINTS

- The natural variations in antioxidant activities and phenolics in barley impact bread quality parameters.
- The total contents of phenolics in breads with added barley flours are higher than those of the starting flour mixtures.

- The total antioxidant properties of breads with added barley flour depend on the barley variety, the extraction rate of the flours, and the baking process.
- Substitution of wheat flour with barley flour increases the overall antioxidant properties in breads.
- Barley breads are a healthier food choice because barley contains a significant amount of dietary fiber.

Acknowledgment

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CHAPTER 33

Partial Substitution of Wheat Flour with Chempedak (*Artocarpus integer*) Seed Flour in Bread

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

CSF Chempedak seed flour
EGI Estimated glycemic index
GI Glycemic index
HI Hydrolysis index
IDF Insoluble dietary fiber
RS Resistant starch
SDF Soluble dietary fiber
TDF Total dietary fiber

This chapter is adapted with permission from Zabidi, M. A., and Aziz, N. A. A. (2009). *In vitro* starch hydrolysis and estimated glycaemic index of bread substituted with

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different percentage of chempedak (*Artocarpus integer*) seed flour. *Food Chem*. 117, 64−68.

INTRODUCTION

Chempedak (*Artocarpus integer* (Thunb.) Merr.) belongs to the Moraceae family. Chempedak is native to Southeast Asia and is widely distributed and cultivated in Indonesia, Peninsular Thailand, and Peninsular Malaysia, particularly in Perak and Kedah (Jansen, 1991).

In Malaysia, a number of chempedak selections have been cloned, including the CH29 cultivar, which produces an attractive orange flesh, and CH26, CH27, and CH28, which are high-yielding cultivars (Jansen, 1991). Young chempedak fruits are cooked in coconut milk and eaten as curried vegetable or soup. The chempedak seeds are normally discarded or eaten either roasted or boiled in salt water. Chempedak seeds, the underutilized by-product from the fruit industry, have promising commercial value because they contain an appreciable amount of carbohydrate, protein, dietary fiber, minerals, and various vitamins, such as B_1, B_2, B_3, and C. A novel way of exploiting the underutilized chempedak seed is by processing it into flour.

Chempedak seed flour is a good source of total dietary fiber (TDF) and resistant starch (RS). The importance of dietary fiber is increasing due to its beneficial effects on the reduction of cholesterol levels and the risk of colon cancer. The important amount of starch that escapes digestion and absorption in the small intestine is commonly referred to as RS. Higher RS content in food has a protective effect against colonic diseases (Bingham, 1990). RS is another functional material that has a similar effect to some dietary fibers. In the small and large intestines, RS increased the indigestible carbohydrate ingested, thus lowering the glycemic index (GI) value of food products (Yamada *et al*., 2005). Foster-Powell *et al*. (2002) reported that addition of fiber to food products reduced their GI value. The addition of non-wheat flour to conventional wheat bread helps to improve the nutritive value in terms of dietary fiber. This is attributed to the starch digestion rate, which subsequently releases glucose into the bloodstream at a slower rate, resulting in reduced glycemic and insulinemic postprandial responses in food products containing high amounts of dietary fiber (Tovar *et al*., 2003). *In vitro* determinations have shown that common flour-based bread contains limited quantities of RS (i.e., <2%, starch basis) (Englyst *et al*., 1992). Wheat flour was substituted with barley at different levels in bread formulation by Cavallero *et al*. (2002).

Currently, new fibers from different sources are being studied that are potentially useful for making high-fiber bread while diminishing the inherent problems associated with the use of fibers. Thus, chempedak seed flour has potential application to exert health benefits particularly in bakery products, especially bread. Much effort has been made to enrich bakery products, especially bread, with non-wheat flour so as to increase the dietary fiber content yet maintain the desirable eating qualities. The challenge is to create high-fiber bread without the undesirable grainy texture. Chempedak seed flour, a by-product of the fruit-based industry, thus provides a new source of fiber for utilization in bakery products.

NUTRITIONAL VALUES OF CHEMPEDAK SEED AND CHEMPEDAK SEED FLOUR

Chempedak seed and chempedak seed flour consist of valuable nutrients such as carbohydrates, protein, dietary fiber, and resistant starch. Chempedak seed flour shows potential application as a value-added ingredient in various food products. Chemical compositions of chempedak seed and chempedak seed flour are presented in Table 33.1.

The amount of protein and ash content in chempedak seed flour is significantly lower ($p < 0.05$) than that of chempedak seed due to the hydrothermal process employed during processing, which caused hydrolysis and denaturation of the protein compound and leaching

TABLE 33.1 Mean Values for Chemical Composition of Chempedak Seed and CSF (g/100 g Dry Weight)[a]

Sample	Chempedak Seed	CSF
Moisture	56.57 ± 0.12[a]	5.93 ± 0.01[b]
Protein	12.88 ± 0.47[a]	8.78 ± 0.46[b]
Crude fat	0.99 ± 0.05[a]	0.96 ± 0.07[a]
Total dietary fiber	14.82 ± 0.44[b]	26.48 ± 0.25[a]
Insoluble dietary fiber	12.44 ± 0.34[b]	23.93 ± 0.05[a]
Soluble dietary fiber	2.37 ± 0.10[a]	2.55 ± 0.20[a]
Ash	2.57 ± 0.14[a]	2.21 ± 0.09[b]
Carbohydrate[b]	12.17	55.64
Resistant starch	29.72 ± 0.45[a]	14.77±0.14[b]

CSF, chempedak seed flour.

[a]*Results expressed on a dry weight basis (mean value ± standard deviation; n = 3). Values followed by a different letter in the same column are significantly different (p < 0.05).*

[b]*The carbohydrate was calculated by difference (= 100 − protein − lipid − total dietary fiber − ash).*

out of a small amount of protein and ash content into the water. This was attributed to the diffusion concentration gradient of water and chemical elements in chempedak seeds, which led to reduction of certain nutritional components of chempedak seeds. Furthermore, processing of chempedak seeds in alkaline solution promoted denaturation of protein compound. The accessibility of peptide bonds to proteolytic enzymes was increased, and thus the amount of protein content in chempedak seed flour was reduced. Denatured proteins tend to bind more water through exposure of the interior hydrophilic groups (Bhagya and Shamanthaka Sastry, 2003).

In contrast, processing of chempedak seeds into flour did not significantly affect ($p > 0.05$) the crude fat and crude fiber contents. Results showed that carbohydrate content in chempedak seed flour increased substantially ($p < 0.05$) by more than threefold compared to that of chempedak seeds. This indicated that chempedak seed flour was a rich source of starch. However, processing of chempedak seeds did not alleviate the major differences in most of the chemical compositions of chempedak seed flour (Bhagya and Shamanthaka Sastry, 2003).

Insoluble dietary fiber (IDF) content in chempedak seed flour is significantly higher ($p < 0.05$) than that in chempedak seed. This may be attributed to the conversion of starch in chempedak seed into an indigestible form during processing, which involved heating followed by subsequent cooling and/or dehydration (Vasanthan *et al.*, 2002).

The soluble dietary fiber (SDF) in chempedak seed and chempedak seed flour comprises 16.0 and 9.6% of the total amount of dietary fiber, respectively. Dietary fiber promotes beneficial physiological effects, including the prevention of diseases due to its potential in reducing the risks of cancer and coronary heart diseases (Larrauri, 1999).

Resistant starch content in chempedak seed flour was significantly lower ($p < 0.05$) than that in raw chempedak seed (see Table 33.1). This is attributed to the processing of starchy foods, which promotes disintegration and/or microstructural damage of the seeds and affects the water absorption capability (Niba, 2003).

COMPOSITION OF BREAD SUBSTITUTED WITH CHEMPEDAK SEED FLOUR

Wheat flour in bread was partially substituted with chempedak seed flour at different levels (0, 10, 20, and 30%) with addition of maltodextrin (DE15) and α-amylase. The chemical composition of the bread is shown in Table 33.2. An increase in chempedak seed flour substitution level significantly increased ($p < 0.05$) the moisture content due to the water

TABLE 33.2 Mean Values for Proximate Composition of Bread Substituted with Different Levels of CSF (g/100 g Dry Weight)[a]

Composition	Bread 1 (Control)	Bread 2 (10% CSF)	Bread 3 (20% CSF)	Bread 4 (30% CSF)
Moisture	34.64 ± 0.09^{d}	37.15 ± 0.10^{c}	39.75 ± 0.20^{b}	43.53 ± 0.14^{a}
Protein	13.29 ± 0.16^{a}	12.91 ± 0.75^{a}	12.88 ± 0.05^{a}	12.69 ± 0.04^{a}
Crude fat	1.50 ± 0.05^{a}	1.10 ± 0.03^{b}	0.84 ± 0.03^{c}	0.75 ± 0.05^{d}
Total dietary fiber	7.97 ± 0.32^{d}	10.92 ± 0.69^{c}	13.22 ± 0.13^{b}	16.34 ± 0.60^{a}
Insoluble dietary fiber	7.43 ± 0.09^{d}	10.08 ± 0.47^{c}	12.00 ± 0.16^{b}	13.89 ± 0.22^{a}
Soluble dietary fiber	0.54 ± 0.22^{c}	$0.84 \pm 0.22^{b,c}$	1.21 ± 0.02^{b}	2.45 ± 0.38^{a}
Ash	1.43 ± 0.01^{d}	1.57 ± 0.02^{c}	1.74 ± 0.02^{b}	1.79 ± 0.00^{a}
Carbohydrate[b]	41.17^{a}	36.35^{b}	31.57^{c}	24.90^{d}
Calorie value (Kcal/100 g)	256 ± 0.63^{a}	241 ± 0.73^{b}	226 ± 0.76^{c}	208 ± 0.81^{d}

CSF, chempedak seed flour.

[a]*Results expressed on a dry weight basis (mean value ± standard deviation;* n = *3). Values followed by a different letter in the same column are significantly different (*p < *0.05).*

[b]*The carbohydrate was calculated by difference (= 100 − protein − lipid − total dietary fiber − ash).*

absorption in chempedak seed flour. Heat treatment and milling reduced the particle size and subsequently altered the fiber matrix structure. According to Wang *et al.* (2002), addition of fiber mainly modified the water absorption, and the extent of the absorption depended on the structure of the fiber added. The fibrous material is very hydrophilic and thus requires higher addition of water with higher chempedak seed flour substitution level.

Protein content in bread exhibited no significant difference ($p > 0.05$) with increased chempedak seed flour substitution level. This is due to the presence of higher dietary fiber in chempedak seed flour, which diluted the protein content, particularly gluten content, in bread. Dilution of gluten content in bread samples at higher chempedak seed flour substitution levels interfered with gas retention ability and hence caused detrimental effects on the loaf volume (Hung *et al.*, 2007).

Bread samples substituted with a higher level of chempedak seed flour had a substantial decrease ($p < 0.05$) in crude fat compared to the control. Crude fat content in 30% chempedak seed flour bread decreased by twofold compared to the control. Crude fat in the 10 and 20% chempedak seed flour breads decreased significantly ($p < 0.05$) by 26.7 and 44%, respectively, compared to the control. However, the crude fat content in the bread samples at higher chempedak seed flour substitution levels was considered low. Hathorn *et al.* (2007) postulated that the lower fat content at higher chempedak seed flour substitution levels was due to the presence of a higher amount of fiber that is able to bind and absorb substances such as lipid and enzymes.

Hung *et al.* (2007) proposed that the significant decrease in crude fat content was attributed to the decrease in amylose molecule content in the bread samples at higher chempedak seed flour substitution levels. Lower content of amylose molecules was associated with the formation of amylose–lipid complexes in starch granules in the bread system.

Results showed that ash content in bread samples increased significantly ($p < 0.05$) at higher chempedak seed flour substitution levels (see Table 33.2). Ash content in 10, 20, and 30% chempedak seed flour breads increased by 9.8, 21.7, and 25.2%, respectively, compared to the control. The significant increase ($p < 0.05$) in ash content may be attributed to the increased amount of minerals such as calcium and iron at higher chempedak seed flour substitution levels in the bread samples (Hathorn *et al.*, 2007).

Carbohydrate content was significantly decreased ($p < 0.05$) in bread samples as chempedak seed flour substitution level increased. The decrease of carbohydrate content in 10, 20, and 30% chempedak seed flour breads ranged from 5.3 to 20.7% compared to the control. The

significant decrease ($p < 0.05$) of carbohydrate in the samples at higher chempedak seed flour substitution levels was due to the higher amount of moisture, crude fiber, and ash contents and lower amount of crude fat content. Reduction of carbohydrate content in the bread samples at higher chempedak seed flour substitution levels corresponded with reduced caloric value of the food, which is beneficial for diabetic patients. The calorie value of the bread samples at higher chempedak seed flour substitution levels exhibited a reduction ranging from 5.9 to 18.9% compared to the control.

In contrast, higher chempedak seed flour substitution levels in samples resulted in a significantly increased ($p < 0.05$) amount of crude fiber. The result corroborated those of previous studies with the addition of non-wheat flour and different fibers in bread formulation (Krishnan *et al.*, 1987; Wang *et al.*, 2002). Crude fiber in bread with 10 and 20% chempedak seed flour increased substantially ($p < 0.05$) by 36.6 and 71.4%, respectively, compared to the control. The 30% chempedak seed flour bread had a twofold increase in crude fiber compared to the control. The substantial increase in crude fiber at higher chempedak seed flour substitution levels in bread samples is attributed to the higher amount of fiber in chempedak seed flour.

Insoluble and soluble dietary fiber serve different functional characteristics that influence the physiological effects and the approach used in baking (Oakenfull, 2001). Bread with higher substitution levels of chempedak seed flour had a significantly higher ($p < 0.05$) amount of dietary fiber. SDF in bread increased significantly with the increase in chempedak seed flour. SDF has several beneficial effects, such as delaying gastric emptying, lowering serum cholesterol levels, and lowering postprandial blood glucose and insulin response by slowing glucose absorption (Cavallero *et al.*, 2002; Oakenfull, 2001).

IDF is the predominant fiber fraction in bread, constituting 85–93% of the total amount of dietary fiber. This is in agreement with the conclusion of Dreher (2001), who stated that most common dietary fiber found in foods is IDF. Furthermore, IDF showed a positive correlation with the amount of RS (i.e., water-insoluble dietary fiber) in bread with higher chempedak seed flour substitution levels (Figure 33.1). This indicated that IDF content was based on the amount of RS. IDF and RS have been extensively reported to have beneficial effects in lowering the glycemic index (GI) and postprandial blood glucose (Schnell *et al.*, 2005).

RS content in bread increased significantly ($p < 0.05$) with increased chempedak seed flour substitution levels. RS content increased by 16.3% in the 10% chempedak seed flour bread compared to the control. RS content in chempedak seed flour bread was significantly higher than that in the control bread. However, RS content in the 20 and 30% chempedak seed flour bread increased almost twofold compared to that of the control. RS formation was associated with retrogradation of starch, particularly amylose molecules, through formation of enzyme-resistant amylose–amylose linkages (Onyango *et al.*, 2005). Åkerberg *et al.* (1998) stated that

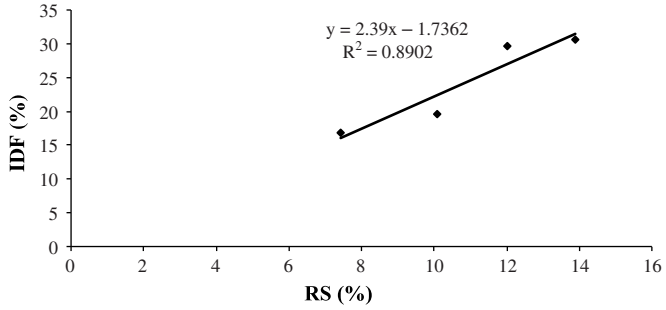

FIGURE 33.1
Correlation between insoluble dietary fiber (IDF) value and resistant starch (RS) content in bread substituted with different levels of chempedak seed flour.

part of the amylose content, amylopectin, played a significant role in RS formation in bread. They suggested that linear molecules of amylopectin might participate in starch retrogradation, which increased the RS content in bread samples. Heating treatments increased the interactions between starches and polymers and contributed to higher formation of RS even though amylose/amylopectin concentration was closely related to starch retrogradation and RS formation. Other factors, such as the presence of other food components and the type of processing, contributed to the RS content in food products (Lehmann *et al.*, 2002).

According to Yamada *et al.* (2005), indigestible dextrin substances in the bread samples contributed to the RS content, which significantly reduced the postprandial insulin secretion and blood glucose responses. High-molecular-weight (HMW) molecules of maltodextrin reassociated to form double helices among themselves or with starch molecules. Therefore, at low maltodextrin concentration, the presence of starch in bread may help the reassociation of HMW molecules of maltodextrin and further increase the RS content in the bread samples (Wang and Jane, 1994).

Foster-Powell *et al.* (2002) reported that addition of fiber to food products reduced their GI value. RS is another functional material that has a similar effect as some dietary fibers. The results of RS, hydrolysis index (HI), and estimated glycemic index (EGI) for bread substituted with different levels of chempedak seed flour are presented in Table 33.3. The results show that RS increased significantly ($p < 0.05$) by 16.3% in the 10% chempedak seed flour bread compared to the control. An elevated amount of chempedak seed flour further increased the amount of RS by almost twofold compared to the control bread. The extent of RS formation is closely related to the degree of starch retrogradation, the amount of dietary fiber, the amylose/amylopectin concentration, other food components in the product, and the type of processing.

At a higher chempedak seed flour substitution level, HI value was found to decrease significantly ($p < 0.05$) compared to the control bread. The significant decrease ($p < 0.05$) in HI value with increase of chempedak seed flour substitution levels directly corresponds with the significant decrease ($p < 0.05$) in EGI in bread with higher chempedak seed flour substitution levels. SDF in food products reduced the postprandial blood glucose response (Behall and Scholfield, 2005). However, Wolever (1990) asserted that IDF inhibits starch digestion, which is more important in the further reduction of the GI. Kinetics of *in vitro* starch hydrolysis for bread with different substitution levels of chempedak seed flour exhibited significantly lower ($p < 0.05$) *in vitro* starch hydrolysis compare to the white bread and control bread. At higher chempedak seed flour substitution levels, *in vitro* starch hydrolysis increased gradually as the time intervals increased (Figure 33.2).

Products with a high content of RS and dietary fiber have been reported to have lower glycemic and insulinemic response in healthy human subjects (Behall and Schofield, 2005). Thus, further studies on the development of food containing high dietary fiber and low GI are of significant interest to improve nutritional quality and reduce the GI value upon consumption.

TABLE 33.3 Model Parameters, Resistant Starch, Hydrolysis Index, and Estimated Glycemic Index of Bread Substituted at Different Levels of CSF[a]

Sample	RS (% db)	K_c	k	Calculated HI	EGI
Bread 1 (control)	16.86 ± 0.88^{a}	15.48 ± 1.17^{a}	0.09 ± 0.03^{a}	43.23 ± 2.28^{a}	63.44 ± 1.25^{a}
Bread 2 (10% CSF)	19.60 ± 0.05^{b}	14.81 ± 0.92^{a}	0.06 ± 0.01^{a}	$40.28 \pm 1.81^{a,b}$	$61.83 \pm 0.99^{a,b}$
Bread 3 (20% CSF)	29.63 ± 0.20^{c}	$14.19 \pm 0.32^{a,b}$	0.07 ± 0.01^{a}	$38.96 \pm 0.67^{b,c}$	$61.10 \pm 0.37^{b,c}$
Bread 4 (30% CSF)	30.69 ± 0.75^{c}	12.90 ± 0.65^{b}	0.11 ± 0.07^{a}	36.24 ± 2.77^{c}	$59.61 \pm 1.52^{b,c}$

CSF, chempedak seed flour; EGI, estimated glycemic index; HI, hydrolysis index; k, kinetic constant; K_c, equilibrium constant; RS, resistant starch.
[a]*Mean value ± standard deviation;* n = *3. Values followed by a different letter(s) in the same column are significantly different* ($p < 0.05$).

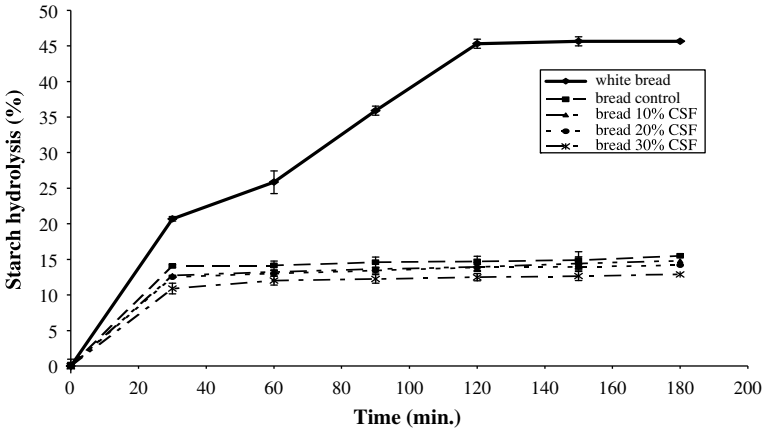

FIGURE 33.2
Profile of *in vitro* starch hydrolysis (%) in white bread (reference) and bread substituted at different levels of chempedak seed flour (CSF). All tested bread samples exhibited gradual increases in *in vitro* starch hydrolysis as time increased. White bread underwent higher starch hydrolysis during 30—120 min compared to the other bread samples. Results are expressed as mean ± standard deviation; $n = 3$.

QUALITY OF CHEMPEDAK SEED FLOUR BREAD

Volume

Loaf volume was an important indicator for identifying bread characteristics because it provided quantitative measurement of baking performance (Hathorn *et al.*, 2007). Bread samples at higher chempedak seed flour substitution levels elicited lower loaf volume compared to the control (Figure 33.3). A pronounced decrease ($p < 0.05$) of loaf volume (49.1%) was exhibited in 30% chempedak seed flour bread, followed by 20% chempedak seed flour bread (22.3%) and 20% chempedak seed flour bread (8.1%), compared to the control. The significant decrease in loaf volume at higher chempedak seed flour substitution levels was attributed to the gluten dilution effect (Krishnan *et al.*, 1987), which was associated with the low protein quality network in the dough (Rosell *et al.*, 2001) and indicated weak interaction between starch and gluten of flour (Oates, 2001).

Differences in protein content in the flour of only 1 or 2% marked a significant decrease in bread volume. According to Haglund *et al.* (1998), baking of bread with a protein content of flour at 8% or lower resulted in detrimental effects to acceptable loaf volume. Furthermore, substitution of chempedak seed flour, which contains dietary fiber, particularly IDF (Pollard *et al.*, 2002), and nongluten protein networks (Oates, 2001) into the bread formulation caused an adverse effect on carbon dioxide gas production and retention during dough proofing that exerted lower loaf volume (Zhang *et al.*, 1998).

Substitution of chempedak seed flour with wheat flour subsequently weakened coherence and continuity of the protein matrix in the dough due to the tight interaction between starch granules and gluten. Hence, gas cells in the dough at higher chempedak seed flour substitution levels were not able to expand due to less gas retention (Oates, 2001).

The specific volume of bread samples decreased significantly ($p < 0.05$) at higher chempedak seed flour substitution levels. Bread with 30% chempedak seed flour had a substantial decrease ($p < 0.05$) in loaf volume of 54.3%, followed by the 20% chempedak seed flour bread (27.7%), compared to the control. Hathorn *et al.* (2007) reported a significant decrease in specific volume with the increased substitution level of hazelnut testa and sweet potato flour, respectively, into the bread formulation.

The lower value of specific volume was directly related to the lower loaf volume of the bread samples. The occurrence of lower loaf volume and specific volume was due to the higher amount of amylopectin, which increased the water retention (Hung *et al.*, 2007) and decreased the gas retention (Lee *et al.*, 2001) of loaf during baking, thus resulting in reduced loaf volume.

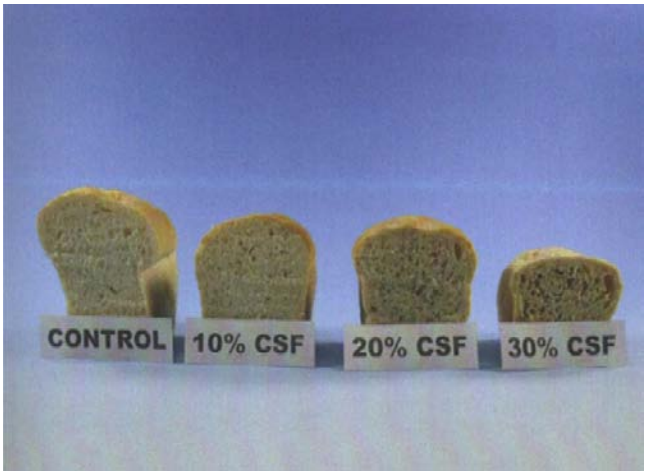

FIGURE 33.3
Cross-section of the control and 10, 20, and 30% chempedak seed flour (CSF) breads.

SENSORY EVALUATION OF CHEMPEDAK SEED FLOUR BREAD

Crust color

The score for crust color decreased significantly ($p < 0.05$) as chempedak seed flour substitution level increased. However, no significant difference ($p > 0.05$) was observed for the control and 10% chempedak seed flour bread. Bread with 30% chempedak seed flour had the lowest score for crust color, indicated that the crust color of the bread was not attractive at higher chempedak seed flour substitution levels.

Crumb texture

Crumb texture was observed to reduce significantly ($p < 0.05$) with increased chempedak seed flour substitution level. Addition of non-wheat flour into the bread formulation resulted in coarser structure and increased the crumb pore size. Bread with 10% chempedak seed flour has a significantly higher score ($p < 0.05$) for crumb texture upon storage compared to the other samples. However, no significant difference ($p > 0.05$) for crumb texture was found between control and 10% chempedak seed flour bread.

Taste

The taste characteristic of bread is vital in determining the overall acceptability of the product. At higher chempedak seed flour substitution levels, the bread had slightly bitter taste and imparted the chempedak seed flour flavor in 30% chempedak seed flour bread, which resulted in a significant reduction ($p < 0.05$) on the taste score characteristic. The taste decreased significantly ($p < 0.05$) as chempedak seed flour substitution level increased. The control and 10% chempedak seed flour breads did not differ significantly ($p > 0.05$) in taste.

Overall acceptability

The score for overall acceptability decreased significantly ($p < 0.05$) as chempedak seed flour substitution level increased. The control received the highest score for overall acceptability, followed by the 10 and 20% chempedak seed flour bread. The 30% chempedak seed flour bread was unacceptable because it obtained the lowest score for overall acceptability. Thus, bread substituted with up to 20% chempedak seed flour was considered acceptable. The 10% chempedak seed flour bread was comparable with the control in terms of the overall acceptability of the bread.

TECHNOLOGICAL ISSUES

- Chempedak seed flour is a good source of TDF and RS because the flour contains 26.48% TDF and 14.8% RS.
- Bread incorporated with 10% chempedak seed flour provides 13.0% protein, 1.10% fat, 19.60% RS, 10.92% TDF, 10.08% IDF, and 0.84% SDF.
- Bread substituted with chempedak seed flour has a lower GI than the control. This has potential for utilization in diabetic patients.
- High RS content in chempedak seed flour bread has potential to prevent/control colon cancer, to reduce constipation, and for slow release of glucose in blood.
- Chempedak seed flour has commercial potential as a dietary fiber source in bakery products due to its ability to maintain the acceptable eating qualities of white bread while providing dietary fiber.
- Chempedak seed flour contributes to the development of value-added food that is currently in demand, such as bread.

SUMMARY POINTS

- At higher chempedak seed flour substitution levels, bread samples had significant increases ($p < 0.05$) in moisture, crude fiber, ash, resistant starch, and total dietary fiber.
- Crude fat and carbohydrate content decreased significantly ($p < 0.05$) with higher chempedak seed flour substitution level in bread.
- The estimated glycemic index value of bread at higher substitution levels of chempedak seed flour was found to decrease significantly ($p < 0.05$).
- Loaf qualities (loaf volume and specific volume) were significantly decreased ($p < 0.05$) at higher substitution levels of chempedak seed flour.
- Sensory evaluation of bread substituted with different percentage levels of chempedak seed flour showed that the 10% chempedak seed flour bread was not significantly different ($p > 0.05$) from the control in terms of crumb color, crumb texture, taste, and overall acceptability.

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CHAPTER 34

Effect of Starch Addition to Fluid Dough During the Bread Making Process

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

RS Resistant starch
SDS Slowly digestible starch

INTRODUCTION

Starch is one of the most abundant carbohydrates in plants. The cereals, in particular, are composed of approximately 75% carbohydrates. Starch occurs in granules in the endosperm. Starch granules differ in size (i.e., in rice they have a diameter of only 5 μm, whereas in wheat the diameter may be 25–40 μm) and shape (either large, lens-shaped granules or small, spherical granules). Cereals differ greatly in starch content, with maize and wheat having the highest starch content, followed by barley and oats (Atasoglu and Yurtman, 2007). In plants, starch is packaged in granules and can be fractioned into two glucose homopolysaccharide macromolecules: amylose and amylopectin.

According to their origin, starches differ in the content of these two polymers. Amylose is a linear glucan with α-1,4 glycosidic linkages, composed of long chains that take mainly a helical conformation, whereas amylopectin is a branched polymer of higher molecular weight than that of amylose (Thomas and Atwell, 1997). Because each glucose residue has three hydroxyl groups, the individual starch chains are held close together by hydrogen bonds. When starch granules in water are heated at a given temperature, water is forced in between the

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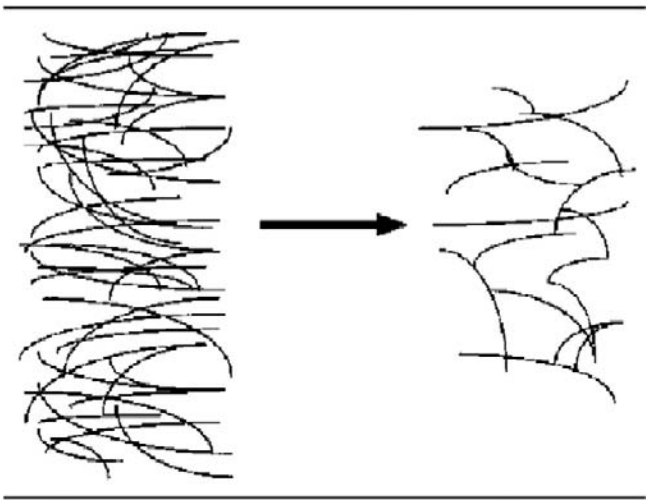

FIGURE 34.1
Starch gelatinization illustrated schematically. *Source: Reproduced with permission from Hart, B. (1997). Technology and food production.* Nutr. Food Sci. *2, 53–57.*

starch molecules. The starch granules expand dramatically, and if the ratio of starch to water is sufficiently high, the granules will be unable to easily move past one another. This causes a rise in viscosity. The process of starch gelatinization is illustrated in Figure 34.1. This difference in their conformation grants them different properties. Several studies have analyzed the properties of starch-baked films and have demonstrated that variations in the amylose:amylopectin ratio lead to products with different mechanical properties. Due to the presence of sugars and different molecular conformations, studies have reported interesting results in terms of crystallinity, water absorption, and mechanical behavior.

The ratio of amylose to amylopectin depends on the cereal nature and origin. Within common varieties of cereals, 25–75% of starch is present as amylose, whereas in waxy varieties (i.e., rice and corn) most of the starch is amylopectin (Figure 34.2). The chemical and physical structure of starch determines its availability, degradability, and the interactions with other system components. Most starches contain approximately 20–35% of amylose. The heating of starch in water leads to swelling of the granules: The extent of solubilization and disintegration of starch granules depends on the processing conditions. Dispersed or gelatinized starch is a ternary system polymer based on amylose/amylopectin and water, with the latter being a poor solvent for starch and particularly for the amylose.

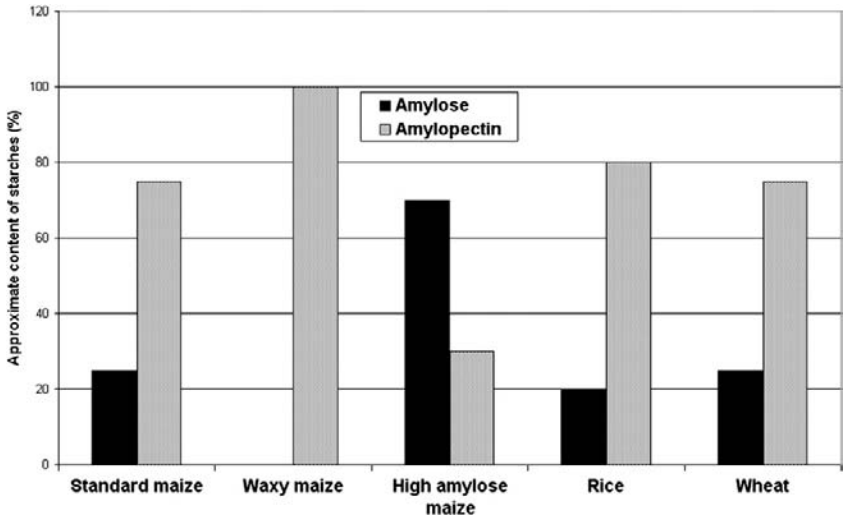

FIGURE 34.2
Approximate amylose and amylopectin content in standard maize, waxy maize, high amylose maize, rice, and wheat. *Source: Adapted from McKevith, B. (2004). Nutritional aspects of cereals.* Nutr. Bull. *29, 111–142.*

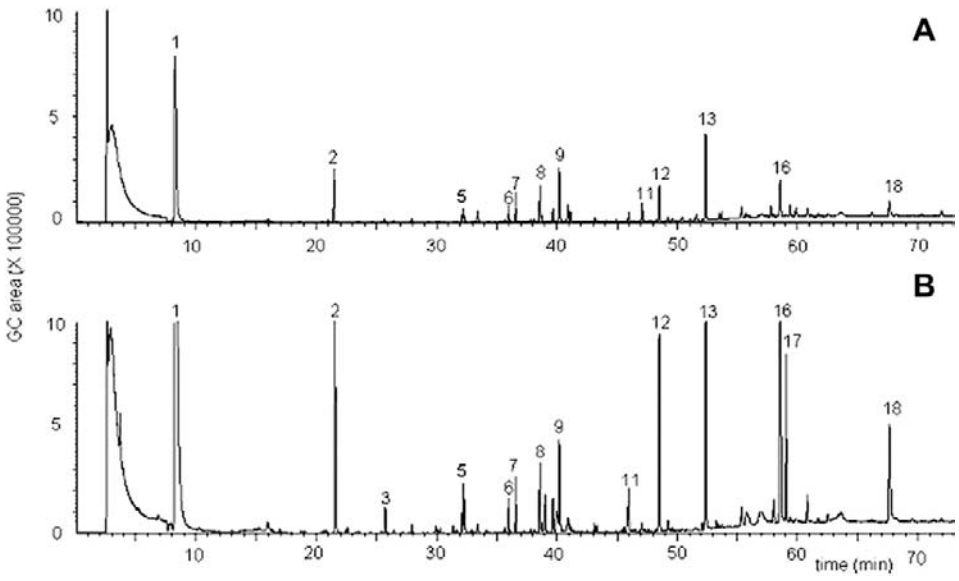

FIGURE 34.3
Gas chromatography–mass spectrometry/headspace solid-phase microextraction profiles of the co-culture of *Saccharomyces cerevisiae, Candida milleri*, and *Lactobacillus sanfranciscensis* in the liquid dough without (A) and with (B) soluble addition of starch. The peaks reported are (1) ethanol, (2) isoamyl alcohol, (3) acetoin, (4) ethyl octanoate, (5) acetic acid, (6) 1-octanol, (7) isobutyric acid, (8) butyric acid, (9) isovaleric acid, (10) ethyl-9-decenoate, (11) hexanoic acid, (12) phenylethanol, (13) octanoic acid, (14) γ-octalactone, (15) γ-decalactone, (16) decanoic acid, (17) ethyl-9-hexadecenoate, and (18) dodecanoic acid. *Source: Adapted from Vernocchi, P., Ndagijimana, M., Serrazanetti, D., Gianotti, A., Vallicelli, M., and Guerzoni, M. E. (2008). Influence of starch addition and dough microstructure on fermentation aroma production by yeasts and lactobacilli.* Food Chem. *108, 1217–1225.*

STARCH USE IN BAKERY APPLICATIONS

Starch contributes greatly to the texture properties of many foods. Both native and modified starches are characterized by different properties that enable the development of products with different structural and sensory features. The differences between the amounts of amylose and amylopectin of the various starches determine their functional use in food technology. For example, a high amylose starch might be used in an edible coating, whereas a waxy corn starch with a high amylopectin:amylose ratio forms viscous pastes when heated. In sweet foods, the addition of exogenous starch has a number of useful functions. Exogenous starch can help to replace eggs, reduce spread, and increase the number of useful functions.

Starch and starch-based ingredients are made in many countries from many different starchy raw materials, such as wheat, barley, maize, rice, white or sweet potatoes, and cassava. Although they have similar chemical reactions and are usually interchangeable, starches from different sources have a different granular structure that affects their physical properties.

Starch and starch products are used in many food and non-food industries and also as chemical raw materials for many other purposes, such as in plastics and tanning of leather. However, the food industry is the largest consumer of starch and starch products. Starch is a major component in bread making and plays an important role in texture and quality of dough and bread, and bread making depends to a large extent on the selection of flour with the proper gluten and starch characteristics. As reported by Miyazaki *et al*. (2006), starch competes with other components for available water in the system. Thus, starch determines the structure of the dough system and contributes to the texture of the final baked products. It is used in biscuit making to increase volume and crispness. Moreover, exogenous starch and modified starches are also used in many types of candies. Innovative new starch-derived specialities from

TABLE 34.1 Effects of Native and Modified Starches on Bakery Quality Features and Bakery Health Properties

	Interactions	Effects of Starch Addition
Bakery quality features	Chemicophysical interactions with food components Competition with the components for the available water Interaction of starch of gluten-free flours with gums influences the food matrix Interaction with microbial metabolites and microorganisms during fluid fermentation processes in bakery and retention of a wide spectrum of ligand molecules (i.e., butanol and *n*-pentanol)	Improvement of quality and texture of bakery products (including gluten-free), bread, biscuits, and candies; lipid and egg replacing; etc. Entrapping of flavoring agents and antimicrobial compounds produced by starters with consequent enhancement of their production Reduction of antimicrobial activity of lactones as free molecules with consequent overproduction
Bakery health properties	Interaction with microorganisms Interaction with gut microbiota: a fraction of starch (RS) escapes digestion and plays an important role in human health	Ability to trap molecules and microorganisms and particularly pathogens (i.e., in medicine, therapy for *Vibrio cholerae*) Prebiotic effect

tapioca are reported to enable a 75% reduction in the butter, margarine, or shortening used in cakes.

Starches from different sources, in addition to having variable amylase:amylopectin contents, possess specific shapes and gelatinization temperatures (Miyazaki *et al.*, 2006). The native starch of the flour as well as the various added modified starches can play different roles in all phases of bread making, including fermentation, as well as in all phases of human consumption due to their ability to interact with micro- and macromolecules as well as with microbial cells.

In this chapter, the following interactions are considered:

- Starch interactions with microorganisms and microbial metabolites during fermentation
- Starch interactions with gut microbiota
- Starch interactions with dough components.

Modified flours and starches, also called swelling starch, are especially characterized by their modified water absorption and solubility properties. The viscosity of flours (starch) and water suspensions is adjusted to suit the product-specific needs. In the bakery, modified flours and starches are applied as water binders, freshness extenders, and binders.

The ability of the various native or modified starches to interact with molecules or microbial cells can be exploited in industrial fermentation processes for bakery and food products and for functional food formulations in order to obtain the desired quality and health properties as summarized in Table 34.1.

STARCH INTERACTION WITH MICROORGANISMS AND MICROBIAL METABOLITES DURING FERMENTATION

Extensive research has been carried out to study the essential role of food ingredients and their properties in the modification of the microstructure and textural properties of starches as well as their physicochemical interactions with food aroma compounds (Terta *et al.*, 2006). In particular, it has been shown that in several food systems, polysaccharides such as starch are involved in the retention of a wide spectrum of ligand molecules, such as flavor compounds (Guichard, 2002; Heinemann *et al.*, 2003). Linear ligands are thought to be located in the hydrophobic cavities of the amylose helix, whereas bulky ligands such as *n*-butanol and *n*-pentanol may be located between the amylose helices (Helbert and Chanzy, 1994). The partitioning of the molecules between different phases of a food system is based principally on

two mechanisms (Terta *et al.*, 2006): (1) diffusion decrease, as predicted by the Stokes–Einstein equation in which diffusion is inversely proportional to viscosity, and (2) specific molecular interactions of ligands/matrix with consequent complexation, encapsulation, and hydrogen bonding. The importance of these phenomena on flavor retention, release, and perception by consumers has been extensively investigated (Heinemann *et al.*, 2003). In fact, according to Boutboul *et al.* (2002), the retention of *d*-limonene, ethyl hexanoate, octanal, and 1-hexanol increased with the polarity of the flavor molecules. Maltodextrin appeared to be the most efficient matrix for flavor retention, followed by pregelatinized starches, extruded high amylose starch, and finally granular starches (native and acetylated starches). Retention has been explained as mainly dependent on specific area. Moreover, another potentially important factor for consumer perception of flavor is the presence of the enzyme α-amylase in saliva, which can degrade starch, thus reducing the viscosity of the pastes. It has been hypothesized that the viscosity signal reaching the brain may modulate the processing of the taste and flavor signals.

A viscosity reduction could be expected to contribute to enhanced flavor perception (Cook *et al.*, 2003). Ferry *et al.* (2006) compared starches with similar viscosity. They hypothesized that perceived taste, and hence flavor, from viscous solutions is related to the efficiency with which the solution mixes with saliva in the mouth. Starch pastes, in which the granular structure is largely retained, mix efficiently, and this is responsible for their different mouthfeel and increased flavor perception in comparison with random coil polysaccharides.

An additional effect of starch matrix/ligand interactions should be involvement in the regulation of the fermentation processes in solid or viscous systems. In fact, generally in industrial liquid fermentation, the production of metabolites by starter and nonstarter microorganisms follows dynamics that are described as exponential in the presence of a limiting factor. The antimicrobial activity of the major part of the flavor metabolites such as alcohols, esters, and aldehydes is a limiting factor for fermentation performance. The trapping of metabolites and their removal from the system as free molecules should play a role in fermentation that is as important as that of temperature or pH (Vernocchi *et al.*, 2008). A new and promising application of a strategy based on metabolite trapping concerns the addition of starch to liquid dough or sourdough used at the industrial level as a prefermented inoculum to accelerate the hand making process (Vernocchi *et al.*, 2008). Different sourdough processes including liquid fermentation have been developed at the industrial level (Carnevali *et al.*, 2007).

Vernocchi *et al.* (2008) studied the effect of soluble starch addition to liquid sourdough in order to evaluate whether the chemicophysical interactions of the microbial metabolites with the starch matrix affect the metabolic activity of the microbial consortium and increase flour production. The results obtained indicate that when, particularly under osmotic stress, *Lactobacillus sanfranciscensis*, *Saccharomyces cerevisiae*, and *Candida milleri*, which are frequently associated with sourdough, were inoculated together (Table 34.2), many key metabolites such as ethanol and acetic acid significantly increased. Moreover, the most interesting effect of starch addition, particularly under osmotic stress, was the production of relevant levels of flavoring agents such as γ-decalactone accompanied by a minor level of γ-octalactone, as show in Figure 34.2. Lactones, particularly γ-decalactone, are volatile compounds that contribute to the natural flavor of many fruits and are usually generated through the action of yeast cells on lipid metabolites in several fermented foods. γ-Decalactone results from lactonization of 4-hydroxydecanoic acid. Many studies have dealt with the biotranformation pathway that involves β-oxidation steps (Aguedo *et al.*, 2004). The principal problem for the industrial production of these odorants is that their accumulation is strongly limited by their toxicity against producing cells (Aguedo *et al.*, 2004). In fact, the trapping of lactones *in situ* by hydrophobic solvents is generally regarded as the unique method to maintain viability of the fermenting agent during the industrial production of lactones. In liquid sourdough, the observed overproduction of lactones in the presence of soluble starch can be attributed to the

TABLE 34.2 Effect of Liquid Dough with and without Starch and Liquid Dough with and without 40% Sucrose on Metabolites Released by *L. sanfranciscensis*, *S. cerevisiae*, and *C. milleri*

	***L. sanfranciscensis*, *S. cerevisiae*, and *C. milleri* Inoculated in Liquid Dough with and without Starch (mg/l)**			
	LD	**LDS**	**LDO**	**LDOS**
Ethanol	3952 ± 345	4680 ± 421	6147 ± 593	6,728 ± 622
Isoamyl alcohol	453 ± 41	344 ± 31	226 ± 20	310 ± 27
Acetic acid	152 ± 13	312 ± 27	414 ± 38	462 ± 40
Isobutyric acid	362 ± 35	248 ± 22	271 ± 24	273 ± 24
Isovaleric acid	780 ± 72	824 ± 81	539 ± 51	743 ± 72
Hexanoic acid	120 ± 11	70 ± 7	61 ± 6	60 ± 6
Phenylethanol	n.d.	763 ± 75	662 ± 57	715 ± 70
Octanoic acid	1024 ± 99	765 ± 75	539 ± 49	663 ± 64
γ-Octalactone	n.d.	n.d.	n.d.	19 ± 2
γ-Decalactone	n.d.	n.d.	n.d.	14,422 ± 1,435
Ethyl-9-hexadecenoate	18 ± 2	39 ± 3	27 ± 3	13 ± 1
Decanoic acid	540 ± 48	843 ± 82	826 ± 81	785 ± 76

LD, liquid dough; LDO, liquid dough with sucrose 40%; LDOS, liquid dough with sucrose 40% and starch; LDS, liquid dough with starch; n.d., not determined.
Source: Adapted from Vernocchi *et al.* (2008).

TABLE 34.3 Effect of Modified Starch Addition on Acetic and Lactic Acid Production during *In Vitro* Fermentation with *Bifidobacterium breve*[a]

	Acetic Acid (mol/100 ml)	**Lactic Acid (mol/100 ml)**	**Total (mol/100 ml)**
Glucose (control)	394.1 ± 4.9	536.4 ± 5.7	930
Native starches			
Wheat	411.6 ± 7.8	125.1 ± 3.5[b]	536
Potato	249.3 ± 2.1[a]	132.3 ± 1.4[a]	381
Modified starches			
Wheat	300.2 ± 5.7[a]	52.5 ± 2.8[b]	352
Potato	294.5 ± 3.5[a]	73.0 ± 4.2[b]	367

Source: Adapted from Wronkowska *et al.* (2006).
[a]*Mean ± SD. Significant difference between groups and the control medium containing glucose:* [a]$p \leq 0.01$; [b]$p \leq 0.001$.

ability of this hydrocolloid to entrap the lactones and reduce their antimicrobial activity as free molecules.

Starch has another use associated with its chemicophysical properties. In medicine, the oral rehydration therapy for *Vibrio cholerae* disease has been improved with starch integration due to its ability to abduct or trap molecules and microorganisms (Gancz *et al.*, 2005). *Vibrio cholerae* adheres to the included starch granules, accelerating the therapy effects. These findings support significant exploitation of starch's chemicophysical attributes.

STARCH INTERACTION WITH GUT MICROBIOTA

Dietary starch has different physiological effects in humans depending on its rate and the extent of digestion. The digestion of starch occurs predominantly in the small intestine, where pancreatic α-amylase is released into the lumen and amyloglucosidase, α-glucosidase, and maltose are embedded in the brush border of the intestinal wall. Approximately 10% digestion of starch is catalyzed by salivary α-amylase (Sang and Seib, 2006). The rate of starch digestion in food is altered by factors that are both extrinsic and intrinsic to starch. Extrinsic factors

include the food particle size, viscosity of the digest, α-amylase inhibitors, and the individual level of α-amylase, whereas intrinsic factors include the swelling and solubilizing of starch granules, the extent of branching, and the physical association of starch chains. Part of starch is slowly digested or escapes digestion in the small intestine. Starch has been classified as rapid digestible, slowly digestible (SDS), and resistant starch (RS). Resistant starches are not adsorbed in the small intestine and have an important role in human health. For example, white and whole wheat bread, rolled oats, and cornflakes were found to be 87–98% digested in ileostomy patients (Sang and Seib, 2006). The average American ingests approximately 12–17 g of fiber each day, but the National Cancer Institute recommends daily introduction of 20–40 g. Starch ingredients with high levels of SDS and RS are recommend to improve the nutritional profiles of grain-based foods.

Normally, the bacteria that ferment carbohydrates that escape small intestine digestion belong to the large bowel ecosystem. The gut bacterial profile reflects the supply of fermentable sugars and oligosaccharides in the diet. RS appears to act as dietary fiber. Four categories of resistance have been defined (Baghurst *et al.*, 1996):

- RS1 refers to starch that is physically inaccessible for digestion because it is "trapped" (i.e., intact whole grains and partially milled grains).
- RS2 refers to native resistant starch granules (i.e., found in high amylose maize starch).
- RS3 refers to retrograded starch (i.e., found in cooked and cooled potatoes, bread, and some types of cornflakes).
- RS4 refers to chemically modified starch (i.e., commercially manufactured starches).

RS is regarded as butyrogenic. In fact, predominant substrates for bacterial fermentation in the large intestine include RS, nonstarch polysaccharides and oligosaccharides, undigested proteins, and endogenous compounds such as pancreatic secretions and mucins (Arrigoni *et al.*, 2002). The fermentation process of these substrates provides the colon with metabolic end products and supplies the metabolizable energy for the growth or maintenance of the intestinal microbiota. The principal bacterial end products are short-chain fatty acids, mainly acetic, propionic, and butyric ones. Some gases, such as hydrogen, carbon dioxide, and methane, are also produced to a lesser extent (Velazques *et al.*, 2000).

Butyrate appears to be of great physiological significance because it is the major fuel for colonic epithelial cells even when competing substrates, such as glucose and glutamine, are available. Moreover, RS, especially high amylose maize starch, is sometimes referred to as bifidogenic, or even prebiotic, because it is able to promote the survival of bifidobacteria and lactic acid bacteria during passage through the gastrointestinal tract (Topping and Clifton, 2001). Bifidobacteria generate L(+)-lactic acid, which is rapidly absorbed in the colon, whereas lactic acid bacteria also produce D(−)-lactic acid, which increases the acidity in the colonic lumen, thus protecting it from the growth of putrefactive bacteria. Weak acids display potent antimicrobial activity as their undissociated form passes freely through the cellular membrane of pathogenic microorganisms (Cotter and Hill, 2003).

The other end products of the *Bifidobacterium* metabolism include acetic acid, formic acid, and ethanol, which are generated in different proportions depending on carbohydrate availability and form (Biedrzycka, 2003). Acetate is another product of *Bifidobacterium* metabolism—more inhibitory than lactic acid against yeasts and molds—that is absorbed from the hindgut and represents a secondary fuel for the tissues (Cummings, 1997). It is rapidly cleared from the blood and metabolized by skeletal and cardiac muscle and brain. On the basis of changes of *Bifidobacterium* metabolism and of microbiota composition of rat cecal digesta, Wronkowska *et al.* (2006) evaluated the susceptibility of new, modified starch preparations to fermentation (studied *in vitro*) in comparison with native starches. The modified starches appeared to function as substrates for selected *Bifidobacterium* strains. Generally, they stimulated the growth and acidifying properties of bifidobacteria better than did the native starches. Although

glucose is the preferentially utilized source of carbon and energy for bifidobacteria, the promising properties of native starches and modified starches pave the way for innovation in the bakery sector. Moreover, formulations with resistant or modified starch can result in different flavor or sweetness perceptions.

INTERACTIONS WITH DOUGH COMPONENTS AND ADDITIVES

Wronkowska *et al.* (2006) demonstrated that the thermal characteristics of modified starches, determined with differential scanning calorimetry, were completely different from those of native starches. The peak temperatures for the native starches of wheat, potato, and pea were 61.7, 64.3, and 63.3°C, respectively, whereas for modified starches they were much higher at 140.1, 128.1, 136.1°C, respectively.

Starch—xanthan gum interaction plays an important role in bakery products. The use of xanthan gum in the preparation of gluten-free starch bread has been described (Sudhakar *et al.*, 1996). Xanthan gum and starch interact to form a matrix that during baking allows the development of a structure similar to that obtained in normal breads.

Starch—gum combinations have been used for the past 40—45 years in the food industry. The main advantage of using these combinations, compared to using starch alone, is the ability to reduce starch content up to 50%, thereby reducing the calorific value of the food (Glicksman, 1969). Modified and substituted starches that are widely used as thickeners in foods impart the sensory characteristics typically associated with sauces, soups, gravies, and fillings. However, starches have limitations because they do not achieve adequate viscosity at low concentrations. They also have a very limited ability to control sineresis. When starch alone is used to thicken, water separation occurs in frozen foods during thawing and preparation. Thus, many processors combat sineresis by adding food gums because they can successfully control this problem. Hydrocolloids have been known to affect the pasting properties and amylolysis (Christianson *et al.*, 1981). The synergistic effect in the case of starch—guar gum combinations is explained as follows. In guar gum, which is a non-ionic hydrocolloid, the alternate galactose branches inhibit the formation of intramolecular hydrogen bindings, thus keeping the molecule in an extended form, which can readily interact with the amylose molecule present in the system through noncovalent hydrogen bondings, resulting in a more extended conformation. This in turn increases the degree of pseudoplasticity.

TECHNOLOGICAL ISSUES

Starch plays an important role in the textures of many kinds of food products and serves as a major source of energy for humans. In some cases, native starch does not have the functional properties for food processing requirements such as thickening and stabilization. Therefore, starches used in the food industry are often modified to overcome undesirable changes in product texture and appearance caused by retrogradation or starch breakdown during processing and storage.

Starch contains abundant hydroxyl groups. Each anhydroglucose unit contains two hydroxyls (at C_2 and C_3), and some anhydroglucose units contain other hydroxyls (at C_6). These hydroxyls are potentially able to react with any chemicals having reactivity with alcoholic hydroxyls. This includes a wide range of compounds, such as acid anhydrides, organic chloro compounds, aldehydes, epoxy, and ethylenic compounds. Thus, starch can be modified in various ways to improve its functionality depending on the purpose of its application. Commercially modified starches include hydroxypropylated and/or cross-linked and acetylated and/or cross-linked starches, which are widely used in the food, textile, and paper industries:

- Cross-linking: Cross-linked starches are widely used as thickeners in foods, particularly when a high and stable viscosity is required. Cross-linking minimizes granule rupture, loss of viscosity, and formation of a stringy paste during cooking.

- Hydroxypropylation: Hydroxypropylated starch derivative formed by reaction of starch with propylene oxide is primarily used in the food industry. This modification improves the shelf life, freeze–thaw stability, cold-storage stability, clarity, and texture properties of starch paste. Cross-linking of hydroxypropylated starch imparts viscosity stability and a desired short-textured property of the paste. Swollen but intact starch granules are usually desired in most food starch applications to maintain rheological properties. However, for each application, there is an optimum level and balance between hydroxypropyl substitution and cross-linking.
- Acetylation: Acetylated starch derives from a low-degree substitution of three free hydroxyl groups at C_2, C_3, and C_6 of native starch in the presence of an alkaline catalyst and by esterification with acetic anhydride (acetylation) in aqueous medium.

In general, treatments with both hydoxypropylation and cross-linking, or acetylation and cross-linking, might be more suitable for bread making than native starch whatever its origin. In fact, by using modified starches, bakers can control the texture of products and develop unique bread that has a different texture from that of wheat flour bread. Among various modified starches, hydroxypropylated starches are the most effective for retarding staling. As a result, a suitable amount of modified starches could be used for bread making to improve the functionality and quality of breads. Costs of modified starches are generally lower than those of wheat flour because of their low tariffs. Modified starches are valuable for improving bread from both a functional and a commercial standpoint (Miyazaki *et al.*, 2006).

SUMMARY POINTS

- Starch is one of the most abundant carbohydrates in plants. The cereals, in particular, are composed of approximately 75% carbohydrates. The granules in plants are packaged and can be fractioned into two glucose homology saccharide macromolecules: amylose and amylopectin.
- Starch contributes greatly to the texture properties of many foods. Both native starch and various modified derivative forms of it provide a wide scope for the development of various food products having diverse textures and mouthfeel, and they determine their functional use in food technology.
- In several food systems, polysaccharides such as starch are involved in the retention of a wide spectrum of ligand molecules, such as flavor compounds. An additional effect of matrix/ligand interactions is the involvement in the regulation of the fermentation processes in solid or viscous systems.
- Dietary starch has different physiological effects in humans depending on its rate and extent of digestion. Some of the starch ingested by humans escapes digestion in the small intestine.
- Modified starches and combination starch–xanthan gum play an important role in bakery products, particularly in the development of desirable structure in gluten-free breads.

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CHAPTER 35

Fermentation as a Tool to Improve Healthy Properties of Bread

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

EPS Exopolysaccharides
GI Glycemic index
LAB Lactic acid bacteria
WSB-DF Fermented wheat with multispecies sourdough starters
WYB Wheat yeasted bread

INTRODUCTION

The idea that modern humans have diverged too far from the lifestyle of our prehistoric ancestors and with consequent reduced resistance to diseases is an interesting proposition suggested by Eaton and Konnor (1985).

The most dramatic difference between ancient and modern food has been attributed to a remarkably reduced microorganism intake from processed foods with respect to the billion times higher level of lactic acid bacteria (LAB) occurring in unprocessed natural foods. *Lactobacillus* species are the main components of the microbial consortium, which originated from the natural selection occurring during spontaneous fermentation of cereal flours and water. The microbiota of usual cereal sourdough is based on the stable associations of LAB and

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yeasts selected by the process conditions and environmental variables (i.e., humidity, temperature, and oxygen availability). The LAB typically isolated in wheat and rye sourdough belong to the species *Lactobacillus sanfranciscensis*, *Lactobacillus plantarum*, *Lactobacillus pontis*, *Lactobacillus acidophilus*, *Lactobacillus alimentarius*, *Lactobacillus fermentum*, *Lactobacillus curvatus*, *Lactobacillus brevis*, etc. (De Vuyst and Vancanneyt, 2007).

EFFECT OF CEREAL FERMENTATION ON HEALTH PROPERTIES

Traditionally, sourdough is used in baking applications to acidify and leaven bread dough. The various traditional sourdough fermentations were prevalently replaced by the use of baker's yeast in the twentieth century (Gänzle *et al.*, 2007). As observed by Gänzle *et al.*, the current renaissance of sourdough applications in bread making is motivated by the beneficial effect of sourdough on the flavor, texture, shelf life, and nutritional properties of bread and other baked goods. According to Batifoulier *et al.* (2005), due to the reduction in bread consumption and of whole cereal flours, a significant percentage of the European population is marginally deficient for dietary riboflavin and other B vitamins. Due to the heterogeneity of the ecological determinants (e.g., flour composition, temperature, pH and redox potential, dough yield, number of refreshments, and baker's yeast addition), mature commercial sourdoughs differ in microbial species complexity and metabolic activity (Gänzle *et al.*, 2007; Guerzoni *et al.*, 2007; Serrazanetti *et al.*, 2009). The various species and/or strains coexist and interact through nutritional or trophic relationships (Serrazanetti *et al.*, 2009). Several investigations on sourdough fermentation have described the link between metabolites produced and microbial diversity (sourdough and/or bakery products) (Gänzle *et al.*, 2007; Guerzoni *et al.*, 2007; Serrazanetti *et al.*, 2009). The metabolic activities associated with these complex ecosystems give rise to important modifications of the micro- and macromolecular array of the doughs. In addition to carbohydrate catabolism and gluten proteolysis, as well as organic acid production and flavor formation, important changes regarding nutritional and health properties of the dough and bread can be attributed to LAB and yeast co-fermentation. The impact of sourdough fermentation on functional properties of sourdough-based products can be summarized as follows: (1) enhancement of nutrients, organoleptic properties, and antimicrobial activities; (2) synthesis of exopolysaccharides; (3) increase of bioactive compound availability; (4) decrease of antinutritional compounds; (5) gluten detoxification by proteolysis; (6) enrichment in vitamins, amino acids, and peptides; and (7) glycemic index lowering due to acidification.

Katina *et al.* (2005) described the biochemical changes occurring during sourdough fermentation and the baking process that affect nutritional quality as well as texture and flavor. Levels of folate and easily extractable phenolic compounds have been shown to increase and levels of phytate, alkylresorcinols, and tocopherols decrease during the sourdough process (Kariluoto *et al.*, 2004; Katina *et al.*, 2007). Furthermore, results demonstrate that sourdough fermentation can improve the texture and palatability of whole grain or gluten-free products. This process can also stabilize or increase the levels of various bioactive compounds and improve mineral content.

The principal actions of sourdough fermentation related to healthy properties, such as polymerization, solubilization, and increased bioavailability, reduction of allergen and antinutritional compounds, and acidification, are described in this chapter.

MICROBIAL POLYMERIZATION ACTIVITY

Many microorganisms have been reported to produce exopolysaccharides (EPS) in the form of either capsules or slime secreted into the extracellular environment. LAB have been reported to be involved in a diverse range of fermentation processes. Some LAB are reported to be capable of producing EPS, which would naturally make them potential candidates for industrial use because they are natural food microorganisms. Two classes of EPS from LAB can be

distinguished: extracellular synthesized homopolysaccharides and heteropolygosaccharides with (ir)regularly repeating units that are synthesized from intracellular sugar nucleotide precursors. Hetero-exopolysaccharides are produced in small amounts, usually less than 0.5 g/l (Tieking *et al.*, 2003), as shown in Table 35.1.

The production of glucan and levan from sucrose by *Lactobacillus reuteri* and the production of a levan-type fructan by *L. sanfranciscensis* have been described by van Geel-Shutten *et al.* (1998). As suggested by Di Cagno *et al.* (2006), the synthesis of the previously mentioned EPS by sourdough LAB could be considered as a useful tool to replace the commercial additives used for improving the texture of baked goods. The fructan from *L. sanfranciscensis* has been found to positively affect dough rheology and bread texture. Moreover, glucan, fructans, and gluco- and fructooligosaccharides have potential gut health-promoting properties (Poutanen *et al.*, 2009). Fructose oligosaccharides and inulin are increasingly used as prebiotic additives in baked goods. They are not digested by pancreatic enzymes and thus are available for metabolism by intestinal microorganisms, mainly *Bifidobacteria* (Tieking *et al.*, 2003). In general, EPS are reported to be able to replace hydrocolloids currently used for texturizing, anti-staling, cholesterol-lowering, immunomodulating, antitumoral, and prebiotic activities (Tieking *et al.*, 2003).

MICROBIAL SOLUBILIZATION AND INCREASED BIOAVAILABILITY

Sourdough fermentation can both increase and decrease the levels of bioactive compounds depending on the nature of the molecules and the type of sourdough process. The presence of yeasts is reported to favor the formation of folates (Kariluoto *et al.*, 2004) and thiamin. On the other hand, according to Liukkonen *et al.* (2003), the pH decrease due to lactate and acetate production can both increase the solubilization of bioactive compounds (e.g., phenolic compounds) and decrease the levels of some compounds (e.g., thiamin, ferulic acid dehydrodimers, tocopherols, and tocotrienols). The mechanisms that regulate these phenomena during sourdough fermentation are mostly unknown. The role of acidity has been partly illustrated by Liukkonen *et al.* (2003), who studied the influence of rye sourdough fermentation, using two different rye varieties (Amilo and Akusti), on several bioactive compounds, including phenolic acids, sterols, folates, tocopherols and tocotrienols, and lignans. The fermentation phase more than doubled the levels of folates and easily extractable phenolic compounds. The same trend was observed by Guerzoni *et al.* (2010), where different LAB species individually inoculated in a complex maltose matrix produced remarkable amounts of folic acid (vitamin B_9) after a long fermentation time (Figure 35.1).

TABLE 35.1 Esopolysaccharides (EPS) Formed from Lactic Acid Bacteria

Strain	EPS Formed	Origin
L. sanfranciscensis 1	Fructan	Sourdough
L. sanfranciscensis 2	Fructan	Sourdough
L. sanfranciscensis 3	Fructan	Sourdough
L. frumenti 1	Fructan	Sourdough
L. frumenti 2	Fructan	Sourdough
L. frumenti 3	Fructan	Sourdough
L. pontis 1	Fructan	Sourdough
L. pontis 2	Fructan	Sourdough
L. reuteri 1	Fructan	Sourdough
L. reuteri 2	Fructan	Intestinal isolate
L. reuteri 3	Glucan	Duck colon
Weissella confusa 1	Glucan	Sourdough
W. confusa 2	Fructan	Sourdough

Source: Adapted from Tieking *et al.* (2003).

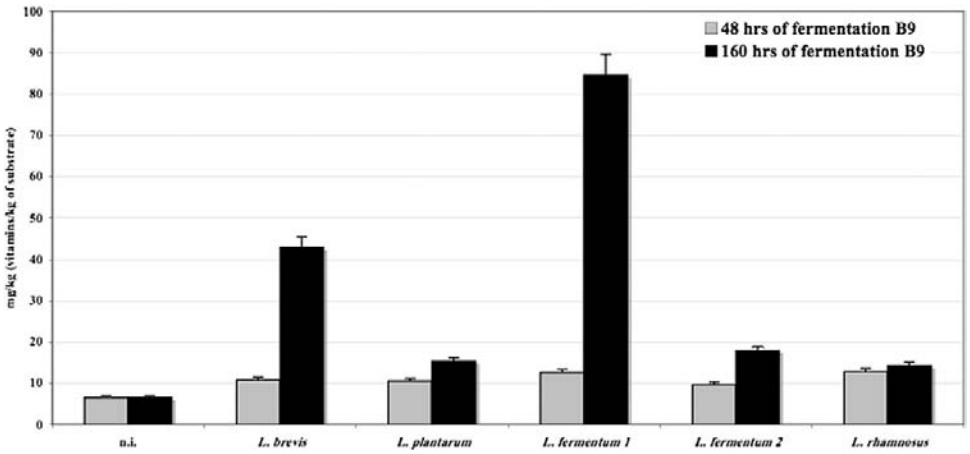

FIGURE 35.1
Folic acid produced by different lactic acid bacteria species during a complex maltose-based matrix fermentation after 48 and 160 hr. The reported values are the mean of three independent experiments. The concentration of folic acid produced by the lactic acid bacteria species increased after 48 and 160 hr, particularly by *Lactobacillus fermentum* 1. *Source: Guerzoni* et al. *(2010).*

The maintenance of total phenolic compounds, produced during kamut sourdough fermentation, was described by Gianotti *et al.* (2010) (Figure 35.2). The amount of total phenolic compounds after baking was similar to that detected in the correspondent kamut flour. These results suggested that sourdough fermentation might counteract the thermal loss of phenolic compounds due to heat process.

The levels of tocopherols and tocotrienols decrease during sourdough fermentation, probably due to contact with air, whereas the amounts of sterols, alk(en)ylresorcinols, lignans, phenolic acids, and alkaline extractable phenolic compounds change very little. The fermentation stage can also increase the antioxidant activity (DPPH radical scavenging activity) in the methanol-extracted fraction, probably due to increased levels of easily extractable phenolic compounds (Liukkonen *et al.*, 2003).

Solubilization of pentosans of flour, especially transformation of water-unextractable arabinoxylan to water-extractable arabinoxylan, has been reported to improve bread volume and texture during baking of wheat and high-fiber bread (Katina *et al.*, 2007). Sourdough fermentation increased the level of total extractable phenols and of free ferulic acid (Katina *et al.*, 2007).

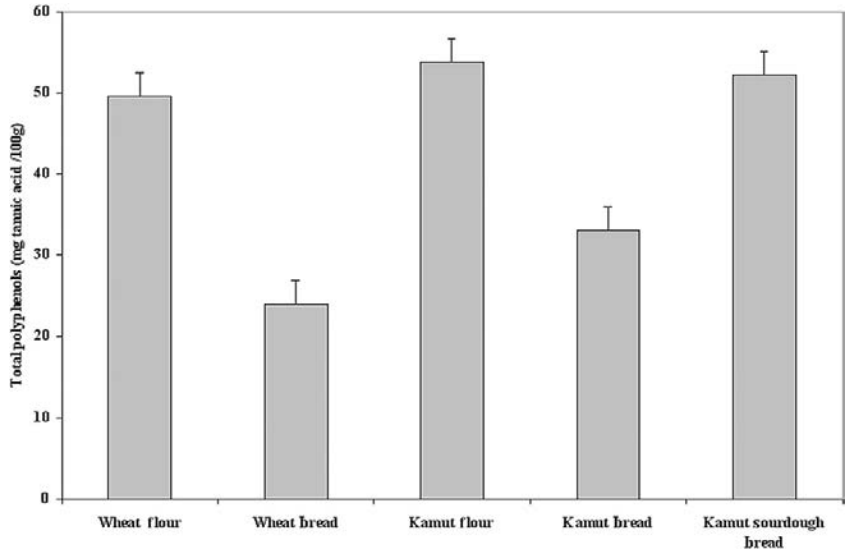

FIGURE 35.2
Total phenolic compounds produced in flour (wheat and kamut) and different dough systems. The reported values are the mean of three independent experiments. With respect to the flour (wheat or kamut), the fermentation by baker's yeast gave rise to lower disposable phenolic compounds in comparison to the kamut flour sourdough fermentation. *Source: Gianotti* et al. *(2010).*

An important advantage provided by sourdough is that this fermentation process can be used to bring about the biotransformation of inorganic selenium (Se) to organic Se forms (Bryszewska *et al.*, 2007). Nutritionally, this is significant because such organo-Se-rich sources are known to be more effective than either selenite (SeO_3^{2-}) or selenate (SeO_4^{2-}) in altering the individual long-term Se status (Bryszewska *et al.*, 2007). The potential of Se-enriched rye/wheat sourdough bread as a route for supplementing dietary selenium intake is reported by Bryszewska *et al.* (2007). According to these authors, the speciation of Se in foods is an important area of research that reflects our understanding of how its chemical form dictates the way in which it is used by the body. Once in the body, Se is reported to play an important role in a number of physiological and metabolic processes and functions—that is, cancer prevention, immune function, anti-aging, and male fertility (Bryszewska *et al.*, 2007).

MICROBIAL REDUCTION OF ALLERGEN AND ANTINUTRITIONAL COMPOUNDS

In addition to carbohydrate and peptide utilization, organic acid production, and flavor formation, sourdough LAB and yeasts possess numerous metabolic activities that can be of interest during sourdough fermentation (Gänzle *et al.*, 2007). For instance, controlled proteolysis of gluten can result in bread suited for celiac patients (Gobbetti *et al.*, 2007; Katina *et al.*, 2005). Biodegradation of phytate, an antinutritional factor that retains certain minerals and hence does not make them bioavailable, has been reported to be activated by endogenous cereal phytase activity through sourdough fermentation (Katina *et al.*, 2005; Lopez *et al.*, 2001). Lopez *et al.* showed that prefermentation of bran with lactic acid bacteria improved phytate breakdown (up to 90%) and increased magnesium and phosphorus solubility.

Proteolysis by sourdough fermentation has been found to be higher than that in yeasted and unstarted doughs. During dough fermentation, the proteolysis by LAB releases small peptides and free amino acids, which are important for rapid microbial growth and acidification and as precursors for the flavor development of leavened baked products (Rollán *et al.*, 2005). Furthermore, this proteolytic activity might be used as a tool to reduce certain allergen compounds.

Investigations carried out with fragments of α-gliadin or with homologous synthetic peptides to this α-gliadin amino acid sequence and the sequence 44−55 confirmed the toxicity of these proteins and peptides for celiac patients. *Lactobacillus plantarum* strains were able to hydrolyze the chemically synthesized 31−43 fragment of α-gliadin. Di Cagno *et al.* (2002) showed that this fragment was also hydrolyzed by *L. alimentarius* and *L. brevis* in a range of 54−50%, and it was also hydrolyzed by *L. sanfranciscensis* (43%) and *Lactobacillus hilgardii* (35%) (Rollán *et al.*, 2005).

From a practical standpoint, baked cereal goods are currently manufactured by highly accelerated processes in which long-time fermentations by sourdough, characterized by a cocktail of acidifying and proteolytic LAB with yeasts, have been almost totally replaced by the indiscriminate use of chemical and/or baker's yeast leavening agents. In these technological circumstances, cereal components (e.g., proteins) are subjected to very mild or absent degradation during manufacture, resulting in lower digestible foods compared to traditional and ancient sourdough baked goods (Gobbetti *et al.*, 2007).

MICROBIAL ACIDIFICATION: GLYCEMIC INDEX-LOWERING ACTIVITY

The metabolic effects of carbohydrates, particularly glucose, are related to the rate of carbohydrate intake in human body absorption after a meal. A common measurement to evaluate

these effects is the glycemic index (GI). From an analytical and instrumental standpoint, GI is defined as the incremental area, under the blood glucose response curve, after an intake of a standard amount of carbohydrates from a test food relative to a control food (glucose or white bread) (Ludwig, 2000). Information about the glycemic response of typical portion sizes of different foods, and thus the total glycemic effects of dietary patterns, can be obtained by the glycemic load. It is defined as the product of the GI and the total dietary carbohydrate in a food or meal (Scazzina *et al.*, 2009). Although there is an ongoing debate about the clinical implications of the GI, it offers a tool for selecting and classifying foods according to their fate during digestion (Ludwig, 2000). Jenkins *et al.* (2002) stated that low GI diets are associated with decreased risk of diabetes and cardiovascular diseases. Positive associations were found between dietary GI and risk of colon and breast cancer (Jenkins *et al.*, 2002).

It has been observed that bread containing lactic acid, produced during sourdough fermentation or added directly, can lower the postprandial glucose and insulin responses in humans (Katina *et al.*, 2005). Table 35.2 presents the titratable acid of different types of breads (Scazzina *et al.*, 2009). This fermentative activity increases the synthesis of short-chain fatty acids such as acetic, propionic, and butyric acids. De Angelis *et al.* (2009) compared wheat flour enriched with fibers and fermented wheat with multispecies sourdough starters (WSB-DF) with wheat yeasted bread (WYB) and wheat sourdough bread (Figure 35.3). They demonstrated that, on 20 volunteers, the value of GI for WSB-DF was approximately 41% with respect to that of WYB. WSB-DF bread manufactured at the industrial plant combined low GI with a physiologically significant supply of dietary fiber and high standard structure and sensory features.

TECHNOLOGICAL ISSUES

The current market trend is clearly moving toward tastier breads. With the industrialization of bakeries, the electromechanical technician became the key person ensuring that the automatic lines would be as operational as possible. Unskilled bakers were sought, and untrained people were hired to perform the work. Returning to traditional fermentation in the industry can only be successful if it is linked to an investment in skilled people (Decock and Cappelle, 2005). Thus, the sourdough production previously practiced with baker's yeast will disappear from bakeries and sourdough will be produced by specialized companies supplying stabilized or dried sourdough products to the bakery industry. The traditions are being maintained by artisan bakers who believe that the culture of their mother dough guaranties a superior quality and that the greater value of their end product can be appreciated. This tradition should be encouraged as a source of microbial biodiversity. Moreover, the industrialization of the process should enhance and standardize the functional properties associated with sourdough fermentation. The improvement and standardization of the process require specific and scientific selection of microbial strains suitable to predominate under the various process conditions and cereal types. In addition to these technological attributes, the strains must be

TABLE 35.2 Percentage Composition and Titratable Acidity of Breads[a]

	TS	RS (%TS)	Protein	Fiber	Moisture	Titratable Acidity
Wholemeal *S. cerevisiae*	45	3.3	8.2	10	31	85
Wholemeal sourdough	39	4.7	7.9	9.8	37	122
White *S. cerevisiae*	51	6.1	8.5	2	30	53
White sourdough	52	7.7	7.8	2.2	30	77

Source: Adapted from Scazzina *et al.* (2009).

[a]*Acidity values are expressed as equivalents of ions H^+/kg. The composition in terms of protein, fiber, moisture, total starch (TS), and resistant starch (RS) is different depending on titratable acidity due to sourdough fermentation.*

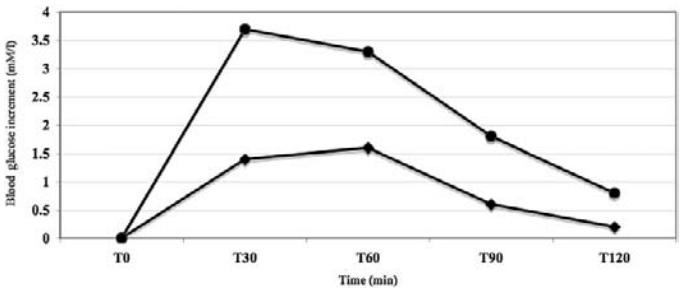

FIGURE 35.3
Blood glucose increment (mM/l) in healthy volunteers following ingestion of anhydrous glucose (reference; circles) and wheat sourdough bread enriched with oat and rye fibers (diamonds). The increase of blood glucose is higher after ingestion of anhydrous glucose compared to wheat sourdough bread enriched with oat and rye fibers. Values are means, $n = 20$ ($p < 0.05$). *Source: Adapted from De Angelis* et al. *(2009).*

selected on the basis of their ability to produce polyphenols, EPS, acidifying power, and vitamins. Only these individual attributes can confer nutritional added value to bakery products and compensate the high costs of the sourdough process management. At the same time, this approach allows for the production of functional cereal-based products with features that correspond to the promised health claims.

CONCLUSIONS

Scientific evidence concerning the potential health and quality benefits provided by sourdough fermentation has been reported in the literature. Considering the implications associated with sourdough microbial consortium activity, multistrain inoculum can be implemented and extended to other products such as prefermented whole grain formulations, used as functional snacks, or dried prefermented flour to be used for pasta and bakery products with low pH ingredients. This technological application enhances the development of new food from cereal by-products (bran). The potential challenges associated with cereal sourdough fermentation are summarized in Figure 35.4.

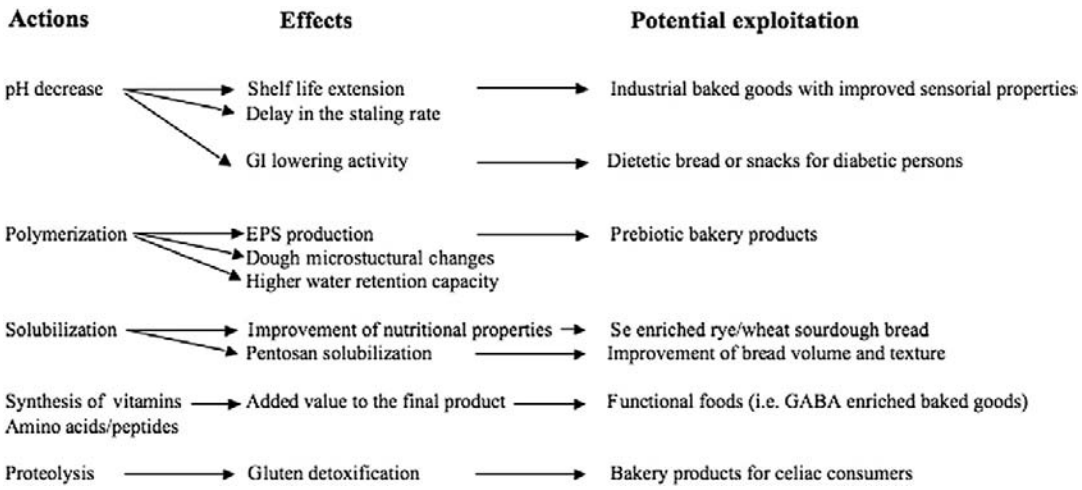

FIGURE 35.4
Potential effects by which lactic acid bacteria and yeasts contribute to baked goods production at the nutritional level. pH decrease, polymerization, solubilization, synthesis of nutritional and functional compounds, and proteolysis are the potential actions by which lactic acid bacteria and yeast contribute to baked goods production at the nutritional level.

SUMMARY POINTS

- The added value of sourdough fermentation, in comparison with baker's yeast fermentation, is mainly dependent on the lactic acid bacteria strains involved.
- Lactic acid bacteria release macromolecules that have prebiotic activities (e.g., exopolysaccharides) in the dough.
- The interactions between microorganisms and their environment generate important biotransformations of the dough ingredients whose effects can confer functional properties to the baked goods.
- Lactic acid bacteria act in terms of degradation of antinutritional compounds.
- These actions improve the texture, the flavor compounds, and the shelf life of the baked goods.

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CHAPTER 36

Apple Pomace (By-Product of Fruit Juice Industry) as a Flour Fortification Strategy

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

AK Ambari Kashmiri
DDAP Drum-dried apple pomace
DPPH 1,1-Diphenyl-2-picrylhydrazyl
FBC Fat binding capacity
FDAP Freeze-dried apple pomace
GD Golden Delicious
GS Granny Smith
HPLC High-performance liquid chromatography
IDF Insoluble dietary fiber

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L Liberty
OH Hydroxyl
RD Red Delicious
RG Royal Gala
SDF Soluble dietary fiber
WA Wine Asp
WHC Water hydration capacity

INTRODUCTION

Apples, with 7500 varieties, are grown mainly in China, the United States, Turkey, Poland, and Italy due to their temperate climatic conditions. Apples are usually processed into products such as juice and cider, dried or frozen, and canned as fresh slices and cubes, baby foods, apple butter, or jelly and vinegar. Pomace is the solid remains of any fruit, such as apples, grapes, and olives, after pressing for juice or oil, which mainly contain the skin, pulp, seeds, and stalk of the fruit. Apple pomace is the major by-product of the apple juice industry, representing 25%, whereas approximately 75% of the fruit weight is extracted as juice.

By-product utilization in industry

Conventionally, apple pomace is used as animal feed. It contains a larger amount of moisture, allowing it to ferment faster and thus causing serious problems for its disposal. Jewell and Cummings (1984) studied the feasibility of producing biogas by anaerobically digesting the pomace, wherein approximately 80% of the organic matter present was successfully converted into a substitute for natural gas having an energy value of 110–530 kcal per metric ton. Also, operating a full-scale bio-drying system was achieved on low energy consumption as an alternative to recover dry and biologically stabilized solids in less than 5 days. Molasses, a sugar industry by-product, is the only substrate for the production of baker's yeast. Because of the presence of a higher amount of fermentable sugars and growth inhibitory compounds in apple pomace, which are absent in molasses, it is an alternative source or suitable substrate for the production of baker's yeast (Joshi and Shashi Bhushan, 2003). Extraction of 10–15% of pectin (dry weight basis) from apple has an effect on the yield and quality of pectin (May, 1990). Several studies have been carried out on the extraction process. Wang *et al.* (2007) optimized the use of the microwave for extraction of pectin from dried apple pomace, wherein 0.315 g of pectin was extracted from 2 g of dried pomace in 21 min.

APPLE POMACE AS A DIETARY FORTIFICANT

Apple pomace, a dietary-rich ingredient, is considered as a potential food ingredient for food products because of its well-balanced proportion of soluble and insoluble fractions of dietary fiber. Apple pomace has better quality dietary fiber due to the presence of bioactive compounds such as polyphenols, flavonoids, and carotenes. Concentrates from apple pomace as such and after processing have been evaluated for their functional properties.

Particle size

The particle size of pomace has a distinct effect on surface properties, which depend on the variety of apple used, storage conditions, and pomace preparation involved. Dried apple pomace ground in a hammer mill was passed through 30- and 50-mesh sieves and characterized. With a decrease in particle size, water holding capacity (WHC) and fat binding capacity (FBC) decreased, whereas the emulsifying activity and emulsifying stability increased significantly (Figure 36.1). The whipping properties as indicated by foam expansion increased with a decrease in particle size, whereas the foam stability was unaffected by particle size, indicating that the pomace can function better in the preparation of products in which foam stability is required instead of eggs (Grover *et al.*, 2003). The blends prepared using wheat flour and

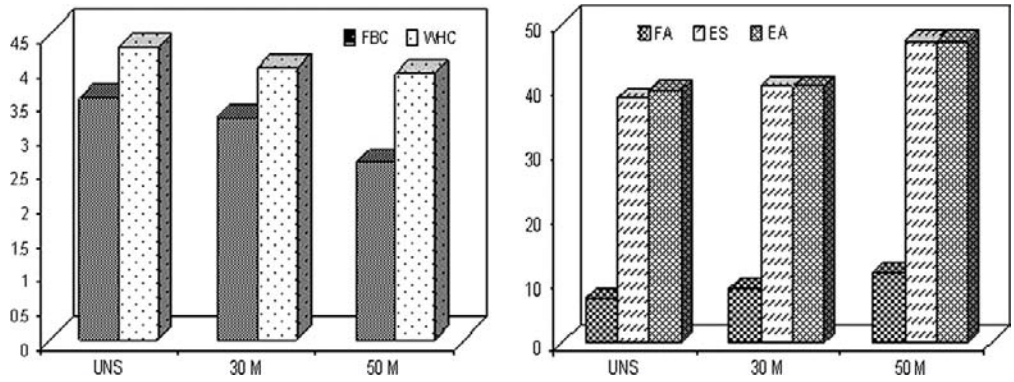

FIGURE 36.1
Effect of particle size on the properties of apple pomace. With a decrease in particle size, water holding capacity (WHC) and fat binding capacity (FBC) decreased, whereas the emulsifying activity (EA) and emulsifying stability (ES) increased significantly. FA, foam ability; UNS, unsieved. *Source: Masoodi* et al. *(2001).*

pomace of varying particle size at different levels showed an increase in viscosity with decreasing particle size. The addition of apple fiber and cellulose on wheat gluten showed that with an increase in the apple fiber concentration, the WHC did not increase linearly, indicating that there may be an interaction between fibrous materials and gluten (Chen, Rubenthaler, and Schanu, 1988). The water sorption isotherms of spray-dried apple, wheat, and oat brans showed that apple fiber was more hygroscopic, which could be due to either the structural difference of cell wall materials between bran of grain and fruit fiber or the lower fiber content in cereal brans, or the smaller particle size of apple fiber, thus indicating that it can function as a humectant (Chen, Rubenthaler, Leung, *et al.*, 1988).

PROXIMATE COMPOSITION

Freshly pressed apple pomace has a pH of approximately 4.1, and its physical characteristics change even when stored in refrigerated conditions (8°C), during which the pH decreases to 3.0. Pomace of Tennessee-grown cultivars such as Golden Delicious (GD), Red Delicious (RD), and Wine Asp (WA) are composed of 70–75% flesh, 2–3.3% seeds, and 0.4–1% stalk. The color of the pomace differs with the cultivar and is important when used as an ingredient in foods. GD had the highest *L* value (lightness) and least red (lowest *a*) value, whereas WA was darkest and deep red. There was slight variation in the yellowness (*b* value) among the cultivars. The *a*/*b* ratios showed that GD was most yellow, whereas WA was deep red (Carson *et al.*, 1994). The apple pomace from GD, RD, WA, Ambari Kashmiri (AK; Southern Citrus Products, Gudur, India), Royal Gala (RG), Granny Smith (GS), and Liberty (L) cultivars of Chile was chemically characterized. The ash content (Table 36.1) in the AK and L varieties was similar, whereas varieties GD and RD had slightly higher ash content (5.5–6.7%) compared to that of RG and GS (1.24–1.88%). The lipid content of the ether extract was in the range of 1.00–4.46%, whereas the crude protein content was in the range of 1.1–3.68% (Carson *et al.*, 1994; Figuerola *et al.*, 2005; Sudha *et al.*, 2007).

DIETARY FIBER

Dietary fiber, also called roughage, is the indigestible component, which plays an important role in nutrition because of its beneficial physiological effects. Cereal fibers have more insoluble dietary fiber (IDF), whereas fibers from fruit and vegetables have a high amount of soluble dietary fiber (SDF). They show some functional properties, such as WHC and FBC, due to the porous structure formed by polysaccharides, which can hold water through hydrogen bonds (Dawkins *et al.*, 2001). The TDF for GD, RD, and WA varieties was in the range of

TABLE 36.1 Proximate Composition of Apple Pomace from Different Varieties[a]

Variety	WHC (g H_2O/g Solid)	Ash (%)	Protein (%)	Ether Extract (%)	TDF (g/100 g)	SDF (g/100 g)	IDF (g/100 g)	SDF:IDF
GD	3.38	5.50	1.90	1.00	35.50 ± 1.50	03.00 ± 0.30	32.50 ± 1.70	1:10.80
RD	3.96	6.70	1.10	2.50	41.10 ± 0.63	03.10 ± 0.50	38.30 ± 0.10	1:12.35
WA	3.38	4.80	2.20	1.10	33.40 ± 0.50	03.50 ± 0.10	29.90 ± 0.40	1:8.54
AK	8.39	0.50	2.06	2.70	51.10 ± 1.86	14.60 ± 0.14	36.50 ± 1.14	1:2.50
RG	1.62	1.88	3.12	1.57	78.20 ± 0.60	14.33 ± 0.63	63.90 ± 0.16	1:4.45
GS	1.78	1.24	3.68	4.46	60.70 ± 0.23	04.14 ± 0.21	56.50 ± 0.20	1:13.64
L	1.87	0.56	3.64	2.44	89.80 ± 0.24	08.20 ± 0.15	81.60 ± 0.23	1:9.95

AK, Ambari Kashmiri; GD, Golden Delicious; GS, Granny Smith; IDF, insoluble dietary fiber; L, Liberty; RD, Red Delicious; RG, Royal Gala; SDF, soluble dietary fiber; TDF, total dietary fiber; WA, Wine Asp.
Source: Data from Carson *et al.* (1994), Figuerola *et al*. (2005), and Sudha *et al*. (2007).
[a]*Apple pomace has a high water retention capacity. It is low in fat and protein but rich in dietary fiber.*

33.4–41.1%. Variety AK had a TDF of 51.1%, whereas the varieties of Chile had higher TDF of 60.7–89.8% (Table 36.2). The SDF for varieties GD, RD, and WA was similar, and AK and RG had the highest SDF among all the varieties. However, the ratio of SDF to IDF is important for dietary and functional properties. Fiber, with an SDF:IDF ratio close to 1:2, is generally accepted as a food ingredient (Schneeman, 1987).

Functional properties

The WHC or hydration properties of dietary fiber refer to its ability to retain water within its matrix. The WHC (see Figure 36.1) was approximately 8.39 g water/g solid for variety AK, whereas varieties RG, GS, and L had WHC in the range of 1.6–1.9 g water/g solid and GD, RD, and WA varieties had similar WHC in the range of 3.3–3.9 g water/g solid (Carson *et al.*, 1994; Figuerola *et al.*, 2005; Sudha *et al.*, 2007). Higher WHC helps to increase the stool weight and potentially slows the rate of nutrient absorption from the intestine (Gallaher and Schneeman, 2001) and enhances the viscosity of added food. The low WHC in varieties RG, GS, and L was attributed to the higher ionic strength of phosphate buffer solution in comparison with either distilled water or tap water. Swelling capacity, which is related to the amount of insoluble fiber present, was in the range of 6.6–8.3 ml water/g. The FBC was quite low (0.6–1.45 g oil/g). These results suggest the effectiveness of fortifying with apple pomace in the development of food products rich in dietary fiber and in low-calorie foods.

TABLE 36.2 Fiber Components of Processed Apple Pomace[a]

Component	Apple Pomace	DDAP	FDAP
Cellulose (%)	31.00	16.44	16.67
Lignin (%)	15.00	8.87	8.44
Hemicellulose (%)	12.00	4.09	4.69
Pectin (%)	9.00	3.91	6.09

Source: Data compiled from Chen, Rubenthaler, Leung, *et al.* (1988) and Wang and Thomas (1989).
[a]*On a dry weight basis. Cellulose was the major component of fiber from apple pomace. Drum drying or freeze drying of pomace showed no significant difference for hemicellulose, cellulose, and lignin contents. Pectin was significantly lower in drum-dried apple pomace (DDAP) than in freeze-dried apple pomace (FDAP), which was due to the heat degradation of pectin during the drying process.*

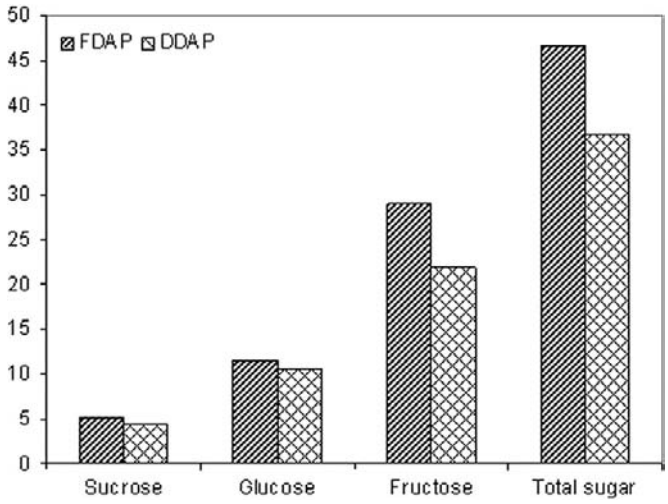

FIGURE 36.2
Effect of drying process on the sugar content of apple pomace. Sucrose and glucose were marginally lower in drum-dried apple pomace (DDAP; $p < 0.05$). Fructose was significantly lower in DDAP than in freeze-dried apple pomace (FDAP; $p < 0.05$) and was lost to a greater extent due to the higher temperature involved in the drum drying process compared to glucose and sucrose. *Source: Wang and Thomas (1989).*

Fiber components

Apple pomace on mild alkaline degradation yielded an α-cellulosic fraction of approximately 26%, water dispersible uronide fraction of 10–18%, which was extractable; using various aqueous solvents, this resulted in an approximately 44% yield. These fibers had differential viscometric behavior and were capable of providing a high concentration of solid matter to an aqueous food system without altering the viscosity of the system (Walter *et al.*, 1998). Cellulose was the major component of the different pomaces studied for their components (see Table 36.2). In apple fiber, water-soluble hemicelluloses comprised approximately 19.2%, and lignin, which is water-soluble fiber, comprised 15.3% (Chen, Rubenthaler, Leung, *et al.*, 1988). Drying of apple pomace either by drum drying (DDAP) or freeze drying (FDAP) showed no significant difference ($p < 0.05$) for hemicellulose, cellulose, and lignin contents. Pectin was significantly lower ($p < 0.05$) in DDAP (3.91%) than in FDAP (6.09%), which was due to the heat degradation of pectin taking place during the drum drying process (Wang and Thomas, 1989). Analysis of sugar (Figure 36.2) determined by high-pressure liquid chromatography (HPLC) and detected by Waters differential refractive index detector showed that sucrose and glucose were marginally lower in DDAP ($p < 0.05$). Fructose was significantly lower in DDAP than in FDAP ($p < 0.05$). Compared to glucose and sucrose, fructose was lost to a greater extent due to the higher temperature involved in the drum drying process. Thus, sugar content in the formulation of confectionery products could be reduced by incorporation of apple pomace.

POLYPHENOLS

Apple polyphenols have been investigated mainly for their physiological and physical properties, which cause problems during juice processing and oxidation that causes browning of apples. There has been interest in natural food polyphenols because of their potential to function as free radical scavengers.

Antioxidant activity

Natural antioxidants from fresh fruits are more effective than dietary supplements. Fresh apple extracts had antioxidant activity equivalent to 1500 mg of vitamin C and were seen to inhibit the

growth of colon and liver cancer cells *in vitro*. Upon acetone extraction, the RD variety gave 290 and 219 mg of phenolics and 142 and 97 mg of flavonoids from 100 g of apples with and without skin, respectively, indicating more phenolic compounds in skin than in pulp. The total antioxidant activity measured as total oxyradical scavenging capacity was higher in apples with skin than in apples without skin. The antioxidant value from 100 g of apples was equivalent to 1500 mg of vitamin C (Eberhardt *et al.*, 2000). The total phenolics was 7.24 g/kg, followed by dihydrochalcone glycosides (1.86 g/kg), catechins (0.64 g/kg), and cinnamic acids (0.28 g/kg) (Lu and Foo, 1997). Phenolic compounds from GD, RD, and GS varieties determined by HPLC with diode array detection showed higher amounts of phenolic compounds such as catechins and flavonol glycosides in apple peel than in pulp, and the recovery of phenolics ranged between 95 and 105% irrespective of the variety of apples used (Escarpa and Gonzáles, 1998). Recovery of pectin and phenolic compounds was carried out simultaneously using HPLC. Phloridzin, chlorogenic acid, and quercetin glycosides were the compounds isolated (Schieber *et al.*, 2003). Scavenging activities on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl (OH) radicals were measured by obtaining the erythrocyte sedimentation rate and the activity was stable on DDPH and OH radicals (Gordana *et al.*, 2008). In view of the radical scavenging activity, apple pomace would be beneficial in preventing unwanted free radical-induced oxidative reactions and thus function as a value-added ingredient for functional foods. Polyphenolic compounds such as phloridzin, 3-hydroxy phloridzin, chlorogenic acid, epicatechin, and quercetin glycoside were evaluated by antioxidant activity of the β-carotene/linoleic acid system. These compounds showed 2 or 3 times the DPPH scavenging activities and 10—30 times the scavenging activities of vitamin C or E (Lu and Foo, 2000).

RHEOLOGICAL CHARACTERISTICS

Dough characteristics

Farinograph water absorption increased from 60.1 to 70.6% (Figure 36.3) when apple pomace was incorporated at 0 and 15% (Sudha *et al.*, 2007). Chen, Rubenthaler, and Schanu (1988) reported an increase in water absorption determined using 10-g mixograph from 66.5 to 72.5% when apple pomace was incorporated at 0 and 12%. The farinograph water absorption of blends containing 30-mesh apple pomace increased from 59.1 to 69.4% with an increase in pomace content from 0 to 11%. With reduction in particle size (50 and 60 mesh), the water absorption also increased to 70.2% upon increase in the addition of pomace content in the blend. The increase in water absorption was attributed to the increase in surface area of pomace, which would hold more water (Masoodi *et al.*, 2001). Dough development time, which indicates the rate of hydration and development of gluten, increased from 1.5 to 3.5 min with an increase in pomace content from 0 to 15%. The increase was predominant in

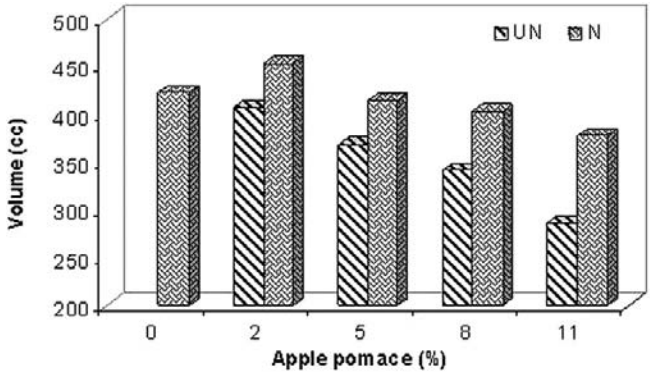

FIGURE 36.3
Effect of apple pomace on bread volume. The loaf volume of breads with neutralized fiber (N) was comparatively higher than that of breads containing unneutralized fiber (UN). *Source: Masoodi and Chauhan (1998).*

blends containing pomace of larger particle size, indicating that the increase in fiber content in the blends slowed the rate of hydration and development of gluten. Dough stability decreased and mixing tolerance index value increased irrespective of the particle size, indicating higher resistance to weakening of dough during mixing (Masoodi *et al.*, 2001; Sudha *et al.*, 2007). Chen, Rubenthaler, and Schanu (1988) reported that due to the interaction between fibrous material and gluten, which strengthens the gluten, fibrils were probably bound with the gluten, thus affecting the dough mixing properties. The elastic properties of the dough measured by extensograph showed an increase in resistance value from 336 to 742 BU and decrease in extensibility values from 127 to 51 mm when apple pomace constant increased from 0 to 15% in the blends (Sudha *et al.*, 2007). The dough became stiff at 15% levels, which may be due to dilution of gluten proteins or interactions between polysaccharides and proteins as reported by Chen, Rubenthaler, and Schanu (1988). These results indicated that higher energy was required for dough mixing and more time was required for dough development.

Pasting characteristics

The pasting characteristics of wheat flour—apple pomace blends as measured by viscoamylograph showed a marginal increase in pasting temperature from 60 to 63°C (see Figure 36.3). The difference in pasting temperature was due to the effect of varying gelation temperatures on the fiber fractions. Peak viscosity decreased from 950 to 730 BU with increase in the apple pomace from 0 to 15%, indicating that the swelling power, which is the ability of the starch granules to swell freely before their breakdown, decreased (Sudha *et al.*, 2007). Masoodi *et al.* (2001) observed that an increase in pomace initially decreased the peak viscosity values, and further increases in apple pomace content decreased the peak viscosity values. At the 5% pomace level, the peak viscosity values were 510, 495, and 480 BU for blends with 30-, 50-, and 60-,mesh pomace, and the values at 11% were 640, 645, and 695 BU, respectively. The initial decrease in peak viscosity was attributed to dilution of starch and gluten, whereas at higher levels it was attributed to the gelling effect of pectin present in pomace. The hot paste viscosity or the viscosity at 95°C showed a similar trend and was higher at higher levels of pomace, which was again due to the pectin participating in gel formation, and the increase in fineness resulted in more binding of water. The breakdown values increased from 90 to 240 BU with increase in pomace from 0 to 15%. Higher breakdown values at higher concentration of pomace indicated the interaction of fiber with starch and thus made the starch granules more fragile. Cold paste viscosity decreased from 1760 to 970 BU, and setback values decreased from 810 to 240 BU (Sudha *et al.*, 2007).

BAKERY PRODUCTS

Bread

Chen, Rubenthaler, Leung, *et al.* (1988) prepared bread from hard wheat flour and partially replaced it with dry apple fiber and hydrated apple fiber (hydration 1:7 for 12 h). As the concentration of fiber increased, water absorption, mixing time, and bread weight increased, whereas the loaf volume decreased. The increase in water absorption was due to the strong water binding ability of fibers. On the other hand, the addition of hydrated apple fiber had a less deleterious effect on the bread making quality. When dry fiber was used, the dough was slack in the early mixing stages and on prehydration of apple fiber provided less competition for water between fiber and dough components than are activated by water during dough development. Apple pomace, as such (unneutralized) and neutralized, was used at 0, 2, 5, 8, and 11% in the preparation of bread. Neutralization did not show any significant effect on water absorption. The loaf volume decreased from 525 to 300 and 385 cc for the bread containing unneutralized fiber and neutralized fiber, respectively (see Figure 36.3). Bread weight increased due to retention of moisture, and breads were softer with neutralized pomace (Table 36.3). Bread containing 5% apple pomace was acceptable (Masoodi and Chauhan, 1998).

TABLE 36.3 Effect of Apple Pomace on Quality Characteristics of Bread[a]

Pomace (%)	Loaf Weight (g)	Bread Firmness (N)	Total Sensory Score (100)
0	141.0	3.0	86.1
Unneutralized			
2	146.0	8.3	79.6
4	147.2	10.2	68.3
8	148.4	11.1	58.0
11	151.0	12.0	50.8
Neutralized			
2	142.0	6.3	76.1
4	143.4	7.8	70.4
8	144.1	9.2	59.4
11	145.5	10.0	52.1

Source: Data from Masoodi and Chauhan (1998).
[a]*Neutralization did not show any significant effect on water absorption. Bread weight increased due to retention of moisture, and breads were softer with neutralized pomace.*

Cookie and pie filling

Oatmeal cookies with apple pomace as an ingredient were prepared. The cookies showed a reduction in diameter by 23%, whereas the thickness increased to approximately 116% with increase in the level of apple fiber (0–12%). The WHC of apple fiber rendered the cookie dough drier in appearance; as a result, the cookie did not spread during baking. Apple fiber at 4% replacement level, which did not adversely affect the cookie quality, was advisable (Chen, Rubenthaler, Leung, *et al.*, 1988). Moon cookies were prepared by substituting 40% (w/w) of flour with flaked apple pomace (DDAP) in the crust and 40% (w/w) of the quick cooking oats in the filling. Sugar contributed by the pomace was taken into consideration, and accordingly sugar in crust and filling was reduced 36.7% (w/w) from the control cookie formulation. The color and appearance of the moon cookie were judged to be better than those of the control cookie (Wang and Thomas, 1989).

Carson *et al.* (1994) used unrefined apple pomace from three cultivars in pie filling and oatmeal cookies. The dimensions of the cookies did not change during baking when formulated with different levels of apple pomace. The sensory scores were similar for cookies with the three levels of pomace. There were differences for flavor ($p < 0.05$) and for all other attributes ($p < 0.01$). Cookies were moderately liked (Carson *et al.*, 1994). There was no color difference between the pie fillings with two levels of pomace of different cultivars, which was measured and reported as Hunter values *L*, *a*, and *b*. There was no difference in sensory scores, and the cookies were moderately liked. Pomace causes white products to darken upon baking, but it can be used in dark products.

Muffins

Apple fiber was used in the muffin formulation on a flour replacement basis. The muffins were baked at 196°C. With increase in the apple fiber concentration, the densities of muffins increased (0.452–0.580 g/cm^3). Muffins prepared with apple pomace at 4% replacement level were acceptable. Because the process of drum drying is more economical than freeze drying, DDAP would be a cheaper source of dietary fiber in the preparation of bakery products. Due to the presence of high dietary fiber and fruit sugar, it can serve as an alternative source of dietary fiber and is an option for reducing the external sugars in the formulation (Wang and Thomas, 1989).

Cakes

Apple pomace and pomace of different particle sizes (sieved through 30, 50, and 60 mesh) were used in the preparation of cake (Masoodi *et al.*, 2002). Batter viscosity increased with

TABLE 36.4 Nutritional Composition of Bakery Products Containing Apple Pomace[a]

Parameter	Moon Cookies		Muffins		Cakes	
	Control	Experimental	Control[b]	Experimental	Control	Experimental
Fat (%)	22.4	23.2	8.4	8.4	19.3	20.50
Protein (%)	11.2	10.0	6.6	5.9	8.5	8.46
Carbohydrates (%)	57.2	46.8	29.7	30.8	—	—
TDF (%)[b]	11.6	18.4	17.9	18.7	0.47	14.20

TDF, total dietary fiber.
Source: Data from Wang and Thomas (1989) and Sudha *et al.* (2007).
[a]*Bakery products incorporated with apple pomace have higher dietary fiber content compared to products without pomace.*
[b]*Contained wheat bran.*

increase in apple pomace content, and also with reduction in particle size, a significant increase ($p < 0.05$) was observed, which was due to the WHC of the fiber. The weight of the cakes increased significantly with increase in pomace content, which is due to the higher retention of moisture by pomace. Finer particle size had higher volumes. Volume index decreased. The uniformity index, which relates to the symmetry of the cake, increased. The volume of cakes decreased from 850 to 620 cc with increase in pomace level from 0 to 30%, and the density of the cakes increased from 0.48 to 0.67 g/cc. This increase in density may be due to the strong water binding properties of apple fiber. The texture of cakes measured objectively showed that cakes became harder, as indicated by the increase in the values from 1.03 to 1.46 kg force. Sensory evaluation scores were low for crumb color and grain because the cells were compact and dense. The creamish yellow color of the control cake changed to brown with pomace content. However, with increase in the pomace levels, cakes had a pleasant fruity flavor. Cakes with 20% replacement had acceptable quality (Sudha *et al.*, 2007). Apple pomace can thus be a natural colorant and flavorant in the preparation of cakes, thus avoiding the use of artificial sources of colorant and flavorant.

NUTRITIONAL BENEFITS

Moon cookies prepared by replacing 62.5 g of all-purpose flour in the crust and 26.7 g of quick-cooking oats in the filling with apple pomace contained 1.7 g more TDF, 2.3 g less carbohydrates, and 10 fewer calories than the control cookies (Table 36.4). Similarly, the fiber content in experimental muffins, in which 52.5 g of wheat bran was replaced with an equal amount of apple pomace, was 7.12% compared to 6.8% in control muffins (Wang and Thomas, 1989). The moisture content in pomace-incorporated cakes (25%) was marginally higher, whereas there was no significant change in total fat content and protein content values. However, the TDF content was significantly higher at 14.2% for cakes prepared by substituting 25% of wheat flour with apple pomace compared to 0.47% for control. The IDF (8.4%) and SDF (5.8%) were significantly higher than that of the control cakes (0.31 and 0.16%). The total phenolics, which was 10 times higher than that of the wheat flour, showed that incorporation of 25% of apple pomace increased both the water and methanol extract phenols compared to those of control cake (Sudha *et al.*, 2007).

SUMMARY POINTS

- Apple pomace represents approximately 25–30% of fruit and is the by-product of the apple juice processing industry.
- Apple pomace has been found to be a better substrate in the production of baker's yeast.
- Apple fiber can be a source of dietary fiber in the preparation of bakery products.
- Because recovery of phenolic compounds in peel and pulp is in the range of 90–95%, apple fiber can be a source of polyphenols.

- The antioxidant property of pomace can function as a natural substitute for synthetic antioxidants.
- Drum drying can be avoided during processing of pomace.
- Particle size of apple pomace affects surface properties such as hydration capacity, fat absorption, and emulsifying properties.
- Apple pomace of finer particle size performed better than that of larger particle size in relation to cake making.
- Neutralization of pomace improved the loaf volume of bread.
- Because apple pomace has a pleasant fruity flavor and brownish color, the addition of an artificial flavoring agent and a synthetic color can be avoided.
- Cakes prepared with apple pomace had higher dietary fiber and phenolic contents than the control product; hence, apple pomace has potential to be a good source of dietary fiber and polyphenols.

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CHAPTER 37

Use of Sweet Potato in Bread and Flour Fortification

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

DPPH 2,2-Diphenyl-1-picrylhydrazyl
RVA Rapid visco analysis

INTRODUCTION

Sweet potato (*Ipomoea batatas Lam.*), grown in tropical and subtropical regions, is a root crop. Asia and Africa produce 95% of the total world output. It is grown and consumed mostly in developing countries, and it is the world's fifth largest food crop. It contains abundant nutritional substances and could become an important food resource to solve the problem of food shortages caused by desertification and population growth (Ishida *et al.*, 2000; Rumbaoa *et al.*, 2009).

Sweet potato quality is an integrated and complicated concept constituted by a number of factors. The demand for different levels of quality of sweet potatoes varies because they are used for different purposes. However, with regard to production and utilization, sweet potato

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TABLE 37.1 Chemical Composition of Three Varieties of Chinese Sweet Potato Roots (w/w, %)[a]

Source	Moisture	Dry Matter	Starch	Protein	Lipid	Ash	Fiber
Xushu18	68.1 ± 1.92	31.9	21.9 ± 1.35 (68.5)	1.6 ± 0.05 (4.9)	0.5 ± 0.03 (1.5)	0.7 ± 0.04 (2.2)	0.6 ± 0.04 (1.9)
Sushu2	63.3 ± 1.21	36.7	27.8 ± 1.47 (75.9)	2.0 ± 0.08 (5.5)	0.4 ± 0.03 (1.0)	0.9 ± 0.07 (2.5)	1.0 ± 0.10 (2.7)
Sushu8	81.4 ± 1.78	18.6	10.7 ± 0.92 (57.8)	1.0 ± 0.10 (5.5)	0.3 ± 0.03 (1.5)	0.6 ± 0.04 (3.1)	0.8 ± 0.05 (4.4)

Source: Zhenghong (2003).
[a]*Values based on dry weight are given in parentheses.*

quality mainly includes nutritional quality, edible quality, and processing quality. These quality indicators are affected by both genotype and environment. Because different genotypes and environmental conditions result in different quality, the nutritional and health values and processing suitability of genotypes grown in different localities vary (Guoquan, 2003). The nutritional composition of three common varieties of Chinese sweet potato is listed in Table 37.1.

Fresh sweet potato is prone to water loss, decay, bud growth, sensory quality changes, and so on, which substantially reduce its shelf life (Rees *et al.*, 2003). However, the processing of sweet potato into a powder not only prolongs the shelf life but also improves the nutritional status of the specialty bread made from sweet potato powder, which acts as a substitute for wheat flour (Hathorn *et al.*, 2008). Preliminary experiments showed that waste liquid produced as a by-product of starch extraction contained approximately 1.5% crude protein, which could serve as a rich source of protein to be utilized.

When added to various food products, spray-dried sweet potato powder has the potential to provide additional functional benefits. Sweet potatoes are rich in carbohydrate, β-carotene, ascorbic acid, and minerals. The powder can enhance natural color and flavor, and it acts as a thickening ingredient, like pregelatinized starch, in food systems. However, with heat treatment, β-carotene, ascorbic acid, and many other nutrients are broken down. Changes in the nature of carbohydrates also occur at high temperatures. For instance, starch is converted to dextrin and sugar by intrinsic amylase. These chemical conversions also change their functions and, as such, make the rheology of reconstituted powder solutions different. Thus, maintaining their form and function in the process of drying and subsequent storage requires further investigation (Grabowski, 2008).

SWEET POTATO PIGMENTS AND THEIR PHYSIOLOGICAL FUNCTIONS

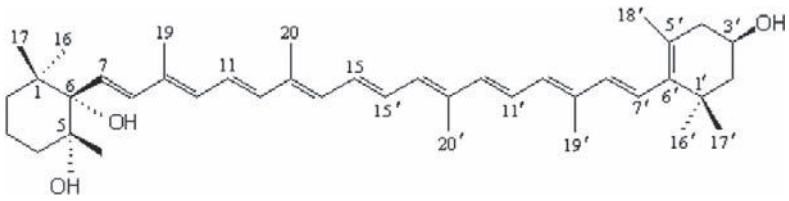
Carotenoids in sweet potato

Different varieties of sweet potato storage roots have a variety of flesh colors, such as white, yellow, orange, and purple. The light flesh color is mainly formed by carotenoids such as β-carotene (Kimura *et al.*, 2006) and luteochrome (de Almeida *et al.*, 1986).

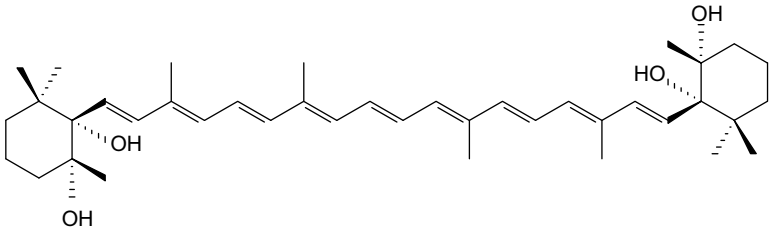
Maoka *et al.* (2007) analyzed the carotenoids of the yellow-fleshed sweet potato variety "Benimasari." The results showed that the total carotenoid content of freeze-dried sweet potato flesh was 3.1 mg/100 g. Seven kinds of known carotenoids were found after separation by analytical high-performance liquid chromatography: β-carotene (10% of the total carotenoid), β-carotene-5,8- epoxide (6.5%), β-carotene-5,8,5′,8′-diepoxide (40.5%), β-cryptox-anthin-5′, 8′-epoxide (10.5%), β-cryptoxanthin- 5,8;5′, 8′-diepoxide (5.5%), auroxanthin (2.2%), and neochrome (4.5%). In addition, four kinds of unknown carotenoids with the 5,6-dihydro-5, 6-dihydroxy-β end group were discovered and named ipomoeaxanthins: ipomoeaxanthin A

(1) (3.2%), ipomoeaxanthin B (2) (0.5%), ipomoeaxanthin C1 (3) (2.5%), and ipomoeaxanthin C2 (4) (2.5%). The structures of these four recently discovered carotenoids are shown in Figure 37.1.

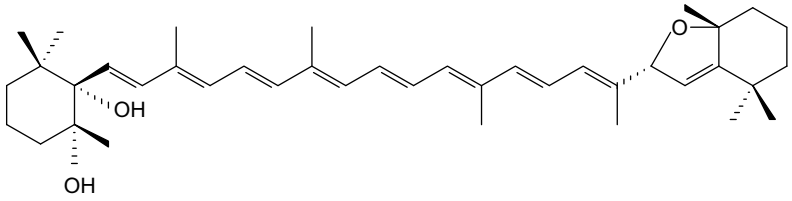
β-Carotenoid is the precursor of vitamin A, the content of which is the most abundant in the orange-flesh and yellow-flesh sweet potatoes. Vitamin A in these varieties was mainly transformed from β-carotene (Rodriguez-Amaya, 1997). People are accustomed to eating sweet potatoes after heat treatments such as boiling and baking. During such processing, in addition to β-carotene degradation, *trans*−*cis* isomerization may also lead to reduced levels of vitamin A. This is because in normal circumstances, the vitamin A activity of *cis*-β-carotene is weaker than that of the *trans*-isomer (Kidmose *et al.*, 2007).



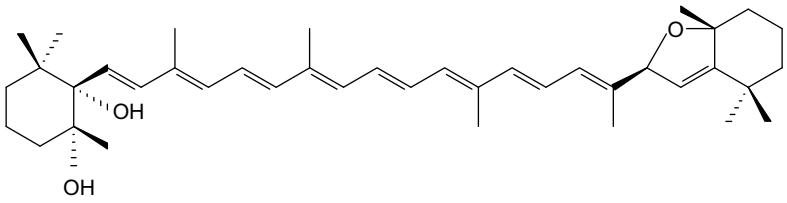
Ipomoeaxanthin A(1)



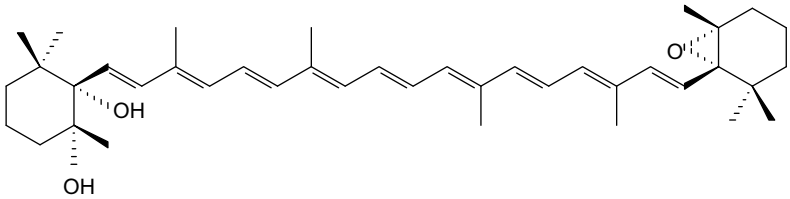
Ipomoeaxanthin B(2)



Ipomoeaxanthin C1(3)



Ipomoeaxanthin C2(4)



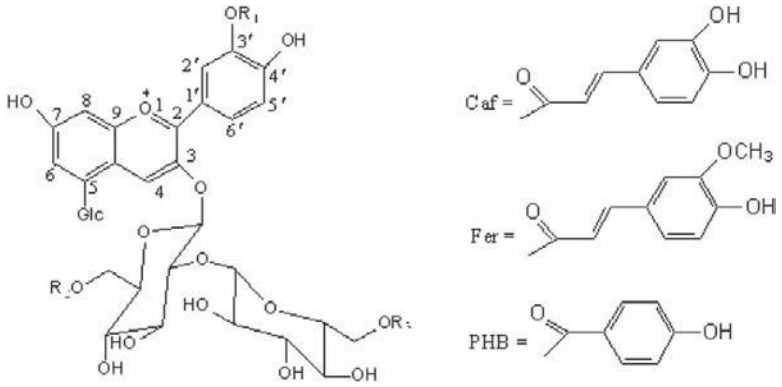
5,6-Dihydroxy-β-carotene-5′,6′-epoxide(5)

FIGURE 37.1
Structures of recently discovered carotenoids. *Source: Maoka* et al. *(2007)*

Anthocyanins of sweet potato

Sweet potato is rich in antioxidants such as polyphenols. For example, the phenolic content of American sweet potato ranged from 0.14 to 0.51 mg chlorogenic acid equivalent/g fresh weight after the storage root was peeled. Chlorogenic acid and isochlorogenic acid are main components in polyphenols of American sweet potato (Walter *et al.*, 1979). Japanese purple sweet potato contains approximately 0.4–0.6 mg anthocyanin/g fresh weigh. For different sweet potato varieties, the antioxidant capacity may vary widely with change of the flesh color from white or yellow to orange and even purple.

In recent years, several reports have indicated that the phytochemicals in sweet potatoes display antioxidative or radical scavenging activities (Konczak-Islam *et al.*, 2003; Rabah *et al.*, 2004; Suda *et al.*, 2003). A red-fleshed sweet potato cultivar grown in the Andean region has been reported to have higher antioxidant activity and phenolic content than a cultivar of blueberry fruit that was thought to be rich in antioxidants (Cevallos-Casals *et al.*, 2003). Kano *et al.* (2005) reported that anthocyanins from a crude extract of the storage roots of purple sweet potato (Figure 37.2) showed stronger radical scavenging activity than anthocyanins from red cabbage, grape skin, elderberry, and purple corn (Table 37.2).



Ingredient	R1	R2	R3	Abbreviation	m/z
A	H	Caf	H	Cy-CafSop-Glc	935
B	H	Caf	Caf	Cy-diCafSop-Glc	1097
C	H	Caf	PHB	Cy-CafPHBSop-Glc	1055
D	Me	Caf	H	Pn-CafFerSop-Glc	949
E	H	Caf	Fer	Cy-CafFerSop-Glc	1111
F	Me	Caf	Caf	Pn-diCafSop-Glc	1111
G	Me	Caf	PHB	Pn-CafPHBSop-Glc	1069
H	Me	Caf	Fer	Pn-CafFerSop-Glc	1125

FIGURE 37.2
Chemical structures of anthocyanins in purple sweet potato extract. Caf, (E)-cafferic acid; Cy, cyaniding; Fer, (E)-ferulic acid; Glc, glucopyranoside; Me, methyl; PHB, *p*-hydroxybenzoic acid; Pn, peonidin; Sop, sophoroside. *Source: Kano* et al. *(2005).*

TABLE 37.2 DPPH Radical Scavenging Activity by Commercial Pigments

Pigment	ED_{50} Value (μg/ml)[a]
Grape skin	89.8 ± 1.3[a]
Elderberry	52.2 ± 4.3[b]
Red cabbage	48.2 ± 1.4[b]
Purple corn	35.1 ± 0.9[c]
Ayamurasaki	24.0 ± 0.9[d]

Source: Kano *et al.* (2005).
[a]*Values are mean ± SD. Mean values with different letters are significantly different at* $p < 0.05$ *by Tukey's test.*

STARCH PROPERTIES OF SWEET POTATOES

Granule properties of sweet potato starch

Specialty bread is made from non-wheat flour, whose nutrient content is higher than that of bread from wheat flour. In addition, it has a flavor and color that most common breads do not have. Due to these properties, specialty bread is becoming increasingly popular among consumers (Hathorn *et al.*, 2008). Researchers hold the view that sweet potato is a highly nutritious crop and has the potential to add value to the human food system, but it still needs to be developed, particularly in the specialty bread market (Walter *et al.*, 2002).

Starch is the most important nutrient in sweet potato roots, as well as the core and key to sweet potato quality. Different demands on starch type, content, and quality have dictated various end uses to which flour could be put.

The starch content of sweet potato flour ranged from 36.4 to 79.8%, and its average value was 63.49% (Guoquan, 2003), which indicates a great potential to breed and cultivate high-starch content varieties. In addition, granule properties of sweet potato starch, such as granule size and distribution, are some of the most obvious features affecting the functional properties of starch granules (Guoquan *et al.*, 2006). Starch granule size is closely related to the major quality traits of bread (Sahlström *et al.*, 1998). Amylose content has a significant impact on bread volume and texture. Sweet potato flour amylose content ranged from 8.5 to 17.32%, and its average value was 14.25% (Guoquan, 2003). Hence, varieties with amylose content more than 20% are appropriate for bread when sweet potato flour is used as the raw material.

The proportion of damaged starch granules

Damaged starch refers to partial starchy endosperm cells whose membranes were damaged during milling of the starch raw material.

The requirement proportion of damaged starch is related to the end use of whole flour. If the flour is used for the processing of nonfermented products, the higher the proportion of whole granule starch, the better solubility of starch. However, for the production of bread, a certain amount of damaged starch granules are needed during dough fermentation. Starch is a main source of energy during dough fermentation. If the starch cells are integrated, amylase cannot penetrate the cell and react with the internal starch. Only when the cell is damaged can part of the starch become free so that amylase can act upon it. Therefore, the more the amount of damaged starch granules, the higher the amylase activity. However, if there are too many damaged starch granules, the size and quality of the baked bread will deteriorate. The permissible amount of damaged starch granules is related to the content of flour protein. The optimum permissible amount of damaged starch is in the range of 4.5 to 8%, in accordance with the flour protein content.

Starch thermal and pasting properties

Starch thermal and pasting properties are the most important features. According to rheology, rapid visco analysis (RVA) can be used to observe changes in starch viscosity.

Zhenghong (2003) isolated starches from three typical varieties of Chinese sweet potato (XuShu18, SuShu2, and SuShu8). The pasting behaviors of starch from these sweet potato varieties were characterized and compared to those of starch isolated from potato and mung bean (Figure 37.3). RVA profiles showed the same tendency of pasting temperature and peak viscosity of all the samples. The gelatinization temperature range of the three sweet potato starches was higher than those of potato starch and mung bean starch. The gelatinization temperature and pasting temperature of the three sweet potato starches were slightly higher than those of mung bean starch and much higher than those of potato starch. The peak viscosity of the three sweet potato starches was higher than that of mung bean starch but much lower than that of potato starch.

A comparison of gelatinization parameters of sweet potato starch and of other crops is shown in Table 37.3, which indicates that the peak viscosity, trough viscosity, breakdown viscosity, final viscosity, and setback value of sweet potato starch were all higher than those of wheat flour.

In general, the binding force within the root starch granules is weak and gel temperature is low; consequently, there is consistent swelling capacity. Furthermore, viscosity spectra and paste transparency also have higher values than those of corn starch. However, root starch is prone to aging (Zaidul *et al.*, 2007).

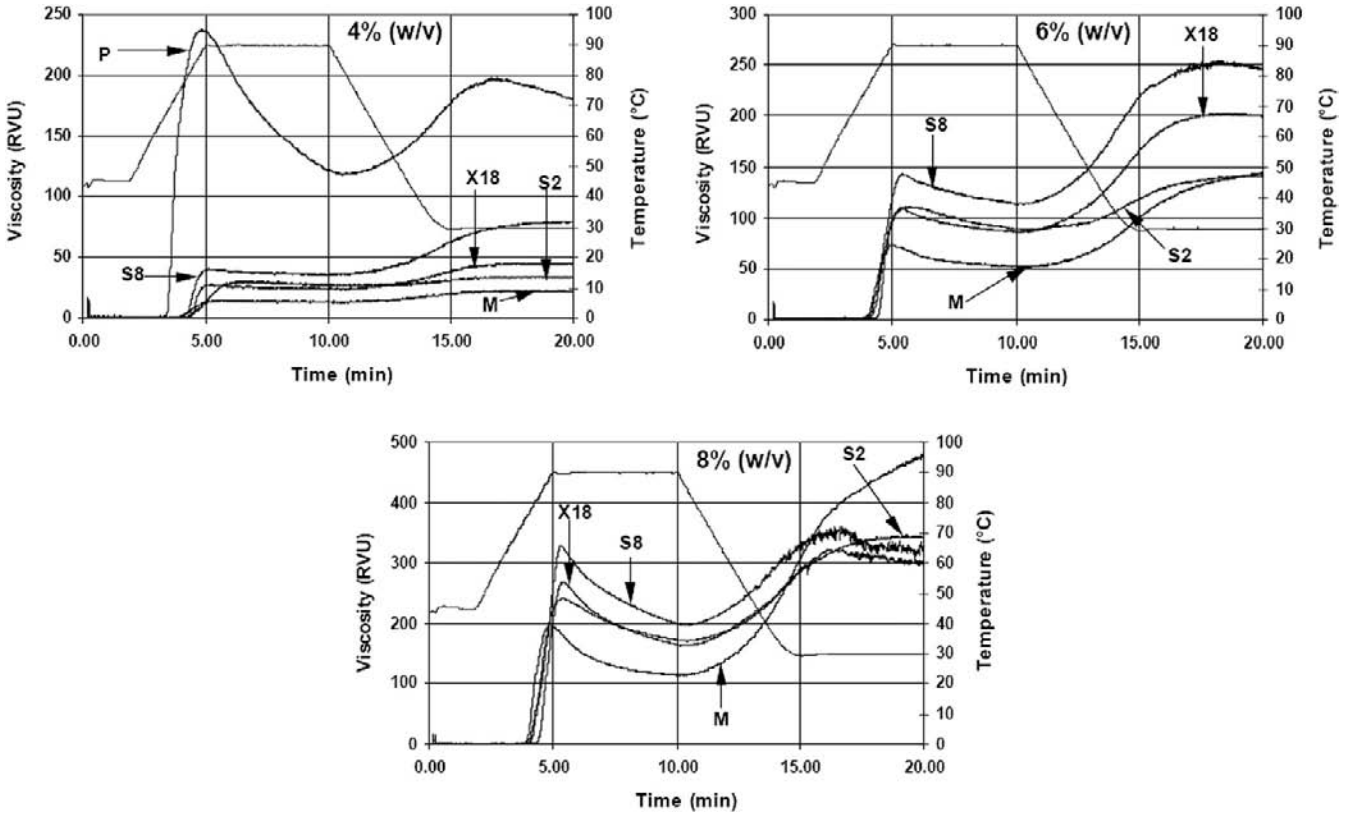


FIGURE 37.3
RVA viscosity profiles of three Chinese sweet potato starches as compared to potato and mung bean starches. M, mung bean; P, potato; S2, SuShu2; S8, SuShu8; X18, XuShu18. *Source: Zhenghong (2003).*

TABLE 37.3 RVA Properties of Control Wheat Flour, Potato Starch, Sweet Potato Starch, Yam Starch, and Cassava Starch[a]

Control Sample	Peak Viscosity	Trough Viscosity	Breakdown Viscosity	Final Viscosity	Setback Viscosity	Peak Time (min)	Pasting Temperature (°C)
Wheat	33.3	21.8	11.5	41.8	20.1	5.0	—
PS	543.9	165.4	378.5	204.8	39.4	3.0	69.1
SPS	132.8	96.8	36.0	139.1	42.3	4.5	81.5
YS	224.1	172.2	51.9	239.1	67.4	4.4	71.1
CS	134.9	75.9	59.0	122.5	46.6	4.3	72.7

CS, cassava starch; PS, potato starch; SPS, sweet potato starch; YS, yam starch.
Source: Zaidul *et al*. (2007).
[a]*RVA parameters are the mean of two determinations of the control samples, where the maximum standard deviation was ±0.08, ±2.62, ±2.14, ±1.88, and ±0.41 for wheat, PS, SPS, YS, and CS, respectively.*

Starch pasting properties are directly related to the content of amylose. Amylose, which limited water movement, reduced the swelling capacity of starch grains by altering the initiation of the amylose—lipid complex, which resulted in inferior quality during dough processing (low peak viscosity, low breakdown viscosity, high minimum viscosity, and high rebound values). Accordingly, it could be inferred that amylose reduced not only water absorption of dough but also the flexibility and extensibility of dough in the process of dough making and bread baking, which ultimately reduced bread volume, structural texture, and palatability. However, the characteristics and the roles of amylopectin and amylose are opposite, and the greater the amylopectin:amylose ratio, the more advantageous the high-gluten food processing quality. The content of amylose in sweet potato starch is lower than that of wheat flour. When sweet potato starch is combined with wheat flour to make composite powders, the pasting properties of wheat flour are optimized and the quality of bread is simultaneously improved. However, Zhenghong (2003) reported that there was no obvious relationship between the peak viscosity and amylose content of the sweet potato starches from different varieties. The differences in the peak viscosities of the different sweet potato starches may partly result from the different phosphorus contents.

Peak viscosity reflects α-amylase activity. High peak viscosity weakens amylase activity. Thus, fermentation performance and the quality of bread are poorly exhibited during the bread making processing. When making noodles, however, a higher peak viscosity value is more desirable. If peak viscosity is too low, the enzyme activity is so severe that the dough will be sticky, which is detrimental to making bread, noodles, and cakes. From this analysis, it can be seen that peak viscosity may appear too high when sweet potato flour is used in the processing of bread. This is regarded as one of the restrictions of sweet potato bread flour processing. When the content of sweet potato starch added to wheat flour gradually increases from 20 to 50%, starch peak time is significantly reduced, whereas the setback value is significantly increased, as is the breakdown viscosity.

Swelling behavior of sweet potato starches

Both single- and two-stage swelling patterns are found for different varieties of sweet potato starches. This is indicative of different mechanisms of interaction forces within the sweet potato starch granules. A first association was relaxed from 65 to 75°C, which was followed by a strong interaction from 80 to 95°C. Swelling power is affected by the extent of chemical cross-bonding within the granules and noncarbohydrate substances such as lipid or phosphate. A high amylose content as well as the presence of higher numbers of stronger intermolecular bonds may also reduce swelling (Zhenghong, 2003).

Effects of activity of sweet potato amylase on bread making

Starch paste is subject to the role of amylase. The lack of amylase activity would make starch gelatinization inadequate and the starch would become too dry and hard, which limits the

appropriate expansion of the dough and makes bread volume and structural texture undesirable. On the contrary, excessive active amylase will make the degree of starch gelatinization too high, which makes it impossible to tolerate the increased pressure, making the small size of the gas cell rupture into the large size of the gas cell and the gas spill. Consequently, the volume of bread becomes smaller and flesh becomes tacky.

Sweet potato roots contain two kinds of amylase: α- and β-amylase. The former has better thermal stability and can hydrolyze starch granules at the optimum temperature of 70–75°C, whereas the latter can only hydrolyze gelatinized starch. Because sweet potato starch gelatinization temperature is 73–75°C, the two kinds of amylase activity remain highest under dry conditions with a temperature range of 70–80°C during sweet potato flour processing (Takahata *et al.*, 1995). Research on 21 sweet potato varieties showed that the temperature was much closer to the amylase optimum temperature by using air drying rather than sun drying of sweet potato flour, which consequently enhanced saccharification by amylase (Guoquan, 2003). Therefore, in addition to the starch content of the different varieties, the effects of processing conditions such as the drying methods on starch saccharification should be taken into consideration in the production of sweet potato flour.

For sweet potato flour used for bread processing, varieties with high starch and low sugar (amylase activity of only 53.5 μg/ml), which are difficult to saccharify, are appropriate. Dough gelatinization requires a certain degree of amylase activity. In addition, the reducing sugar is not only a carbon source of yeast but also the matrix of color, smell, and taste of bread during production. However, the inhibition of amylase activity is required during the bread baking phase, and it loses its activity at approximately 80–85°C. Amylase may still play a role if excessively used during the baking stage, which results in the starch in bread becoming much more liquefied and producing a large amount of dextrin, leading to small bread volume, uneven texture, and the bread being sticky and difficult to slice. Therefore, it is inappropriate to select the varieties with high saccharifying ability. If the varieties with low saccharifying ability cannot meet the requirements, amylase can be added from other sources, such as the fungal amylase. The amylase has low heat resistance, short reaction time, and produces less dextrin, which is a benefit in making bread core nontacky. Even if too much is added, it would not significantly affect the quality of bread.

The function of sweet potato protein during bread processing

Root protein of sweet potato includes mainly storage protein (sporamin) and glycoprotein. Sporamin is the main protein in sweet potato, affecting its nutritional quality.

Sporamin can scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and hydroxyl radical. Research shows that anti-DPPH radical activity is enhanced with increased concentration of sporamin. The mechanism may be that sulfydryl in sporamin reduces DPPH radical and inhibits DPPH radical oxidation. Animal studies have shown that sweet potato glycoprotein has anti-diabetic activity for the diabetic model of insulin deficiency and insulin resistance. However, whether the inhibiting trypsin activity of sporamin adversely affects the human body, and whether it has anti-cancer effects similar to the homology of soybean trypsin inhibitor is unknown, and more in-depth studies are needed.

Wheat gluten protein, including gliadin (prolamine) and glutenin, accounts for 80% of the total protein in wheat flour and combines with water to form gluten. Gluten, which is highly elastic and extensible, can retain CO_2 from dough fermentation so that baking bread is porous and soft. Generally, it is believed that gliadin gives the dough extensibility and glutenin gives the dough flexibility. Dough types and processing suitability are determined by the ratio of gliadin to glutenin. For bread wheat, the higher the protein content, the better the wheat quality. Grain protein content (dry basis) should be higher than 15%.

Therefore, although protein from sweet potato flour is of high nutritional value, as a raw material of processing bread, it will cause the gluten level to be inadequate and reduce bread

volume. As a result, it becomes difficult to accept by consumers. Thus, when sweet potato flour in whole or in part replaces wheat flour to make bread, appropriate food additives are selected to improve bread volume, texture, flavor, shelf life, and its overall quality. Dough enhancer may be a mixture of more additives, such as hydrocolloids, phospholipids, or gluten. Gallagher *et al.* (2003) used milk powder as dough enhancer in gluten-free bread making, which resulted in increased bread volume and more flexible texture of crust and crumbs. For flat bread, lecithin is used as a dough enhancer, resulting in a texture that is more flexible (Hathorn *et al.*, 2008). Therefore, it is an effective way to overcome the defects in gluten-free bread made with sweet potato flour.

SUMMARY POINTS

- Sweet potato, as the world's fifth-largest food crop, has the potential to be a very important food resource for solving food shortage problems.
- Sweet potatoes contain carbohydrate, protein, carotenoids, anthocyanin, and polyphenols; thus, they possess high nutritional value and strong antioxidative ability.
- The processing of sweet potato into flour not only prolongs the shelf life but also increases the intake of many nutrients into specialty bread made from this flour, which acts as a substitute for wheat flour.
- Starch is the most important nutrient in sweet potato tuber, as well as the key to sweet potato quality. Based on the different end uses of the flour, there are different demands on starch type, content, and sweet potato quality.
- The raw materials for bread making, processing methods, the amount of amylose, the amount of damaged starch granules, and pasting properties of sweet potato flour affect bread quality.
- When sweet potato flour in whole or in part replaces wheat flour to form bread, additives are selected to improve bread volume, texture, flavor, shelf life, and overall quality.

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CHAPTER 38

Fortification of Bread with Soy Proteins to Normalize Serum Cholesterol and Triacylglycerol Levels

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

ER Endoplasmic reticulum
GMP Glutenin macropolymer
LDL Low-density lipoprotein
LPs Lipophilic proteins
PC Phosphatidylcholine
SDS Sodium dodecyl sulfate
SPI Soy protein isolate
TG Triacylglycerol
VLDL Very low-density lipoprotein

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is a species of legume that originated in East Asia and has been cultivated for approximately 5000 years in northeastern China. Soybean is used to make traditional foods, including tofu, soybean paste, miso, and soy sauce. Soybean was first

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introduced to Europe in the early seventeenth century and to the United States in the eighteenth century, and it has since become an important global crop. Global soybean production was more than 220 million metric tons in 2007.

Soybeans contain extremely high amounts of protein and oil (30–50% and 13–25% of the total mass, respectively). Soy protein meets the required amino acid composition for humans and animals, except for slightly low sulfur amino acid content. However, soy foods have not been well accepted in areas other than eastern Asia due to their bitter taste, chalky mouthfeel, and the "beany" and "greeny" flavors that are generated primarily from linoleic acid by soy lipoxygenases. For that reason, most fat-free soy meal has been used as the primary source of protein for animal feeds or rations.

In the 1960s, a new method became available to extract food-grade soy protein isolate (SPI) from defatted soy meal under lower temperatures. SPI is used in a variety of foods for its functional properties, including solubility, water and fat absorption, and emulsification. In addition, soy protein has been shown to lower the risk of cardiovascular disease in humans (Anderson *et al.*, 1995). In particular, one of the major storage proteins of soy, β-conglycinin, reduces high serum triacylglycerol (TG) concentration and visceral fat in humans (Kohno *et al.*, 2006). Daily intake of SPI and β-conglycinin is required for this effect; thus, SPI or β-conglycinin supplementation in foods eaten daily is desirable. As such, bread is a convenient vehicle for SPI and β-conglycinin; however, SPI and β-conglycinin produce adverse effects on bread making and bread quality. This chapter reviews the features and the physiological functions of soy proteins as well as the characteristics of bread fortified with soy proteins.

SOY PROTEINS

In the typical soybean, proteins comprise approximately 40% of the total mass; however, both genetic and environmental factors strongly influence seed composition. The nutritional value of soy proteins is high; the protein digestibility-corrected amino acid score of SPI is approximately that of egg white. Furthermore, the biological values (i.e., the ability of the body to absorb and utilize the protein) of whole soybean, soy milk, and SPI are 74, 96, and 91, respectively. These values are the highest among major edible crops. Adding soy protein to foods made from crops such as wheat, maize, and rice increases their nutritional value because soy protein contains relatively high amounts of lysine, which is a limiting amino acid for complete protein in these crops.

Soybean produces exalbuminous seeds. Within the embryo, storage proteins glycinin and β-conglycinin are synthesized and stored in cotyledons, where they are subsequently used as nitrogen, carbon, and sulfur sources in embryonic development (Nielsen and Nam, 1999). These globulin proteins comprise approximately 60% of the total soy proteins (Nielsen and Nam, 1999); the remaining proteins fulfill protective, structural, and metabolic roles.

β-Conglycinin is composed of three main types of subunits designated α, α′, and β, with molecular weights of 50–70 kDa. Random combinations of these subunits form seven heterotrimers and three homotrimers. β-Conglycinin subunits are translated in the rough endoplasmic reticulum (ER) and undergo folding and assembly into trimers in the ER lumen. Subunits are modified by cotranslational N-glycosylation (i.e., one N-glycan on the β subunit and two N-glycans on the α or α′ subunit). The assembled trimers are transported via the Golgi apparatus and accumulate in protein storage vacuoles.

Glycinin is composed of five types of subunits designated A1aB1b, A1bB2, A2B1a, A3B4, and A5A4B3. They are categorized into two groups according to amino acid sequence similarity: group I (A1aB1b, A1bB2, and A2B1a) and group II (A3B4 and A5A4B3). Each glycinin subunit is synthesized in the rough ER as a precursor protein (molecular weights of approximately 50 kDa). They undergo folding, formation of intrachain disulfide bonds, and assembly into trimers in the ER lumen. Several lines of evidence indicate that the folding is performed with

the aid of molecular chaperones and several members of the protein disulfide isomerase family (Kamauchi *et al.*, 2008; Wadahama *et al.*, 2007, 2008). The assembled trimers are transported via the Golgi or directly from the ER to protein storage vacuoles. Glycinin precursor subunits in trimers are cleaved into acidic subunits and basic subunits at a well-conserved Asn—Gly peptide bond by a vacuolar processing enzyme and then assembled into hexamers in the protein storage vacuoles (Nielsen and Nam, 1999). The three-dimensional structures of β-conglycinin and glycinin are very similar, suggesting that these genes evolved from a common ancestor gene (Adachi *et al.*, 2003; Maruyama *et al.*, 2004).

ISOLATION OF SOY PROTEINS

Most edible soy protein products are derived from white flakes made by dehulling, flaking, and defatting soybeans by hexane extraction. These products consist of defatted flour (approximately 50% protein), soy protein concentrate (65—70% protein), and SPI (>90% protein). Recently, the purified soy protein component β-conglycinin isolate has become commercially available. Soy protein products have many important functional properties, including solubility, water and fat absorption, emulsification, and imparting of texture (i.e., gelation, cohesion—adhesion, and elasticity). Soy protein products are used in a variety of foods, including beverages, meat products, bakery items, pasta products, cheeses, and simulated meats.

Commercial SPI is extracted from white soy flakes with water. β-Conglycinin and glycinin are soluble in salt solution, and salts are present in white flakes; therefore, these proteins are easily extracted by adding water. Most proteins can then be isoelectrically precipitated after the extract is acidified (pH 4.3—4.8). Finally, the precipitated protein curd is neutralized, sterilized, and dried (Figure 38.1). For a long time, SPI was believed to be composed primarily of β-conglycinin and glycinin. However, SPI has been found to contain lipids associated with lipophilic proteins (LPs) such as oil body-associated proteins (Iwabuchi and Yamauchi, 1987; Samoto *et al.*, 1998). Because *n*-hexane cannot efficiently extract phospholipids or hydrophobic membrane proteins, acid-precipitated proteins contain LPs associated with membrane

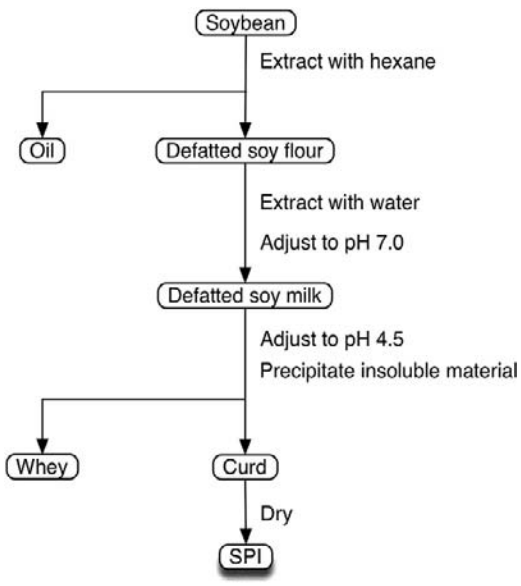

FIGURE 38.1
Schematic diagram depicting isolation of soy protein isolate. Water was added to white soy flakes defatted with *n*-hexane to solubilize proteins. Isoelectric precipitation of most proteins can be performed after lowering the pH. The precipitated protein curd is then neutralized, sterilized, and dried. SPI, soy protein isolate.

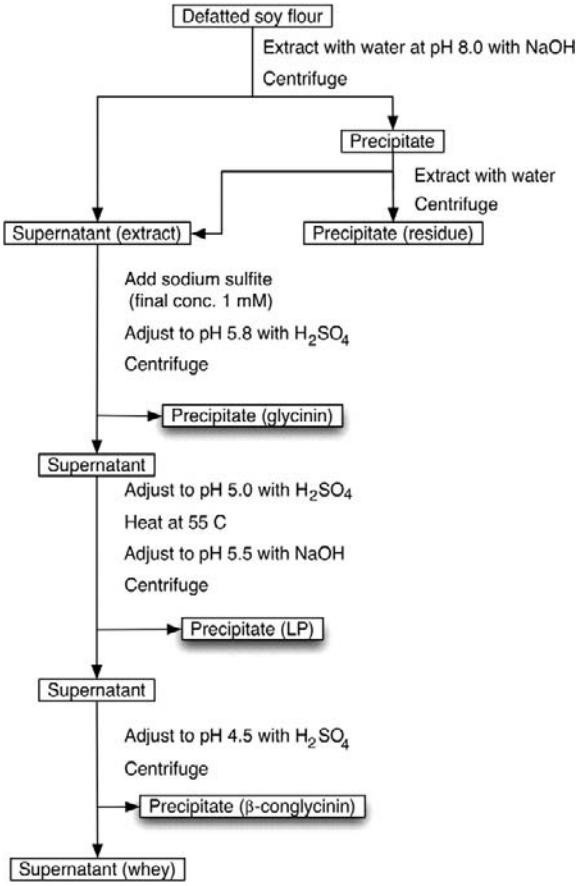

FIGURE 38.2
Schematic diagram depicting fractionation of glycinin, lipophilic proteins, and β-conglycinin. A three-step acidification of the water extract of defatted soy flour separated three proteins with the nitrogen distribution ratio of 23% (β-conglycinin), 46% (glycinin), and 31% (lipophilic proteins). LP, lipophilic proteins.

phospholipids from oil bodies and protein storage vacuoles. Compared with β-conglycinin and glycinin, LPs are difficult to detect by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis due to lower sensitivity to Coomassie brilliant blue staining; thus, the importance of LPs in SPI has been overlooked. Samoto *et al.* (2007) developed a method for fractionating acid-precipitated proteins (Figure 38.2). In that study, the nitrogen distribution ratios for the three separated proteins were 23% (β-conglycinin), 46% (glycinin), and 31% (LPs).

PHYSIOLOGICAL FUNCTION OF SOY PROTEINS

Approximately 100 years ago, the cholesterol-lowering effects of soy protein compared with animal protein were reported in rabbits (Ignatowsky, 1908). Since then, many studies have reported the effects of soy proteins on serum lipids in humans; however, results have been inconsistent, possibly because of different experimental conditions, such as soy protein content in the diet and degree of hypercholesterolemia in the subjects. In a meta-analysis published in 1995, Anderson *et al.* concluded that soy protein consumption significantly decreased serum levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, and TG, corresponding to the degree of hypercholesterolemia. Based on these findings, the U.S. Food and Drug Administration granted the following health claim for soy protein in 1999: "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease."

Most commercially available SPI products contain significant amounts of genistein, daidzein, and glycitein. These isoflavones have been shown to exert strong biological actions in animals, such as serum cholesterol lowering, arterial vasodilation, and atherosclerosis inhibition (Sacks *et al.*, 2006). Hence, these isoflavones were assumed to be largely responsible for the beneficial effects of SPI on hypercholesterolemia in humans. Human studies comparing the effects of casein, animal proteins, and ethanol-washed isoflavone-free SPI on serum cholesterol levels have demonstrated declines in LDL cholesterol with isoflavone-free soy protein consumption (Jenkins *et al.*, 2002; Lichtenstein *et al.*, 2002). Furthermore, studies comparing the effects of SPI with or without isoflavones confirmed that isoflavones are not responsible for the lipid-lowering effects in humans. However, the soy protein component(s) responsible for this effect is not known. Candidates include a peptide derived from glycinin that inhibits reabsorption of bile acid from the intestine (Nagaoka *et al.*, 1997) and LPs that have been shown to reduce serum cholesterol (Kanamoto *et al.*, 2007). However, these studies were performed in rats; the effects of glycinin and soy LPs in humans are unclear. Thus, identification of components responsible for cholesterol lowering remains unsolved.

The effects of LP-free β-conglycinin were assessed by supplementation of the diets of adults with high plasma TG. Intake of β-conglycinin (5 g/day) normalized serum TG and reduced visceral fat in subjects with body mass indices between 25 and 30 (Kohno *et al.*, 2006). Based on these findings, in 2007, soy β-conglycinin was approved as a food for specified health use in Japan.

The plasma TG level is controlled by the amount of very low-density lipoprotein (VLDL) secreted from the liver and the rate of VLDL-TG catabolism in blood. To determine the effects of soy β-conglycinin on lipid metabolism, small peptides were derived from LP- and isoflavone-free β-conglycinin by protease digestion and used to treat the human hepatocellular carcinoma cell line HepG2 (Mochizuki *et al.*, 2009). The findings showed that the β-conglycinin-derived peptides suppressed TG synthesis, thereby suppressing the secretion of VLDL from HepG2 cells into the medium.

ADVERSE EFFECTS OF SOY PROTEIN FORTIFICATION ON RHEOLOGICAL PROPERTIES OF DOUGH AND BREAD QUALITY

The addition of SPI and purified β-conglycinin to foods can increase soy protein consumption and help achieve a physiologically beneficial intake. Fortification of bread with soy protein has a long history due to the relatively high amounts of lysine and valine in soy, which are the limiting amino acids of wheat proteins. Although the nutritive value of the bread rises with the percentage of SPI (Mizrahi *et al.*, 1967), SPI reduces bread quality. SPI-containing bread was judged to be firmer, drier, grainier, less tender, and gummier compared to the ideal bread (Elgedaily *et al.*, 1982). In addition, SPI-containing bread exhibited a strong beany flavor, which curtailed its overall acceptability (Ranhotra and Loewe, 1974). Bread loaf volume also decreased, and bread crumb firmness and firming rate increased proportionally with the level of SPI fortification (Chen and Rasper, 1982; Mizrahi *et al.*, 1967; Ribotta, Arnulphi, *et al.*, 2005). An inverse relationship was shown between bread loaf volume and firmness; therefore, the low specific volume of SPI-containing breads was probably the cause of the increased firmness values rather than the effect of soy protein *per se* (Ribotta, Arnulphi, *et al.*, 2005). A strong correlation between dough volume after fermentation and finished loaf volume has been observed in the presence of SPI. SPI had no effect on CO_2 production by yeast; thus, SPI may decrease the gas retention capacity of dough. Scanning electron microscopy revealed that gluten containing SPI had a much more porous network compared with that of control dough lacking SPI (Roccia *et al.*, 2009), suggesting that SPI makes the gluten network brittle against the pressure of gas. Highly purified β-conglycinin decreases loaf volume more than SPI (Ukai and Urade, 2007; Urade *et al.*, 2003).

TABLE 38.1 Effects of Soy Protein Isolate on Rheological Properties of Wheat Flour Dough

Property	Equipment	Parameter	Effect of SPI	References
Farinographic behavior	Microfarinograph	Water absorption Arrival time Development time Stability	Increase Decrease Decrease Decrease	Mizrahi *et al*. (1967), Ranhotra *et al*. (1974), Ribotta, Arnulphi, *et al*. (2005), Roccia *et al*. (2009), Urade *et al*. (2003)
Dynamic viscoelastic property	Rheograph gel	Storage modulus (E') Loss modulus (E'') tan λ (E''/E')	Increase Increase Decrease	Urade *et al*. (2003)
Large deformation property	Rheoner	Force at failure point Failure point	Increase No change	Urade *et al*. (2003)
	Micro-extensograph	R_m E Area	Increase Decrease Decrease	Ribotta, Arnulphi, *et al*. (2005), Roccia *et al*. (2009)
Creep property	Texture analyzer	J_o J_l μ_o	Decrease Decrease Increase	Roccia *et al*. (2009)

E, maximum extensibility; J_o, instantaneous compliance; J_l, retarded compliance; μ_o, Newtonian viscosity; R_m, maximum resistance to extension; SPI, soy protein isolate.

Changes in the gluten network structure were accompanied by changes in the rheological properties of dough. Farinograph analysis revealed that the addition of SPI reduced arrival time, development time, and stability (Table 38.1) (Mizrahi *et al*., 1967; Ranhotra and Loewe, 1974; Urade *et al*., 2003). SPI also affected the dynamic viscoelastic properties of dough to increase storage (E') and loss modulus (E'') values and decrease tan λ (E''/E') value, which represents the strength of the gluten network as determined by a Rheograph gel (Urade *et al*., 2003). In addition, SPI affected the large deformation properties of dough. The force value at failure point obtained using a Rheoner increased after adding SPI to the dough (Urade *et al*., 2003). When tensile strength test was performed with the Intron Universal Testing Machine, SPI increased the resistance to extension and the relaxation time (Chen and Rasper, 1982). Increased maximum resistance and decreased extensibility by SPI fortification were revealed using a micro-extensograph (Roccia *et al*., 2009). At the same time, area under the extension curve, which is a measure of the energy required for extension, was reduced by SPI, and the area under the extension curve was highly correlated with specific loaf volume. By the creep test, SPI decreased instantaneous compliance, retarded compliance of the dough, and increased Newtonian viscosity of the dough (Roccia *et al*., 2009); the decrease in retarded compliance reflects the loss of elasticity of the dough. Taken together, the results obtained by the rheological assays indicated that SPI increases dough firmness and weakens the gluten network.

SPI appears to weaken the gluten network both indirectly and directly. Soy proteins such as glycinin and β-conglycinin were detected in SDS-insoluble gluten proteins obtained from dough (Ribotta, León, *et al*., 2005), indicating that soy proteins tightly associate with gluten proteins in dough. Hydrogen bonds and hydrophobic interactions between glutenin and gliadin molecules are essential for gluten formation; soy proteins may interfere with these interactions. Dough's elastic properties are related to the quantity of the SDS-insoluble glutenin macropolymer (GMP), which is comprised of high-molecular-weight and low-molecular-weight glutenin subunits linked by disulfide bonds. Although it seems possible that soy protein containing cysteine residues may decrease GMP content by thiol exchange or reduction of disulfide bonds among glutenin proteins, GMP content of SPI-containing dough did not show any significant differences compared with dough lacking SPI (Peréz *et al*., 2008). Even a small amount of soy glycinin (2.5–8.2% of total wheat protein) decreased developing time and stability time (Lampart-Szczapa and

Jankiewicz, 1982); thus, direct interactions with soy proteins may considerably alter the gluten network.

The indirect influence of SPI is related to reduced water availability in the dough. Many studies have reported that SPI increases water absorption in wheat dough as assessed by farinograph (Roccia *et al.*, 2009; Urade *et al.*, 2003). Thus, water binding in the dough is increased and syneresis is decreased (Roccia *et al.*, 2009). Free water is thought to act as an inert filler or lubricant in polymers such as gluten (Masi *et al.*, 1998). Therefore, changes in dynamic viscoelastic behavior and the relaxation phenomena may be due in part to the decrease in free water by SPI fortification.

TECHNOLOGICAL ISSUES

The lower quality of bread due to SPI fortification is a serious problem. The increased firmness and smaller loaf volume may be primarily due to the reduced free water in dough and interference of the gluten network formation. Roccia *et al.* (2009) showed that adding more water to the dough attenuated the SPI-induced increase in maximum resistance, but the additional water had no effect on extensibility or area under the extension curve. Therefore, the problem of increased firmness in SPI-containing bread can be solved by adjusting the amount of water to be added. In addition, adding more water restored normal instantaneous compliance and partially attenuated the decrease in retarded compliance on the creep test, which is an index parameter for elasticity (Roccia *et al.*, 2009).

Several materials, including detergents, have been shown to improve the bread making properties of SPI-containing dough and the quality of protein-fortified bread. Studies have reported the effects of sodium stearoyl-2-lactylate (SSL), nonionic hydrophilic polysorbate 60 (Tween 60), and nonionic lipophilic sodium tristearate (Span 65) on the rheological properties of the SPI-containing dough (Chen and Rasper, 1982). These detergents did not alter water adsorption of SPI-containing dough according to the farinograph assay, nor did they improve the increased resistance to extension or the relaxation time according to the tensile strength test. However, Tween 60 improved gas retention in the dough and increased loaf volume. SSL and Span 65 also improved gas retention in dough, but they did not completely restore the normal loaf volume of bread containing SPI.

Lecithin is a mixture of polar lipids that is permitted for use in all types of bread and bakery products. Lecithin also improves gas retention in dough and sufficiently restores the volume of dough fortified with SPI (Figure 38.3) or β-conglycinin (Mizrahi *et al.*, 1967; Ribotta, Arnulphi, *et al.*, 2005; Urade *et al.*, 2003). Moreover, the addition of lecithin produces a thicker, denser crust that tends to retain its crispness. Most lecithin used in the baking industry is derived from soybeans; soy lecithin is a mixture of phospholipids that includes phosphatidylcholine (PC), phosphatidylethanolamine, and phosphatidylserine. PC is the active constituent in lecithin (Urade et al., 2003), the function of PC cannot be replaced by other major phospholipids, phosphatidylethanolamines, or compounds derived from PC, phosphatidic acid, lysophosphatidylcholine (lysoPC), or choline. Confocal microscopy revealed that soy PC co-localizes with gluten in dough but not the starch granules (Figure 38.4). Farinograph behaviors or viscoelastic properties of dough impaired by SPI were not improved by soy PC. Soy PC exerted little effect on arrival time, development time, and stability. Nor did soy PC change the rheolograph value (E' and E'') or large deformation properties of SPI-containing dough.

Interestingly, the effect of PC on delipidated wheat flour containing β-conglycinin was lower compared with its effect on native wheat flour containing β-conglycinin (Ukai and Urade, 2007). Thus, soy PC alone has the ability to increase dough volume, and wheat flour lipids appear to boost this effect. Among wheat lipids, glycolipids such as monogalactosyl diglyceride and digalactosyl diglyceride best enhance the action of soy PC (Ukai and Urade, 2007).

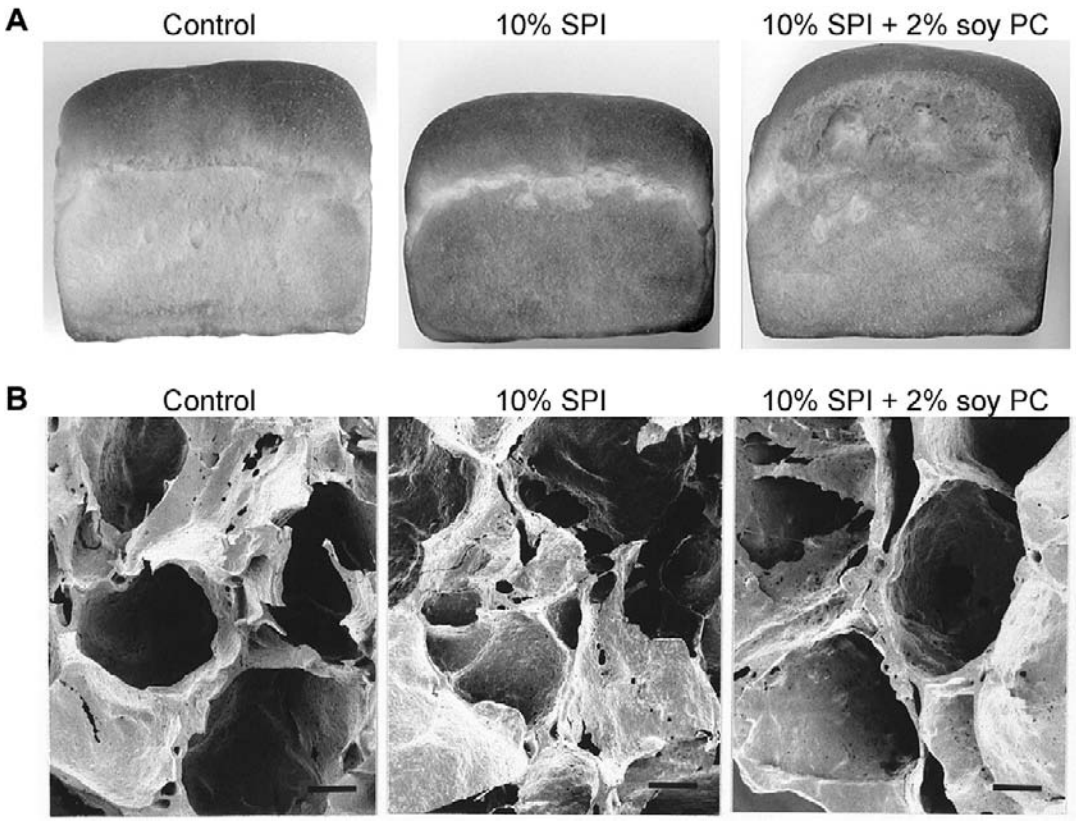

FIGURE 38.3
Effects of soy protein isolate and soy phosphatidylcholine supplementation on bread quality. (A) Unfortified bread (left), bread fortified with soy protein isolate (SPI) (center), and bread fortified with SPI plus soy phosphatidylcholine (PC) (right). (B) Scanning electron micrograph of unfortified bread (left), bread fortified with SPI (center), and bread fortified with SPI plus soy PC (right). For the wheat flour—soy protein combinations, 10% of the wheat flour was replaced with SPI (by weight). PC (2 g) was added to flour (100 g). PC, the active constituent in lecithin, improves gas retention in dough and sufficiently restores the volume of dough fortified with SPI. Scale bars = 200 μm. *Source: Reprinted with permission from Urade, R., Okamoto, S., Yagi, T., Moriyama, T., Ogawa, T., and Kito, M. (2003). Functions of soy phosphatidylcholine in dough and bread supplemented with soy protein.* J. Food Sci. *68, 1276–1282.*

PCs are a class of phospholipids composed of two fatty acyl chains. Fatty acyl chains that are combined in soy PC are linoleic (18:2), palmitic (16:0), stearic (18:0), oleic (18:1), and linolenic (18:3) acids. Many PC molecular species are possible, and the effect of PC on bread loaf volume depends on the particular molecular species (Figure 38.5). At one extreme, 1-palmitoyl, 2-palmitoyl-PC had no effect on dough volume at 36°C during fermentation. This molecular species exists in the gel crystalline form at 36°C because its gel-to-liquid crystalline phase transition temperature is 44.3°C. In contrast, PC molecular species with a liquid crystalline phase transition temperature lower than 36°C increase dough volume, possibly because PC in the liquid crystalline state improves the gas-retaining ability of dough during fermentation. Soy PC is composed of molecular species with liquid crystalline phase transition temperatures lower than 36°C, including dilinoleoyl-PC (35%), 1-palmitoyl, 2-linoleoyl-PC (24%), and 1-oleoyl, 2-linoleoyl-PC (15%) (Urade *et al.*, 2003). Differential scanning calorimetry analysis revealed the phase of soy PC transited at −48.5 to −13.1°C. In its fluid liquid crystalline state, PC self-assembles into a stable bilayer structure and exists as liposomes or multilamellar vesicles (lamellae) in water. The most surface-active property of polar lipids is thought to be a bilayer structure. Thus, a possible mechanism for the beneficial effects of PC may be its direct influence on gas cells in dough by increasing foam stability (Urade *et al.*, 2003).

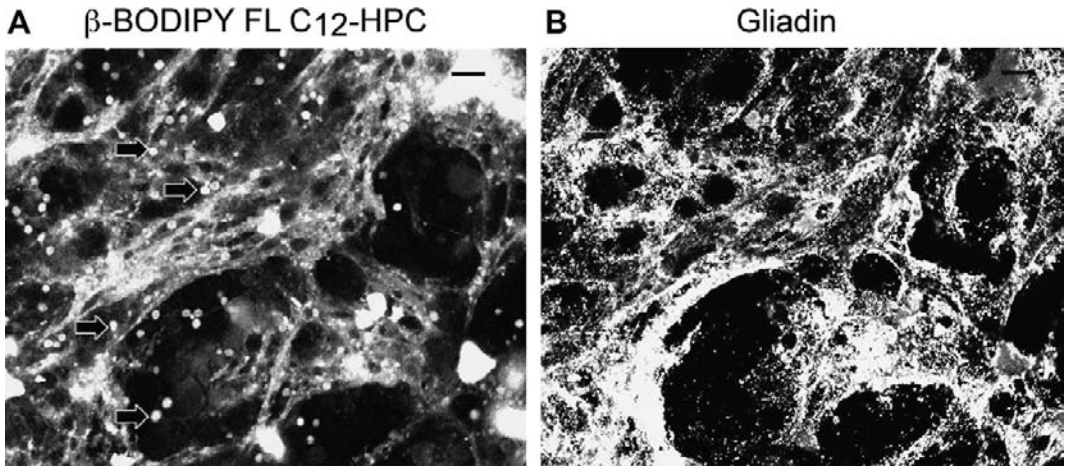

FIGURE 38.4
Distribution of phosphatidylcholine (PC) and gliadin in dough. Dough was made with soy PC containing the fluorescent PC, β-BODIPY FL C12-HPC. Gliadin in dough was immunostained with anti-gliadin rabbit serum, biotin anti-rabbit IgG goat serum, and Cy 5-streptoavidin. The dough was visualized with a laser confocal imaging system showing (A) PC (white) and (B) gliadin (white). The fluorescent PC, β-BODIPY FL C12-HPC was associated with protein fibers containing gliadin and yeast (arrows). Scale bar = 10 μm.

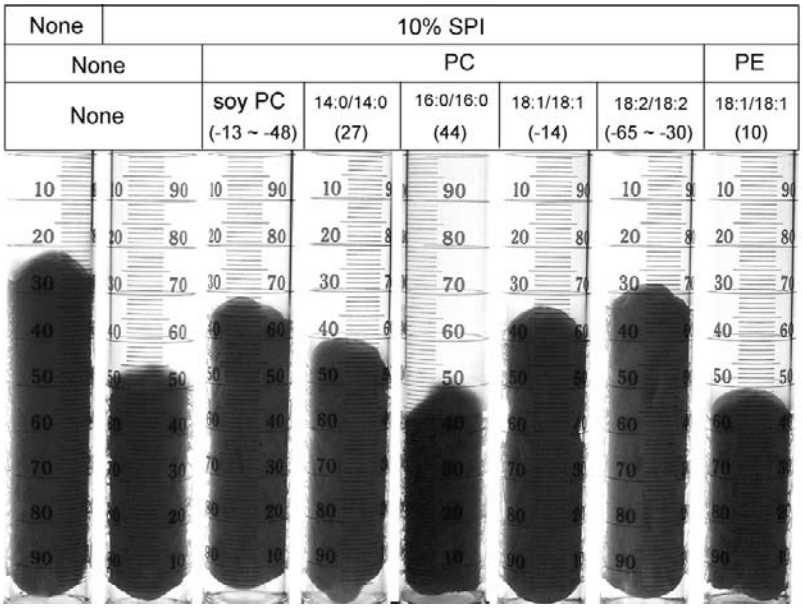

FIGURE 38.5
Effects of phosphatidylcholine (PC) molecular species on volume of soy protein isolate (SPI)-containing dough after fermentation. Dough was made with 100% wheat flour or wheat flour in which 10% was replaced with SPI. In some doughs, the flour (100 g) was further supplemented with 2 g of PC or phosphatidylethanolamine (PE). Dough (15 g) was then incubated at 36°C for 40 min. 14:0/14:0, dimyristoyl PC; 16:0/16:0, dipalmitoyl PC; 18:1/18:1, dioleoyl PC or PE; 18:2/18:2, dilinoleoyl PC. The liquid crystalline phase transition temperature of each PC is shown in parentheses. PC molecular species with a liquid crystalline phase transition temperature lower than 36°C increased dough volume.

SUMMARY POINTS

- Soy protein isolate is composed of three major proteins: β-conglycinin, glycinin, and lipophilic proteins.
- Physiological functions of soy protein isolate include the ability to reduce the risk of cardiovascular disease in humans by decreasing serum cholesterol levels.
- Highly purified β-conglycinin normalizes serum triacylglycerol levels in subjects with high serum triacylglycerol and reduces visceral fat in subjects with body mass indices between 25 and 30.
- Both soy protein isolate and β-conglycinin adversely affect bread making and bread quality by increasing water absorption and interfering with the formation of the gluten network.
- Phosphatidylcholine increases loaf volume without affecting rheological properties of dough.

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CHAPTER 39

Dietary Breads and Impact on Postprandial Parameters

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

GLP-1 Glucagon-like peptide 1
HC High carbohydrate
HDL High-density lipoprotein
LDL Low-density lipoprotein

INTRODUCTION

In parallel with the increase in awareness about the impact of nutrition on human health, the interest in dietary products is growing. This includes a significant interest in breads, which are indispensable to our diets. Wheat is the chief ingredient of bread. Breads can also be made out of ground barley, corn, and rye, or the flours of such grains can be mixed with wheat flour in certain ratios to make bread (Table 39.1).

In the Western world, the majority of whole grain products eaten are based on wheat, whereas the consumption of oats and especially rye and barley is much lower. Oats are mainly consumed in northern Europe, North America, and Australia, whereas rye consumption is essentially limited to northern, central, and eastern Europe (Kamal-Eldin *et al.*, 2007). The grain contains endosperm, embryo, and bran. Through the grinding process, bran and embryo

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TABLE 39.1 Most Consumed Bread Types

Bread Type[a]	White Wheat Bread	Whole Wheat Bread	Rye Bread	Wheat Bran Bread	Soy Bread	Corn Bread
Energy (kcal)	256.3	217	224	216	252	255
Carbohydrate (g)	54.7	44	46	44	54	51
Fiber (g)	0.3	11	4.4	11	0.2	0.9

Source: Reproduced with permission from Istanbul Halk Ekmek (www.ihe.com.tr).
[a]*Nutritional values in 100 g of different kinds of bread.*

are separated from endosperm. Endosperm has a high starch content. Other nutrients are contained mostly in the outer layers of grains.

The grain germinates in wheat embryo (germ). It contains high amounts of vitamins A, E, and B_1; lecithin; essential fatty acids; proteins; and minerals such as zinc, manganese, and chromium.

White bread is made of wheat flour, and the process involves the exclusion of bran and embryo. On the contrary, these are preserved in whole wheat. As a result, processed grains contain more starch, whereas the nutritional ingredients are particularly reduced. Whole grains differ from processed grains by the preservation of bran and embryo (Slavin *et al.*, 1999).

Compared to whole grain bread, most commercial white breads contain little dietary fiber. White bread is often assumed to cause a more drastic rise and fall in blood sugar and insulin levels compared to whole grain bread. For that reason, in both weight-loss diets and hypertension diets, white bread is replaced by rye bread, wheat bran bread, and whole grain bread. Acute and chronic effects of various bread types on postprandial parameters have been investigated, and studies are still being conducted.

POSTPRANDIAL PLASMA GLUCOSE AND INSULIN RESPONSES TO DIFFERENT TYPES OF BREADS

Carbohydrate-rich diets lead to continuous pancreatic stimulation and repeated postprandial insulin secretion. This type of diet has been hypothesized to lead to insulin resistance, β cell dysfunction, and, ultimately, type 2 diabetes (Zammit *et al.*, 2001). Many studies have shown that diets with lower glycemic indices and higher fiber content decrease the development risk of type 2 diabetes and heart diseases, contribute to a higher quality of life for diabetics, and have some preventive and restorative effects against insulin resistance and metabolic syndrome (Amano *et al.*, 2004; Leeds, 2002; Liu *et al.*, 2000). Furthermore, several studies have investigated the short-term effects of different types of breads on glucose metabolism. In a study in which 2-h postprandial effects of white bread, wheat bran bread, and whole grain bread were compared (Mesci *et al.*, 2008), no difference was found among the three types of breads with respect to glycemic effects (Figures 39.1 and 39.2). Hlebowicz *et al.* (2009) found no difference in postprandial blood glucose response or gastric emptying after the ingestion of rye wholemeal bread compared to white wheat bread. Different types of breads do not cause significant differences in the postprandial glycemic response. This also applies for breads that contain different levels of fiber content (Heinonen *et al.*, 1985; Jenkins *et al.*, 1983; Juntunen *et al.*, 2003; Liljeberg *et al.*, 1992). However, different breads show a difference with respect to postprandial insulin response (Juntunen *et al.*, 2002). In particular, rye bread leads to a lower postprandial insulin response compared to other types of breads (Table 39.2).

Rosén *et al.* (2009) found that whole grain rye breads and endosperm rye products induced significantly ($p < 0.05$) lower insulinemic indices compared to white wheat bread. Endosperm and whole grain rye products induced low acute insulinemic responses and improved glycemic profiles. The results also suggested that the rye products possess beneficial appetite-regulating

FIGURE 39.1
Glycemic effects of different kinds of bread. Three different bread types consumed as alternatives to white bread showed similar increases in blood glucose levels as white bread in diabetic patients. Results are expressed as mean ± SD; $n = 121$. *Source: Reproduced from Mesci, B., Oguz, A., Sagun, H. G., Uzunlulu, M., Keskin, E. B., and Coksert, D. (2008). Dietary breads: Myth or reality?* Diabetes Res. Clin. Pract. *81, 68–71.*

properties. Preservation of the intact botanical structure of cereal grains has also been shown to lower the insulin response (Heaton *et al.*, 1988). These effects may be mediated through glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 (GLP-1; Juntunen *et al.*, 2002), which are the most important insulinotropic incretins.

POSTPRANDIAL PLASMA LIPID RESPONSES TO DIFFERENT TYPES OF BREADS

High-carbohydrate (HC) diets are recommended for lowering the risk of coronary heart disease because they decrease plasma low-density lipoprotein (LDL) cholesterol concentrations. In the study by Queenan *et al.* (2007), 75 hypercholesterolemic men and women were randomly assigned to either the 6 g/day concentrated oat β-glucan treatment group or the 6 g/day dextrose (control) treatment group. After 6 weeks, it was observed that 6 g concentrated oat β-glucan per day during this period significantly reduced the total and LDL cholesterol in subjects with elevated cholesterol, and the LDL cholesterol reduction was greater than the change in the control group. In a study comparing the effects of whole wheat bread and β-glucan supplemented oat bread on the lipid profile, the oat-derived β-glucan was found to significantly improve high-density lipoprotein (HDL) cholesterol while diminishing LDL cholesterol and non-HDL cholesterol in overweight individuals with mild

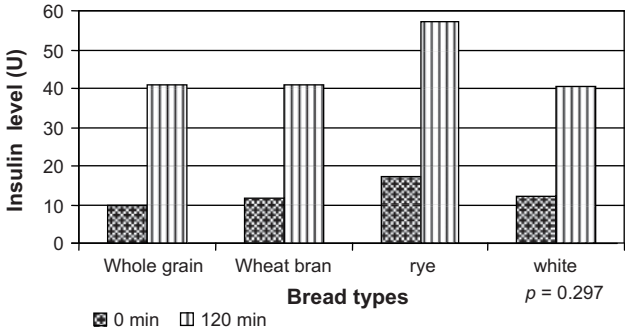

FIGURE 39.2
Insulinemic effects of different bread types. No significant difference was found between insulinemic effects of four different types of breads. Results are expressed as mean ±SD, n= 121. *Source: Reproduced from Mesci, B., Oguz, A., Sagun, H. G., Uzunlulu, M., Keskin, E. B., and Coksert, D. (2008). Dietary breads: Myth or reality?* Diabetes Res. Clin. Pract. *81, 68–71.*

TABLE 39.2 Glucose, Insulin, C-Peptide, Glucose-Dependent Insulinotropic Polypeptide (GIP), and Glucagon-like Peptide 1 (GLP-1) Responses to the Consumption of Test Breads[a]

	Refined Wheat Bread	Endosperm Rye Bread	Traditional Rye Bread	High-Fiber Rye Bread
Glucose (mmol/l)	2.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.1	1.7 ± 0.2
Insulin (pmol/l)	299.2 ± 28.1	206.1 ± 18.8*	220.5 ± 20.8*	222.2 ± 29.1*
C-peptide (nmol/l)	1.9 ± 0.1	1.4 ± 0.1*	1.4 ± 0.1*	1.5 ± 0.1*
GIP (pmol/l)	107.2 ± 7.4	87.7 ± 10.3	59.1 ± 4.6*,**	60.5 ± 5.0*,**
GLP-1 (pmol/l)	28.3 ± 4.7	30.6 ± 6.3	25.9 ± 3.3	26.4 ± 5.4

Source: Reproduced with permission from Juntunen, K. S., Laaksonen, D. E., and Autio, K. (2003). Structural differences between rye and wheat breads but not total fiber content may explain the lower postprandial insulin response to rye bread. *Am. J. Clin. Nutr.* 78, 957–964.
[a]*Rye bread leads to a lower postprandial insulin response compared to the other types of breads. Results are expressed as mean ± SEM;* n = *19.*
**Significantly different from refined wheat bread;* $p < 0.05$.
***Significantly different from endosperm rye bread;* $p < 0.05$.

hypercholesterolemia (Reyna-Villasmil *et al.*, 2007). However, the effects of breads on the blood lipid profile are not as harmless as they were originally thought to be. An unfavorable effect of HC diets is the rise in plasma triacylglycerol concentrations (Coulston *et al.*, 1989; Dreon *et al.*, 1990) resulting from increased rates of very low-density lipoprotein–triacylglycerol secretion (Mittendorfer and Sidossis, 2001). In a study that compared whole wheat brown bread, rice, and roti (a traditional Indian dish) having equal carbohydrate contents with respect to the effects on the blood lipid profile (Ezenwaka and Kalloo, 2005), whole wheat brown bread elicited the highest postprandial triacylglycerol increase among the three foods tested in both diabetic and nondiabetic subjects (Tables 39.3 and 39.4).

EFFECTS OF FIBER CONTENT ON POSTPRANDIAL PARAMETERS

In some studies, low glycemic responses have been attributed to the viscous fiber content of bread such as β-glucan in oat bread (Jenkins *et al.*, 1978), which may slow the gastric emptying rate or the absorption of nutrients in the small intestine (Champ and Noah, 1997; Ellis *et al.*, 1995). Although the fiber content of bread is considered to have positive effects on glucose metabolism, despite their different fiber contents, white and wholemeal breads showed similar postprandial glycemic responses both in healthy volunteers (Heinonen *et al.*, 1985; Liljeberg *et al.*, 1992) and in diabetics (Jenkins *et al.*, 1983; Juntunen *et al.*, 2003). The lower postprandial insulin response to rye bread compared to wheat bread cannot be attributed to total fiber content. Postprandial insulin responses to grain products are determined by the form and botanical structure of food rather than by the fiber content or the type of cereal in the food (Juntunen *et al.*, 2003).

EFFECTS ON SATIETY

Different types of breads are thought to have varying effects on satiety. Isaksson *et al.* (2009) investigated subjective appetite during an 8-h period after intake of isocaloric rye bread

TABLE 39.3 Incremental Glucose and Triglyceride Levels after Ingestion of Test Foods in Diabetic Patients[a]

	Bread	Rice	Roti
Glucose (mmol/min/l)	516.6 ± 44.9	417.6 ± 40.5	675.0 ± 37.2*
Triglyceride (%)	32.4*	8.3	8.9

Source: Reprinted by permission from *Indian J. Med. Res.*, volume 121, pp. 23–31, C. E. Ezenwaka and R. Kalloo, copyright 2005 the IJMR.
[a]*Bread induced the highest triglyceride increase in diabetic patients. Results are expressed as mean ± SD;* n = *38.*
**p < 0.01.*

TABLE 39.4 Incremental Glucose and Triglyceride Levels after Ingestion of Test Foods in Nondiabetic Subjects[a]

	Bread	Rice	Roti
IAUGC (mmol/min/l)	210.9 ± 31.9	180.5 ± 25.3	254.1 ± 34.3
Incremental TG (%)	38.3*	−1.3	14.2

IAUGC, incremental area under the glucose curve; TG, triglyceride.
Source: Reprinted by permission from *Indian J. Med. Res.*, volume 121, pp. 23–31, C. E. Ezenwaka and R. Kalloo, copyright 2005 the IJMR.
[a]*Bread induced the highest triglyceride increase in nondiabetic subjects. Results are expressed as mean ± SD;* n = *27.*
*$p < 0.01$.

breakfasts varying in rye dietary fiber composition and content. This study showed that each of the rye breakfasts resulted in a suppressed appetite during the time period before lunch compared to the wheat reference bread breakfast. Among the rye bread breakfasts, rye bran bread induced the strongest effect on satiety. In the afternoon, the effect from all three rye bread breakfasts could still be seen as a decreased hunger and desire to eat compared to the wheat reference bread breakfast (Isaksson *et al.*, 2009).

However, another study was conducted to test the effect of isocaloric meals based on wholemeal wheat breads and pasta in comparison to similar refined wheat products on postprandial glycemia, appetite, and *ad libitum* energy intake. The results showed that wholemeal breads increased satiety measures compared to their refined counterparts; however, no significant effect on subsequent energy intake was observed (Kristensen *et al.*, 2010).

Because of the widely held conception that dietary breads have a more positive impact on metabolic parameters, in our daily clinical experience we often observe overconsumption of bread by patients on diet. In contrast, as previously stated, the effects of the so-called dietary breads on the metabolic parameters are not different from the effects of white bread. Use of these types of breads without necessary limitation in usual diets is probably an important cause of the recent increased incidence of obesity and in turn leads to the increase in complications related to obesity.

TECHNOLOGICAL ISSUES

Structural characteristics and baking conditions influence the metabolic responses to carbohydrate-containing foods. Postprandial parameters are also affected by the methods used for preparing breads. Food processing processes, such as baking, have been shown to reduce the digestibility of starch (Englyst and Cummings, 1985). In a study involving six different types of French breads prepared using different methods, some varieties of French breads showed lower insulinemic index in healthy subjects and lower glycemic index in type 2 diabetic subjects compared to other varieties, despite the fact that all six had equal carbohydrate contents. These results may be due to differences in bread processing rather than fiber content (Rizkalla *et al.*, 2007). In the study by Najjar *et al.* (2009), 10 overweight volunteers consumed each of the four breads (white, whole wheat, sourdough, and whole wheat barley) containing 50 g carbohydrate in a standard meal. Three hours later, they had another standard meal. Sourdough bread resulted in lower glucose and GLP-1 responses compared to those of whole wheat breads following both meals.

SUMMARY POINTS

- With respect to the effect on increase in blood sugar levels, there is no difference between various types of breads with equal carbohydrate contents. On the basis of lower postprandial insulin responses toward rye bread observed in some studies, rye bread is claimed to give a feeling of satiety for longer periods of time. However, no dietary bread can be recommended for unrestricted consumption in the diabetic diet.

- High-carbohydrate diets reduce the plasma LDL levels, but they increase the triglyceride levels. Therefore, instead of a monotype diet, a more balanced composition will have more desirable effects on lipid parameters.
- Fiber content of the breads does not alter the glycemic response; however, it may have an effect on the feeling of satiety. Studies have reported the positive glycemic and lipemic effects of β-glucan, a different soluble dietary fiber.
- The preparation methods of breads can cause differences in postprandial parameters.
- Regardless of the type, no bread should be consumed in an uncontrolled way.

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CHAPTER 40

Fortification of Vitamin B_{12} to Flour and the Metabolic Response

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

FA Folic acid
FF Flour fortification
MMA Methylmalonic acid
NTD Neural tube defect
RDA Recommended Dietary Allowance

Flour and Breads and their Fortification in Health and Disease Prevention. DOI: 10.1016/B978-0-12-380886-8.10040-6

INTRODUCTION

Screening for vitamin B_{12} deficiency is indicated in patients with relevant signs, such as anemia, neuropathy, or cognitive impairment. Historically, it was believed that most vitamin B_{12} deficiencies resulted from pernicious anemia, and that patients had a higher than 90% probability of having gastrointestinal diseases with malabsorption of vitamin B_{12} (Lindenbaum, 1979).

In the 1990s, the concept of food-bound vitamin B_{12} malabsorption emerged (Carmel, 1995). Approximately 30−40% of patients with subclinical deficiency were suspected to suffer from food-bound vitamin B_{12} malabsorption, with normal free vitamin B_{12} absorption (Carmel, 1995). Food-bound vitamin B_{12} malabsorption has been reported as a frequent condition among elderly people. The prevalence of vitamin B_{12} deficiency was 12% in 548 free-living elderly Americans, surviving members of the original Framingham Study cohort (Lindenbaum *et al.*, 1994).

Pernicious anemia is usually treated with parenteral vitamin B_{12}, but food-bound vitamin B_{12} malabsorption can be treated with oral vitamin B_{12} (Carmel, 1995; Lindenbaum, 1979). Although the vitamin B_{12} Recommended Dietary Allowance (RDA) for adults older than age 51 years is as low as 2.4 μg/day, very low doses (<10 μg/day) have been rarely tested. However, efficacy of oral treatment with low doses gives the opportunity of food fortification with vitamin B_{12}.

Flour fortification (FF) with low doses of folic acid (FA) has been performed since 1998 in the United States and in Canada on the basis of individual trial results, which showed a reduction in the incidence of neural tube defects (NTDs) (Berry *et al.*, 1999; Vergel *et al.*, 1990). Considering these results, one wonders whether higher doses of FA could have a greater effect on the reduction of NTDs. The main concern related to a high daily consumption of FA (>1 mg/day) is a possible masking of the anemia and macrocytosis due to vitamin B_{12} deficiency, which could consequently lead to neurological diseases. FF with both FA and vitamin B_{12} has been proposed to avoid this risk (Ray *et al.*, 2000; Tucker *et al.*, 2004; Winkels *et al.*, 2008). However, an essential question concerning this combined fortification (namely its metabolic response and the proper dose of vitamin B_{12} to be potentially added to flour) remains open.

VITAMIN B_{12} DEFICIENCY-RELATED DISEASES

One of the major issues regarding food fortification with vitamin B_{12} is the awareness of the clinical conditions related to vitamin B_{12} deficiency and the potential of the replacement therapy to prevent them. Also, the so-called FA fortification's masking effect on vitamin B_{12} deficiency status must be considered.

In nature, vitamin B_{12} is primarily found in meat and dairy products, and up to 50% of the ingested quantity can be actively absorbed.

Causes of vitamin B_{12} deficiency

Causes of vitamin B_{12} deficiency are firstly to be attributed to a nutritional deficiency, which is the expression of an inadequate dietary intake and reflects the characteristic of particular populations (elderly people, vegans, and alcoholics). Nonetheless, nutritional deficiencies represent a rare cause of vitamin B_{12} deficiency.

Other major causes of vitamin B_{12} deficiency are malabsorption syndromes, which include classical pernicious anemia (or Biermer anemia). This disease is due to an autoimmune destruction of gastric parietal cells resulting in a variable deficit in the intrinsic factor. Antibodies can be directed against the parietal cells or the intrinsic factor or both, resulting in a huge reduction in vitamin B_{12} absorption.

Food-bound vitamin B_{12} malabsorption is related to all conditions that generate compromised gastric acid production. Approximately 30–40% of patients with subclinical deficiencies were suspected to have food-bound vitamin B_{12} malabsorption, with normal free vitamin B_{12} absorption (Carmel, 1995). This condition is frequent in elderly people, in patients assuming a chronic acid-suppression therapy (proton pump inhibitor and histamine-2 receptor inhibitor), and after subtotal gastrectomy. Chronic atrophic gastritis may also lead to vitamin B_{12} deficiency. Other diseases related to malabsorption are celiac sprue, ileal resection, inflammatory bowel disease, small bowel bacterial overgrowth, tapeworm infestation, and some genetic disorders.

Consequences of vitamin B_{12} deficiency

Megaloblastic anemia is the typical hematologic manifestation of vitamin B_{12} deficiency, although it is well established that it only becomes evident when a very low blood level of vitamin B_{12} is reached. Other hematologic disorders related to vitamin B_{12} deficiency are leukopenia and thrombocytopenia.

Neurologic disorders secondary to vitamin B_{12} deficiency implicate damage to myelin and consequent lesions in the posterior and lateral columns of the spinal cord, in the white matter of the brain, and in peripheral nerves. The major symptomatology is due to peripheral neuropathy, with paresthesia and sensibility disorders. Spastic paresis, as well as autonomic bowel and bladder or sexual symptoms, may occur. Lethargy and weakness may also be present. Moreover, long-term vitamin B_{12} deficiency status can lead to a loss of brain tissue, and together with white matter impairment, it may be responsible for a decline in cognitive status (Smith and Refsum, 2009). Vitamin B_{12} deficiency-related dementia has been known since the 1950s, and there is now good evidence of the correlation between cognitive impairment and low vitamin B_{12} status (Smith and Refsum, 2009). Interestingly, many studies demonstrate that biological markers other than vitamin B_{12} can be predictors of a true vitamin B_{12} deficiency status, and that vitamin B_{12} blood level may not correspond to its actual tissue concentration. Low holotranscobalamin concentrations and high methylmalonic acid (MMA) and homocysteine levels are therefore better associated with cognitive impairment than is the level of vitamin B_{12} (Smith and Refsum, 2009). Other neuropsychiatric manifestations are irritability, depression, impaired memory, and, rarely, psychosis.

It has also been shown that reduced vitamin B_{12} status is a risk factor for NTDs, together with low FA. Indeed, high blood level of homocysteine, which can be caused by vitamin B_{12} deficiency, has been associated with pregnancy complications such as preeclampsia, placental abruption, NTDs, and congenital heart defects (Refsum, 2001).

A possible effect of low vitamin B_{12} status on cardiovascular morbidity has been advocated in relation to increased levels of plasma homocysteine, a well-known cardiovascular risk factor. However, to date, strict evidence for this correlation does not exist.

There is some evidence for interactions among micronutrient intake levels, genotype, and cancer. Both vitamin B_{12} and folates have an effect on DNA repair and thus can prevent the establishment of cancer. At the same time, they could accelerate cellular proliferation if the oncogenic transformation has already begun. Data are controversial and evidence is far from satisfactory.

Treatment of vitamin B_{12} deficiency-related disease

Replacement therapy with vitamin B_{12} aims to treat clinical disorders related to vitamin B_{12} deficiency. Pernicious anemia can benefit from parenteral therapy with vitamin B_{12} and potentially also from oral therapy at high doses, and food-bound vitamin B_{12} malabsorption can be treated and perhaps prevented with oral vitamin B_{12} supply (Blacher *et al.*, 2007). Neurological dysfunction severity as well as the likelihood of recovery are associated with the

duration of symptoms and the degree of disability achieved (Reynolds, 2006). On the contrary, cognitive impairment related to vitamin B_{12} deficiency is not known to be either reversible or preventable with vitamin B_{12} replacement therapy (Malouf *et al.*, 2008; Smith and Refsum, 2009). The available studies provide insufficient data and have too little power and duration.

FORTIFICATION OF FOOD WITH FOLIC ACID COULD MASK HEMATOLOGIC SYMPTOMS IN THE CASE OF VITAMIN B_{12} DEFICIENCY

NTDs are serious birth defects of the spine and the brain. Two of the most common NTDs are spina bifida and anencephaly, which together affect approximately 1 pregnancy out of 1000, resulting in 2500–3000 affected births in the United States annually (Honein *et al.*, 2001).

In numerous studies, FA supplementation has been shown to be effective in the reduction of both the incidence and the recurrence of NTDs, even in populations with low NTD prevalence and in populations with normal folate intakes (Berry *et al.*, 1999). For this purpose, fortification with FA (140 μg/100 g flour) became mandatory in the United States in 1998 (Food and Drug Administration, 1996).

Interestingly, this relatively low level of fortification was chosen to avoid an excess of folate intake in the population. It has indeed been shown that together with a correction of folate deficiency status, fortification was responsible for an excess of FA intake in a consistent portion of the population. This effect can also be due to a contemporary folate intake by other supply products (Morris *et al.*, 2007; Pfeiffer *et al.*, 2005).

Nonetheless, the main concern for a high daily consumption of FA (>1 mg/day) is a possible masking effect on vitamin B_{12} deficiency. Because synthetic FA is reduced directly to tetrahydrofolate, once absorbed, it can escape the metabolic trap caused by lack of vitamin B_{12} and therefore correct the potential condition of anemia and/or macrocytosis. This correction can then mask the clinical/biological marker of vitamin B_{12} deficiency and thus delay the diagnosis of such condition. In this situation, neurologic impairment could establish and even become irreversible before a diagnosis of vitamin B_{12} deficiency can be made. Elderly populations are considered to be at a higher risk of presenting with masked vitamin B_{12} deficiency when they increase their consumption of FA because of their higher average rate of low blood vitamin B_{12} concentration.

IS FLOUR FORTIFICATION WITH BOTH FOLIC ACID AND VITAMIN B_{12} THE SOLUTION TO THE SO-CALLED MASKING PROBLEM?

It has been shown that with FA fortification in the United States, 25% of elderly people and children younger than 11 years have folate concentrations >50 nmol/l and that the percentage of elderly people with both high folate and low vitamin B_{12} status was 4% in the NHANES cohort (Morris *et al.*, 2007; Pfeiffer *et al.*, 2005). Morris *et al.* indicated that a status of low vitamin B_{12} and high folate was associated with anemia and cognitive impairment. Whether such association is to be ascribed to the toxic effect of elevated FA blood levels or to vitamin B_{12} deficiency or both remains to be established. Many studies have focused on the importance of a good equilibrium in vitamin B_{12} and FA levels, and the idea of food fortification with both FA and vitamin B_{12} has become stringent.

So far, two conclusions have become clear. First, a fortification program with 140 μg of FA per every 100 g of flour probably achieved some results in terms of reduction of NTDs, but a greater reduction could probably be achieved with higher levels of FA fortification. Second, the imbalance between vitamin B_{12} and FA levels can produce adverse events, at least in a specific segment of the population.

For these reasons, including vitamin B_{12} in the FA fortification program has been advocated as a feasible way not only to avoid the masking effect and increase the blood levels of vitamin B_{12} in the general population, particularly in the elderly, but also to increase the level of fortified FA and thus help to further reduce the incidence of NTDs. Indeed, two studies have demonstrated that co-fortification of bread or breakfast cereals was associated with increased FA and vitamin B_{12} levels and with decreased homocysteine concentrations in a significant portion of the population (Tucker *et al.*, 2004, Winkels *et al.*, 2008).

TECHNOLOGICAL ISSUES

Crystalline vitamin B_{12} is the classic oral formulation for supplement intake of vitamin B_{12}. To obtain FF with crystalline vitamin B_{12}, it is first necessary that bread/flour proteins do not fix this compound, allowing its maximum absorption. Second, the fortification procedure should not modify color and taste of the final product. Third, crystalline vitamin B_{12} must be stable at high temperatures (e.g., while baking bread): This allows a lower level of destruction of the vitamin. Finally, the correct dose of supplementary crystalline vitamin B_{12} must be calculated in order to obtain the desired absorption amount.

Some experiments have been performed to resolve these issues. With regard to the potential fixing of the vitamin to bread proteins, studies have demonstrated that the quantity of bounded vitamin B_{12} is less than 1%. Moreover, incorporation of vitamin B_{12} in flour, even at high doses (500–1000 μg/100 g flour), has been shown not to modify the aspect of the final product. A total of 45% of crystalline vitamin B_{12} has been found to be destroyed while the fortified bread is being baked. For this reason, and based on results of modeling the mean and the extreme amounts of flour consumption in a variety of subjects, a dose of 10 μg/100 g flour has been proposed as a possible solution. Nevertheless, the correct dose of vitamin B_{12} has still to be determined (Czernichow *et al.*, 2003).

CONCERNS ABOUT AND THE LACK OF DATA ON CO-FORTIFICATION WITH BOTH FOLIC ACID AND VITAMIN B_{12}

In considering the real promise of vitamin B_{12} fortification, we have to focus our attention on some important issues concerning missing data:

1. Although toxic effects of vitamin B_{12} supplement have not yet been proved, larger studies with the primary objective to evaluate such potential adverse events are lacking, particularly those studying long-term effects and cancer.
2. Vitamin B_{12} plasma concentration is known to be a less sensitive marker of vitamin B_{12} deficiency than MMA and homocysteine, and the normal plasma vitamin B_{12} range might be useless in diagnosing early deficiency status.
3. The appropriate dose of vitamin B_{12}, as well as the chemical form and food vehicle, has not been defined definitely.
4. Monitoring programs of the vitamin B_{12} status among the general population and adverse events have not yet been developed.
5. It has not been well established if the vitamin B_{12} deficiency status becomes clinically relevant either in a continuous way or with a threshold level, making the definition of clinical low vitamin B_{12} status challenging.
6. A cause–effect relation regarding the association between vitamin B_{12} deficiency and cognitive impairment has not definitely been proven.

METABOLIC RESPONSE OF FLOUR FORTIFICATION WITH VITAMIN B_{12}

Vitamin B_{12} deficiency is mostly associated with compromised intestinal absorption, and parenteral vitamin B_{12} has been assumed as the standard treatment for low vitamin B_{12} status.

However, it has become clear that even when the mechanism of active vitamin B_{12} absorption is lost, passive vitamin B_{12} absorption is still possible, leading to a 1% absorption of the orally assumed dose. Therefore, although pernicious anemia and states of severe neurologic impairment have to be treated with parenteral therapy, studies have proven the feasibility of oral treatment in certain clinical conditions.

Although high doses of vitamin B_{12} (1000 μg/day) have already been tested, studies show that lower doses, ranging from 10 to 1000 μg/day, can also be effective (Blacher *et al.*, 2007). Moreover, one study tested the effects of 2.5, 100, 250, 500, and 1000 μg oral doses of vitamin B_{12} on sensitive markers of vitamin B_{12} status (Eussen *et al.*, 2005).

Blacher *et al.* (2007) designed the BOSSANOVA study ("*B_{12}* per *os* chez les *s*ujets *a*gés carencés par *no*n absorption de la *v*itamine B_{12} *a*limentaire") to test the ability of a very low oral dose to increase serum vitamin B_{12} in patients with subclinical deficiency (primary goal) and to assess whether there is a dose response. The final objective was to obtain quantitative data to determine the optimal level for vitamin B_{12} FF.

The BOSSANOVA study is a randomized, double-blind trial that evaluated the biological effects of daily oral vitamin B_{12} supplementation at low dosages for 30 days. Patients were randomly assigned to one of six groups of vitamin B_{12} dose (2.5, 5, 10, 20, 40, 80 μg/day) and treated with this daily oral dose for 30 days. Daily doses were administered to the patients in the morning after breakfast under the supervision of the medical staff. The study took place in 2003 and 2004 at the Emile Roux Geriatric Hospital (Limeil-Brévannes, France). Every patient entering Emile Roux Hospital during that period was screened for vitamin B_{12} status. In case of lowered vitamin B_{12} (<162 pmol/l), all the following inclusion criteria had to be fulfilled for inclusion:

- Age >70 years old
- Absence of fatal disease determining a life expectancy <1 month
- Planned hospitalization duration >1 month
- Lack of relevant signs of vitamin B_{12} deficiency (absence of severe cognitive impairment with Mini-Mental State Examination = 23/30, hemoglobin >100 g/l, absence of clinical peripheral neuropathy, and absence of clinical signs of subacute combined degeneration of the spinal cord)
- Absence of known vitamin B_{12} hypersensitivity
- Willingness to give a written informed consent to participate in the study
- Food-bound vitamin B_{12} malabsorption according to Carmel's criteria, excluding Schilling test (Carmel, 1995).

After an inclusion period of 9 months (2004), 1277 inpatients were screened for vitamin B_{12} status. A total of 190 patients (15%) had a serum vitamin B_{12} level <162 pmol/l, and 89 of them fulfilled all the inclusion criteria and were randomized; the mean ± SD vitamin B_{12} serum level was 136 ± 18 pmol/l (n = 89). A total of 67 patients completed the BOSSANOVA study; the remaining 22 randomized subjects who did not complete the study were excluded for one of the following reasons: consent withdrawal, transfer to another hospital for urgent surgery, intensive care, hospitalization shorter than 1 month, or death (Figure 40.1).

Table 40.1 gives the baseline characteristics of the BOSSANOVA study population. As expected, the serum homocysteine level and MMA were increased compared to the usual values, whereas mean plasma folates were still within the normal ranges. It is important to note that 76% (51/67) of the study population had folate deficiency (plasma levels <6.8 nmol/l). This important proportion was not reflected in the mean value that was "normal" because some patients had very high levels in relation to FA supplementation.

1. Low serum vitamin B_{12} level.
2. Normal results of Schilling's test.
3. No dietary vitamin B_{12} deficiencies.
4. At least one of the following conditions:
   - Gastric disease: atrophic gastritis, type A atrophic gastritis, gastric disease associated with *Helicobacter pylori* infection, partial gastrectomy, by-pass gastric surgery (obesity), vagotomy, Zollinger-Ellison syndrome;
   - Pancreatic insufficiency: alcohol abuse, cystic fibrosis;
   - Gastric or intestinal bacterial overgrowth: achlorhydria, sprue tropical, Ogilvie syndrome;
   - Chronic drug treatment: acid-suppressing drugs (cimetidine, ranitidine, omeprazole…), biguanides (metformine);
   - Alcohol abuse;
   - Aging.

FIGURE 40.1
Carmel's criteria. Criteria for the diagnosis of the food-bound vitamin B_{12} malabsorption syndrome.

The biological effects of experimental treatments on serum vitamin B_{12}, homocysteine, and MMA are given in Table 40.2. Baseline concentration of values of vitamin B_{12}, homocysteine, and MMA did not differ among the six groups. A highly significant between-subject effect (baseline values) was obtained for each variable, but a log−dose effect was disclosed only for vitamin B_{12} ($p < 0.001$), with the slope of this effect being higher on Day 30 than on Day 15 (borderline significance, $p = 0.07$).

Figure 40.2 gives the mean values ± SD of serum vitamin B_{12} increase on Day 30 and the estimated linear log−dose relationship (departure from linearity, $F^4_{61} = 0.56$; not significant).

TABLE 40.1 Study Population Characteristics[a]

Age (years)	83.3 ± 7.4
Gender (% male)	43.3
Body mass index (kg/m^2)	24.8 ± 5.0
Mini-Mental State (0−30)	24.7 ± 2.5
Plasma creatinine (μmol/l)	78 ± 29
Hemoglobin (g/l)	121 ± 14
White blood cells (*n*/mm^3)	6,900 ± 2,400
Platelets (*n*/mm^3)	257,000 ± 91,000
Reticulocytes (*n*/mm^3)	46,000 ± 34,000
Mean corpuscular volume (fl)	92.7 ± 6.3
Serum vitamin B_{12} (pmol/l)	120 ± 49
Plasma homocysteine (μmol/l)	18.1 ± 6.9
Plasma folate (nmol/l)	28.7 ± 65.5
Red cell folate (nmol/l)	305 ± 433
Plasma methylmalonic acid (μmol/l)	1.87 ± 0.96
Total folate intake (μg/day)	236 ± 93
Total vitamin B_{12} intake (μg/day)	3.1 ± 0.9

[a]*Clinical and biological values of the population are shown as means ± SD or percentage.*

TABLE 40.2 Relationship between Values of Vitamin B_{12}, Homocysteine, and Methylmalonic Acid[a]

	Vitamin B_{12} (μg/day)						ANOVA *p* Value		
	2.5 (*n* = 8)	5 (*n* = 12)	10 (*n* = 12)	20 (*n* = 12)	40 (*n* = 11)	80 (*n* = 12)	Subject Effect	Dose Effect	Interaction Dose × Time
Serum vitamin B_{12} (pmol/l)							<0.0001	<0.001	0.07
Baseline	111 ± 38	124 ± 44	128 ± 44	103 ± 54	126 ± 40	127 ± 68			
Day 15	137 ± 50	151 ± 55	151 ± 52	155 ± 89	192 ± 47	199 ± 62			
Day 30	163 ± 44	142 ± 33	182 ± 67	171 ± 67	205 ± 56	206 ± 67			
Plasma homocysteine (μmol/l)							<0.0001	0.50	0.23
Baseline	18.6 ± 6.8	19.8 ± 7.3	21.8 ± 7.5	18.0 ± 6.4	14.6 ± 5.3	15.9 ± 7.1			
Day 15	17.8 ± 7.2	17.4 ± 5.4	17.1 ± 7.1	16.7 ± 4.9	14.7 ± 5.0	14.2 ± 3.8			
Day 30	18.5 ± 5.8	18.9 ± 6.7	18.5 ± 7.0	15.8 ± 6.0	16.6 ± 4.6	15.4 ± 6.6			
Plasma methylmalonic acid (μmol/l)							<0.0001	0.19	–
Baseline	1.43 ± 0.90	1.71 ± 0.88	1.95 ± 1.15	2.54 ± 1.04	1.81 ± 0.87	1.63 ± 0.59			
Day 30	1.85 ± 0.94	1.79 ± 0.88	1.96 ± 1.18	2.06 ± 0.92	1.66 ± 1.35	1.48 ± 0.56			

[a]*Serum levels before and after vitamin B_{12} supplementation are shown for each treatment group.*

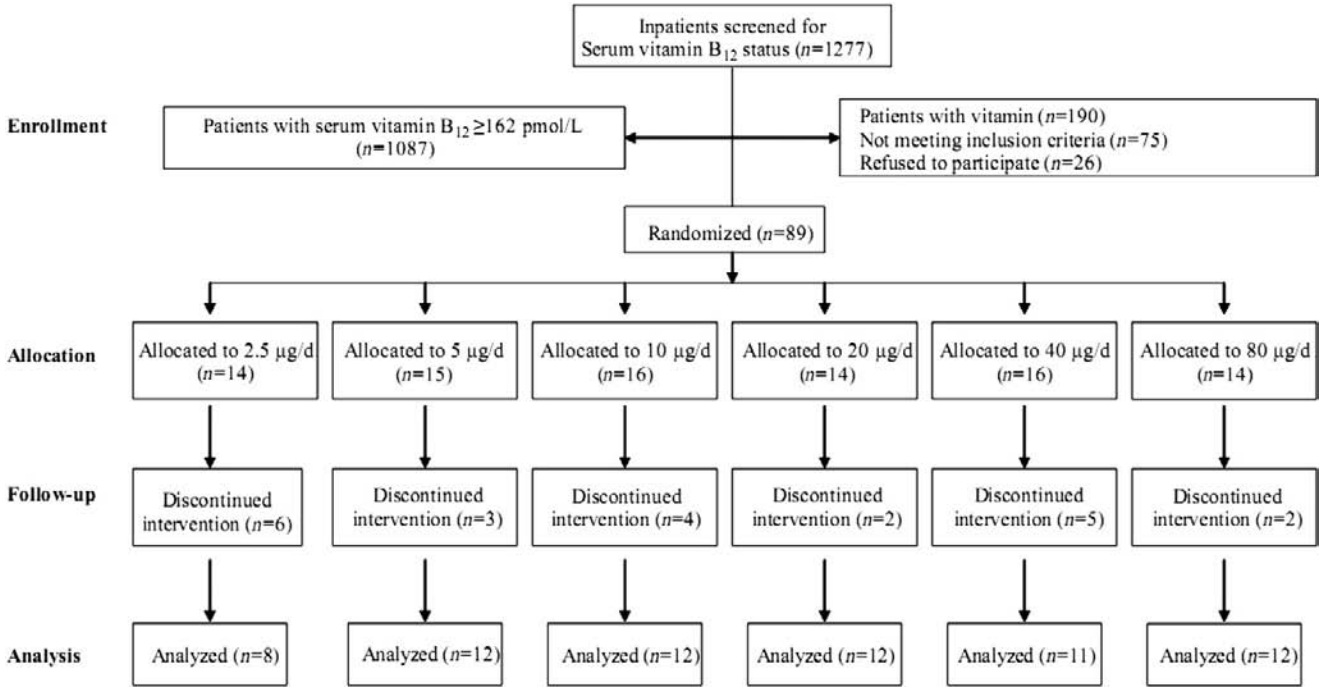

FIGURE 40.2
The BOSSANOVA study. Flow diagram of the progress throughout the study phases.

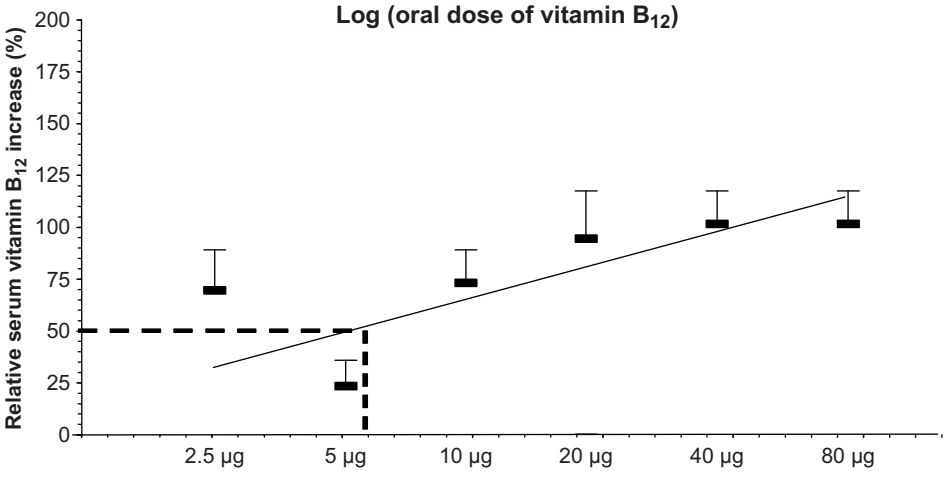

FIGURE 40.3
Response to oral vitamin B_{12}. Relation between the serum vitamin B_{12} concentration increase on Day 30 and the dose of oral vitamin B_{12} after 30 days of supplementation. Values are means + SEM (*n*). Dashed lines: for an increase of 37 pmol/l, a log dose of 1.7766 is needed, corresponding to a dose of 5.9 µg/day (95% confidence interval, 0.9–12.1). *Source: Reprinted with permission from Blacher, J., Czernichow, S., Raphael, M.,* et al. *(2007). Very low oral doses of vitamin B_{12} increase serum concentrations in elderly subjects with food-bound vitamin B_{12} malabsorption.* J. Nutr. *137, 373–378.*

For an increase of 37 pmol/l, a log dose of 1.7766 is needed, corresponding to a dose of 5.9 µg/day (95% confidence interval, 0.9–12.1) (Figure 40.3).

The salient findings of the BOSSANOVA study were that (1) very low oral doses were efficient to increase the serum vitamin B_{12} levels in elderly patients suffering from food-bound vitamin B_{12} malabsorption in a log–dose manner, and (2) 5.9 µg oral vitamin B_{12} per day for 30 days was needed to increase serum levels 37 pmol/l on average.

In this study, only two patients exhibited intrinsic factor antibody: This confirms that among the elderly presenting with low serum vitamin B_{12} levels, food-bound vitamin B_{12}

malabsorption was far more frequent than true pernicious anemia (Carmel, 1995). Furthermore, because serum vitamin B_{12} levels were significantly increased in these two patients after oral supplementation, the causes of vitamin B_{12} deficiency in this case are uncertain. It is important to note that the vitamin B_{12} assays performed for the screening were not used for analysis. Some of the patients included in the BOSSANOVA study on the basis of a low screening dosage presented a normal serum vitamin B_{12} level at the second determination several days later using a more precise method. This study population should thus be described as older patients with low to low–normal serum vitamin B_{12} levels.

There is undoubtedly an oral alternative to parenteral treatment for vitamin B_{12} deficiency, whatever its etiology is (Andres *et al.*, 2003; Carmel, 1995; Eussen *et al.*, 2005; Lindenbaum, 1979; Lindenbaum *et al.*, 1994; Seal *et al.*, 2002; Verhaeverbeke *et al.*, 1997). The present study showed that very low doses of crystalline cyanocobalamin can be efficient to normalize the average levels of serum vitamin B_{12} of the studied population but with an important variability related to low doses: On Day 30, mean vitamin B_{12} level was in the normal range (179 $\pm$ 61 pmol/l), but 29 patients (43%) still exhibited serum vitamin B_{12} $<$162 pmol/l. However, in the BOSSANOVA study, because mean serum vitamin B_{12} level was higher on Day 30 than on Day 15, the maximal biological effect was probably not obtained on Day 30 in this study population. Indeed, a 1-month vitamin B_{12} supplement at these low doses did not translate into a significant decrease in homocysteine and MMA levels. The absence of normalization of these two biological parameters could be related to insufficient doses, insufficient treatment time, or both. Previous studies have shown that different oral vitamin B_{12} doses could be associated with different homocysteine and MMA responses. Seal *et al.* (2002) reported that a treatment with oral vitamin B_{12} at low doses (10 and 50 μg/day for a 1-month period) increased mean serum vitamin B_{12}, but homocysteine was not significantly decreased. Rajan *et al.* (2002) performed an interventional study on a population of 23 elderly adults with food-bound vitamin B_{12} malabsorption receiving sequential daily oral treatment with 25 μg/day followed by 100 and 1000 μg/day crystalline vitamin B_{12}, each dose for a 6-week period. Although serum MMA levels had a similar significant decrease after the two first study periods, a much more important reduction was obtained after the 6-week period with 1000 μg vitamin B_{12} per day. Kuzminski *et al.* (1998) performed a randomized trial comparing the effects of oral versus parenteral vitamin B_{12} treatments; because a very high treatment dose (2000 μg vitamin B_{12} per day) was administered, the majority of the homocysteine and MMA decreases were obtained after only 30 days. Finally, in the BOSSANOVA study, the absence of homocysteine and MMA decrease was probably related to the very low doses. The long-term biological effects of such very low doses, as it could be expected in FF, need further investigation.

Three studies were used to assess/establish the average dose of vitamin B_{12} that should be added to flour to reverse low to normal values of serum vitamin B_{12}, as observed in subjects presenting with protein-bound malabsorption (Andres *et al.*, 2003; Seal *et al.*, 2002; Verhaeverbeke *et al.*, 1997). The first was a randomized trial that assessed the efficacy of oral cyanocobalamin in daily doses of 10 and 50 μg administered for 1 month to patients with serum vitamin B_{12} ranging from 100 to 150 pmol/l (Seal *et al.*, 2002). The second was an open trial measuring the biological effects of a daily dose of 100 μg of vitamin B_{12} on patients with a serum vitamin B_{12} level lower than 162 pmol/l (Verhaeverbeke *et al.*, 1997). Lastly, the third study was an open trial assessing the effects of 250, 500, or 1000 μg daily doses of vitamin B_{12} on patients with a serum vitamin B_{12} level lower than 162 pmol/l (Andres *et al.*, 2003). Based on the data from these studies, the increment from a low value of serum B_{12} (e.g., 111 pmol/l) to 148 pmol/l, which is considered as a normal value, might be achieved with a daily dose of 10 μg of vitamin B_{12}. In the present experiment on individuals, we found that the mean

necessary dose was 5.9 μg/day, with a relatively large 95% confidence interval (0.9–12.1 μg/day), which did not exclude the 10 μg/day dose.

The nutrient intakes measured in the BOSSANOVA study revealed that the patients presented relatively low average folates and vitamin B_{12} intakes in comparison with the RDA level. Those patients would have then benefited from a combined FF with both FA and vitamin B_{12}. FA flour fortification is the best way to prevent pregnancies affected by NTDs (Botto *et al.*, 2005). With respect to FA FF, an option to prevent the potential adverse outcome of vitamin B_{12} deficiency masking could be combined FF with both FA and vitamin B_{12} (Oakley, 2002). In Europe, Hungary is the only country that has started fortification with both vitamins, but no results have been published (Czeizel and Merhala, 1998). Preliminary results from FA fortification in Chile demonstrate an efficient and substantial increase in folate status in women of reproductive age (Hertrampf *et al.*, 2003) without any change, after 6 months, in vitamin B_{12} levels in a sample of elderly subjects with vitamin B_{12} deficiency (Hirsch *et al.*, 2002). Finally, the results of the BOSSANOVA study may have operational implications in the design of a public health program for a safe FF with FA plus vitamin B_{12}. Further research is needed to determine the feasibility and effects of such a combined fortification.

CONCLUSIONS

FA FF is the best way to prevent pregnancies affected by NTDs. With respect to FA FF, a possible method for preventing the potential adverse outcome of vitamin B_{12} deficiency masking would be to combine FF with both FA and vitamin B_{12}. Some studies have shown that low oral doses of vitamin B_{12} have metabolic effects on patients with low or low–normal vitamin B_{12} plasma levels. These data could be used in the design of a public health program for safe FF with FA plus vitamin B_{12}. Further research is needed to determine the feasibility and effects of such a combined fortification.

SUMMARY POINTS

- Vitamin B_{12} deficiency is a common clinical condition, sometimes leading to severe hematologic and neurologic diseases.
- Since folic acid fortification started, the concern of masking anemia and macrocytosis due to vitamin B_{12} deficiency, leading to neurologic disease, has emerged.
- Oral treatment with vitamin B_{12} is effective even at low doses in most cases of food-bound vitamin B_{12} malabsorption, and it has been advocated as a suitable means to prevent B_{12} deficiencies.
- Fortification with vitamin B_{12} is expected to reduce the vitamin deficiency-related diseases and prevent the folic acid masking effect. It may also offer an opportunity to increase folic acid dose in fortified food.
- More studies are needed to verify the appropriate dose and modality of vitamin B_{12} fortification and to examine safety and long-term outcomes.

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CHAPTER 41

Metabolic Effects of β-Glucans Addition to Corn Maize Flour

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

ae Endosperm mutant amylose extender, 50% or higher in amylose
CFA corn maize flour arepa
CFA-BA Corn maize flour arepa with 15 g of C-Trim 20
CFA-BB Corn maize flour arepa with 20 g of C-Trim 20
CFA-BC Corn maize flour arepa with 30 g of C-Trim 20
CMa Corn maize
CMa-F Corn maize flour
DF Dietary fiber
DM2 Diabetes mellitus type 2
GI Glycemic index
GIP Glucose-dependent insulinotropic polypeptide
GLP-1 Glucagon-like peptide-1
LDLc Low-density lipoprotein cholesterol
WWB White wheat bread

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INTRODUCTION

The evolution of the human diet and the emergence of a sedentary lifestyle have led to increased rates of obesity, metabolic syndrome, and chronic diseases such as type 2 diabetes (DM2) and heart disease. Although many factors are involved, metabolic changes caused by the Western diet might be implicated. These pathologies have become a global public health problem, and they have a huge financial impact on society that cannot be ignored. DM2 affects more than 150 million people worldwide, and the number is growing. Indeed, the cost of treatment of such a pandemic of associated conditions by conventional medical means is likely to be prohibitive. Dietary regulation of hyperglycemia and hyperinsulinemia will undoubtedly be more economically viable in the long term than pharmacological intervention, and thus it is likely to be an important method to control diabetes. There is thus an urgent need for broader strategies that influence the nutritive value of generally available foods without changing their popular appeal (Brennan, 2005). One of those strategies could be the addition of functional ingredients to generally consumed products.

Maize (*Zea mays*) is the third most important cereal grain in the world, after wheat and rice, providing nutrients for humans and animals and serving as a basic raw material for production of starch, oil, protein, alcoholic beverages, food sweeteners, and fuel. It is used throughout the world to make a wide variety of food products, such as breakfast cereals, pasta, tortillas, syrups, and bread. The diverse types of maize differ in the chemical compounds stored in the kernel (sweet, pop, dent, starchy or floury, and flint corn maize). The chemical compositions of the main parts of the maize kernel are different as well. The pericarp is high in crude fiber (~87%), mainly the insoluble type. Fiber is also provided by the endosperm and the germ cell walls. The endosperm (70–86% of the kernel weight) contains a high level of starch and is made up of two glucose polymers—amylose and amylopectin. In common maize (either dent- or flint-type endosperm), amylose constitutes 25–30% and amylopectin 70–75%. However, the starch in waxy maize is 100% amylopectin, and amylose in the endosperm mutant called amylose-extender (ae) could be 50% or higher. The germ (7–22% of the kernel weight) is characterized by high crude lipid content and relatively high levels of protein and minerals. On the other hand, the aleurone layer contains protein and crude fiber (Food and Agriculture Organization, 1993).

Factors such as composition of the grain, particle size, pH, amount and type of fiber, viscosity, amylose:amylopectin ratio, and cooking/processing methods affect the absorption of carbohydrates (Granfeldt *et al.*, 2000; Jenkins *et al.*, 1981; Makelainen *et al.*, 2007). Among these factors, the type and level of food processing determine the extent of starch gelatinization, particle size, and the integrity of the plant cell wall. For example, boiling intact cereal grains (rye, oats, barley, or wheat) causes low glucose and insulin responses, but disruption of the granule structure by milling before boiling can enhance the susceptibility to enzymatic degradation and increase postprandial glucose and insulin responses (Alminger and Eklund-Jonsson, 2008; Lehmann and Robin, 2007). In the northern part of South America, particularly Venezuela and Colombia, hard endosperm corn maize is processed with dry milling technology, and it is further converted into precooked flour for traditional maize foods. The process removes the pericarp and the germ; consequently, the flour contains lower amounts of proteins, lipids, fiber, and ash than the whole kernel, and it is relatively enriched in carbohydrates. The proximal composition of this commercial type of flour in Venezuela could be 77.92% carbohydrates, 8.24% proteins, 0.83% lipids, 12.13% humidity, 0.34% ash, and 0.54% fiber; enriched with vitamins (A, thiamine, riboflavin, and niacin) and Fe^{2+} (Hernandez *et al.*, 1999).

The amylose:amylopectin ratio is also very important to consider, and it has been used to explain differences observed in glucose and insulin responses to various starchy foods. Behall *et al.* (1989) and Amelsvoort *et al.* (1992) reported that higher amylose concentration is accompanied by a lowered metabolic response. *In vitro* study results showed that a possible

mechanism is a reduced rate of amylolysis, which could be related to the tendency of amylose to recrystallize or interact with lipids (Granfeldt *et al.*, 1995).

In addition to starch, cereals contain other polysaccharides also called dietary fiber (DF). The term DF is used to describe the edible parts of plants or analogous carbohydrates that resist digestion and absorption in the human small intestine, with complete or partial fermentation in the human large intestine. All DFs are presumed to have physiological effects (Table 41.1) and can be divided into soluble fiber (pectins, β-glucans, gums, mucilages, and storage polysaccharides) and insoluble fiber (cellulose, hemicelluloses, and lignin) (Brennan, 2005; Papathanasopoulos *et al.*, 2010). Among cereal grains, barley and oats are rich in soluble fiber components, especially β-glucan (37% w/w). It is a linear, unbranched polysaccharide composed of 1−4 O-linked (70%) and 1−3 O-linked (30%) β-D-glucopyranosyl units. The 1−3 linkages occur singly, and most of the 1−4 linkages occur in groups of two or three, leading predominantly to a structure of β-(1−3)-linked cellotriosyl and cellotetraosyl units (Butt *et al.*, 2008) (Figure 41.1).

β-Glucan fiber has been shown to improve metabolic responses during oral glucose test or when added to food products (pasta, bread, soup, drinks, cereals, and polenta) in healthy or diabetic subjects (Biorklund *et al.*, 2005; Nazare *et al.*, 2009; Tappy *et al.*, 1996). β-Glucans are also known to slow the rate of lipid absorption, increase bile acid transport toward the lower parts of the intestinal tract, enhance excretion of bile acids (Alminger and Eklund-Jonsson, 2008), reduce plasmatic low-density lipoprotein cholesterol (LDLc), and improve weight management and gastrointestinal function (Butt *et al.*, 2008; Lifschitz *et al.*, 2002; Makelainen *et al.*, 2007). Jenkins *et al.* (2002) also reported that the use of β-glucan can significantly reduce the glycemic index (GI) of foods (4 GI units per gram of β-glucan used) without negatively affecting the palatability of the food product.

TABLE 41.1 Potential Effects of Dietary Fiber Consumption

Type of Fiber	Effects
Soluble	Decrease postprandial glucose response
	Decrease postprandial insulin response
	Reduce total and LDL cholesterol
	Delay gastric emptying
	Delay intestinal absorption
	Higher production of short-chain fatty acids
Insoluble	Increase insulin sensitivity
	Decrease risk of type 2 diabetes
	Increase gut transit time
	Lower production of short-chain fatty acids
	Promote normal laxation
Soluble/insoluble	Low energy density
	Increase bulking effects
	Decrease energy intake
	Decrease weight gain
	Promote satiation and satiety
	Increase/decrease gut hormones release
	Diminish inflammation markers (CRP)
	Increase insulin sensitivity
	Decrease risk of type 2 diabetes
	Decrease risk of cardiovascular disease (reduce levels of PAI-1)
	Improve blood pressure
	Reduce risk of certain types of cancer (colon, rectum, and breast)

LDL, low-density lipoprotein; CRP, C-reactive protein.

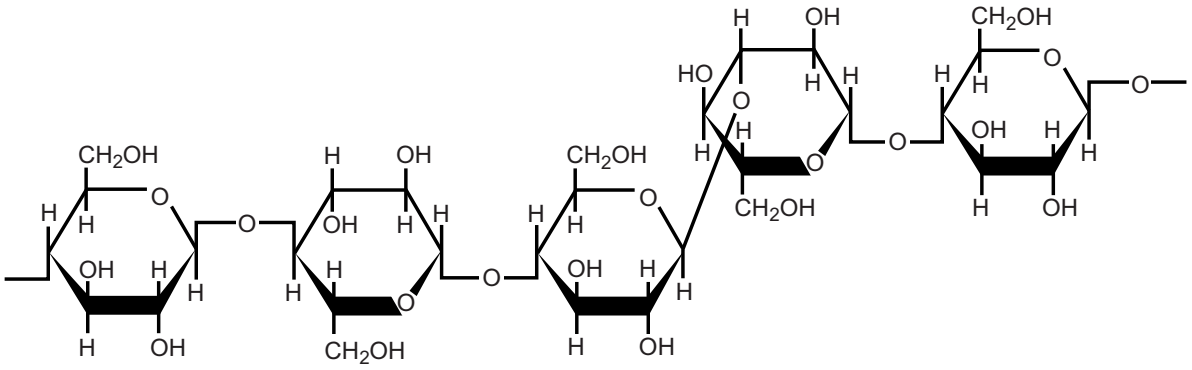

FIGURE 41.1
Chemical structure of β-glucans.

The GI is useful for determining the speed at which carbohydrates in the food are digested and absorbed as glucose (Brennan, 2005). Generally, based on the bread scale, high GI (higher than 90) stimulates insulin secretion because the starch in the majority of these foods (mainly breads and breakfast cereals) is rapidly digested. Medium GI (65–89) foods include all-bran, oatmeal, sweet potatoes, and yam. Finally, low GI foods (lower than 65) cause low post-prandial glucose response; these foods include pumpernickel bread, legumes, nuts, and parboiled rice. Consumption of low GI foods reduces the rate of glucose absorption, which in turn induces a lower rise in circulating insulin and lipids and modulates gut hormones such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (Kendall *et al.*, 2010; Makelainen *et al.*, 2007). Glycemic indices from corn maize (CMa)-based food are shown in Table 41.2.

Corn maize flour (CMa-F) is a product consumed in many different ways in many countries. However, in Venezuela, degermed precooked CMa-F is used to make the staple Venezuelan food "arepas" (a kind of unleavened flat bread that substitutes for white bread). Arepas has a GI higher than 72 and starch hydrolysis index of 85 (Foster-Powell *et al.*, 2002; Granfeldt

TABLE 41.2 Glycemic Indices for Based Corn Maize Food and White Bread

Food	Glycemic Index (%)	Reference Food	Available Carbohydrates (g/Serving)
White bread[a]	100.0	Glucose	14/30
Corn maize bread arepa[b]	72.2	Bread	35.3/100
Corn maize rice bread arepa[b]	86.0	Bread	32.2/100
Corn maize bread arepa[a]	71.5	Glucose	43/100
Corn maize bread arepa (25% amylose)[c]	81.0	Glucose	43/100
High-amylase corn maize bread arepa[c]	45.0	Glucose	43/100
Corn maize meal (boiled in water)[a]	97.0	Bread	13/150
Corn maize flakes[a]	92.0	Glucose	26/30

[a]*Data from Foster-Powell* et al. *(2002).*
[b]*Data from Schnell* et al. *(2005).*
[c]*Data from Granfeldt* et al. *(1995).*

et al., 1995; Schnell *et al.*, 2005). Nonetheless, the addition of β-glucans to the flour used to make this generally consumed food preparation possibly modifies its original metabolic fate without negatively affecting texture or palatably of the product so that it is possible to be used not only by healthy subjects but also by those who require diet regulation (e.g., treatment of dyslipidemias and diabetes) or for prevention of DM2. The aim of this chapter is to evaluate the metabolic effects of the addition of β-glucans to corn maize flour.

MATERIALS AND METHODS

Formulation design

In order to evaluate the metabolic effects of β-glucans addition on precooked CMa-F, a CMa-F-based preparation (arepa) was made. A CMa-F arepa (CFA) was prepared using a standardized method (64 g of flour and 134.4 ml of water) to obtain a 110 g final product with 50 g of carbohydrates, 0.7 g of lipids, and 4.6 g of proteins. An equivalent weight of the commercial white CMa-F was later substituted by 15, 20, and 30 g of β-glucans (C-Trim 20, supplied by the National Center for Agricultural Utilization Research, Peoria, IL), in order to make three different formulations: arepa with β-glucans A (CFA-BA), arepa with β-glucans B (CFA-BB), and arepa with β-glucans C (CFA-BC) (Table 41.3). A mixture of CMa-F or CMa-F with β-glucans and water was prepared, followed by kneading for 5 min until soft dough was achieved. The arepas were cooked in a special toaster for 7 min and allowed to cool.

Sensory evaluation

A sensory test was carried out to select the most acceptable formulation. For this purpose, a total of 20 untrained panelists were recruited, and a 9-point hedonic scale ranging from 1 = dislike extremely to 9 = like extremely was used to evaluate overall acceptance of the formulations (Peryam and Pilgrim, 1957). For the assessment, arepa samples were served in plastic cups and coded with three random numbers. The three samples consisting of CFA-BA, CFA-BB, and CFA-BC were tested on the same days and presented to panelists at random in order to avoid interaction between samples during the test day. The sensory test was replicated three times during different weeks. Every time, the samples were presented with water and paper ballots on a plastic tray. All sensory evaluations were conducted under white fluorescence light at a room temperature of 21 ±1°C. Panelists were instructed to consume the entire samples and to rinse their mouths with water between samples to minimize any residual effect (Table 41.4).

TABLE 41.3 Chemical Composition of the Formulations (Arepas with β-Glucans)

	Formulation		
	CFA-BA	**CFA-BB**	**CFA-BC**
Corn maize flour (g)	57.00	54.00	51.00
Carbohydrates[a]	44.30	41.96	39.60
Lipids[a]	0.63	0.59	0.56
Proteins[a]	4.10	3.88	3.67
C-Trim 20 (g)	15.00 (13%)	20.00 (16%)	30.00 (23%)
β-Glucans	3.14	4.18	5.23
Carbohydrates	6.29	8.38	10.48
Lipids	1.08	1.44	1.80
Proteins	4.05	5.40	6.75
Total weight (g)	115.00	125.00	130.00

CFA-BA, arepa with β-glucans formulation A; CFA-BB, arepa with β-glucans formulation B; CFA-BC, arepa with β-glucans formulation C.

[a]*Data from* Tabla de Composición de Alimentos, para Uso Práctico. *División de Investigaciones en Alimentos, Instituto Nacional de Nutrición Publicación No. 42, Serie Cuadernos Azules, Caracas, Venezuela.*

TABLE 41.4 Sensory Evaluation of Formulations[a]

Formulation	Sensory Score
CFA-BA	6.52 ± 0.8^{a}
CFA-BB	6.02 ± 1.0^{b}
CFA-BC	$3.71 \pm 0.7^{a,b}$

CFA-BA, arepa with β-glucans formulation A; CFA-BB, arepa with β-glucans formulation B; CFA-BC, arepa with β-glucans formulation C.
[a]*Sensory scores are presented as arithmetic mean ± standard deviation. Similar letters indicate statistical difference;* $p < 0.001$.

Subjects

Fourteen healthy medical students between the ages of 18 and 22 years (20.1 ± 1) gave written consent to participate in the study according to the Helsinki ethical guidelines. All subjects were evaluated at the Metabolic and Endocrine Research Center "Dr. Félix Gómez," Faculty of Medicine, University of Zulia, Maracaibo, Venezuela. A detailed background clinical history (which included family history of heart disease, diabetes mellitus, hypertension, dyslipidemia, and obesity) was carried out; a physical examination and laboratory tests were performed for each patient in order to rule out cardiovascular disease and confirm health conditions. Height (Detecto 140-kg balance device), weight, bioimpedance (Tanita Bia body fat analyzer, which incorporates weighing scales; TBF-401, Tanita, Tokyo), skinfold thickness, waist circumference, and blood pressure measurements were carried out as well. Body mass index was also calculated. Total cholesterol, high-density lipoprotein cholesterol, triacylglycerides, and glycemia from all subjects were normal before entering the protocol and were determined using commercial kits (Human Gesellschaft für Biochemica und Diagnoses Mbh). LDLc was calculated by the Friedwald formula. Utilization of any medication was an exclusion criterion. Approval of the study was given by the ethics committee of "Dr. Félix Gómez" Research Center at Zulia University.

Test meals

Three different meals—white wheat bread (WWB), CFA, and CFA-BB (previously selected by sensory test)—were served as breakfast to the subjects, in randomized order, after overnight fasting on three different occasions, with at least a 1-week interval. The WWB and CFA were used as references. The test meals were fed in order to provide 50 g of carbohydrate and were served with 250 ml of water. Basal and postprandial blood glucose concentration was determined by the glucose oxidase method (Human Gesellschaft für Biochemica und Diagnoses Mbh), and enzyme-linked immunosorbent assay was used to measure basal and postprandial insulin levels on serum (DRG Instruments GmbH, Germany).

Statistical analyses

All statistical analyses were carried out using SPSS software version 10.0 for Windows (SPSS, Chicago, IL). The results are shown as arithmetic mean ± standard error (SE). In order to select the more accepted formulation, analysis of variance (ANOVA) was used. One-way ANOVA was carried out as well to compare glucose and insulin responses between test meals, and a student *t* test was performed to compare basal and postprandial (glucose and insulin) levels for each meal. Differences were considered significant at $p < 0.05$.

RESULTS

The chemical composition of the three arepas C-Trim 20 (β-glucans) substituted formulations is shown in Table 41.3. All the resulting formulations had 50 g of carbohydrates, and C-Trim 20 represents 13, 16, and 23% of the total arepa weigh, respectively. Table 41.4 presents the

mean sensory score defined by panelists for the three formulations (arepas with β-glucans) evaluated. There was no significant difference between the CFA-BA and CFA-BB formulations, but there was a significant difference between CFA-BA and CFA-BC ($p < 0.001$) and between CFA-BB and CFA-BC ($p < 0.001$). These results indicate that both formulations with different amounts of β-glucans had the same acceptance by the panelists. However, the CFA-BB formulation was selected to carry out the evaluation of metabolic effects on the basis of its higher β-glucans constitution.

Basal blood glucose and serum insulin concentration as well as the glucose and insulin levels at the postprandial stage after consumption of three meals are shown in Table 41.5. A significant difference was found between CFA and CFA-BB ($p < 0.04$) when postprandial glucose levels were compared and also between WWB and CFA-BB ($p < 0.03$) postprandial insulin levels. Postprandial plasma glucose levels were not significantly different from basal levels after meal intake. However, insulin levels increased significantly after WWB ($p < 0.006$) or CFA ingestion ($p < 0.001$), but subsequent to CFA-BB intake no significant change in insulin levels was observed.

DISCUSSION

Effects on glucose and insulin response

Decreasing the postprandial glucose response is very important to public health. For this reason, many studies have examined the addition of soluble and insoluble fiber to different and commonly consumed food products. Numerous studies have reported inverse relationships between β-glucans content and glucose and/or insulin responses after ingestion of muffins, drinks, pasta, or tempe, with different amounts of β-glucans (Alminger and Eklund-Jonsson, 2008; Behall *et al.*, 2006; Makelainen *et al.*, 2007; Yokoyama *et al.*, 1997).

Nazare *et al.* (2009) reported similar metabolic effects when β-glucans were added to CMa products. In this study, the addition of 5 g of β-glucans to a polenta (Pol + β-glucans) delayed and slowed down the absorption of glucose but did not reduce the absorption of the carbohydrate in overweight subjects or the final quantities of glucose in plasma. They also indicated that the decrease in insulin secretion was delayed during the first 2 h, but after that, metabolic responses were different: The glucose response returned

TABLE 41.5 Postprandial Glucose and Insulin Responses to the Three Different Meals[a]

	White Bread	Corn Maize Arepa	β-Glucans Arepa CFA-BB
n	14	14	14
Fasting glucose (mg/ml)	81.8 ± 7.7	81.8 ± 7.5	87.9 ± 11.1
Postprandial glucose (mg/ml)[b]	82.2 ± 8.1	84.9 ± 8.4[a]	82.6 ± 10.1[a]
Treatment difference (%)[c]	↑ 0.5	↑ 3.8	↓ 6.0
p	NS	NS	NS
Fasting insulin (μU/ml)	13.7 ± 5.7	13.7 ± 4.4	11.8 ± 3.3
Postprandial insulin (μU/ml)[b]	23.4 ± 8.9[b]	20.8 ± 9.2	13.7 ± 2.8[b]
Treatment difference (%)[c]	↑ 70.1	↑ 51.8	↑ 16.1
p	<0.006	<0.001	NS

CFA-BB, arepa with β-glucans formulation B; NS, no significant difference was observed.
[a]*Data are shown as arithmetic mean ± standard deviation. Similar letters indicate statistical difference; a,* $p < 0.04$*; b,* $p < 0.03$.
[b]*Postprandial represents the mean of absolute value in 2 h.*
[c]*Treatment difference (%) = [(postprandial × 100/fasting] − 100.*

to baseline more slowly, and insulin secretion was sustained with a second peak at 150 min. Nevertheless, Battilana *et al.* (2001) showed that the level of exogenous glucose in plasma was 21% lower than that without β-glucans and was associated with a modest decrease in insulin secretion. However, the authors concluded that absorption of carbohydrates is decreased or delayed. This conclusion was supported by the results of Lifschitz *et al.* (2002).

Studies by Tappy *et al.* (1996) and Biorklund *et al.* (2005) on diabetic and healthy subjects have shown that there are decreased postprandial glucose and insulin responses following the ingestion of β-glucans, which are linked to a decreased nutrient absorption rate. However, in the current study as well as in those by Nazare *et al.* (2009) and Juntunen *et al.* (2002), lower glucose and insulin responses were not obtained at the same time. These reported discrepancies between intervention studies could be due to differences in study design, such as the duration of the dietary intervention, the quantity and form of β-glucans, the chosen food matrix, the presence of other nutrients, and the subjects under study (e.g., healthy, obese, or diabetics).

Water-soluble β-glucans exert their effects mainly by increasing viscosity in the small intestine by absorbing fluids, resulting in an extended digestion period. When digestion is delayed, blood sugar increases more slowly, causing a low insulin response. Although the effect has been established, the actions that cause the effect are not fully understood (Battilana *et al.*, 2001). In this respect, two hypotheses have been proposed. One establishes that in the intestine, food is incorporated in the viscous β-glucans solution, making it more difficult for enzymes to degrade the food components and causing lower digestion. Evidence from *in vitro* studies suggests that DF can alter the activity of pancreatic amylase. The effects on enzyme activity were attributed to pH changes, ion exchange properties, enzyme inhibitors, and absorption. Nevertheless, rather than a chemical enzyme–fiber interaction, the presence of fiber, through its particulate viscous nature, has been suggested to impede enzyme–substrate interactions. Furthermore, the presence of fiber in a form that restricts starch gelatinization or the access of the hydrolytic enzymes to starch can slow the rate of its digestion (Alminger and Eklund-Jonsson, 2008) (Figure 41.2).

The other hypothesis states that the β-glucans form a protective layer along the intestinal wall that acts as a viscous barrier or unstirred layer, slowing absorption of nutrient from intestine. In both hypotheses, the viscosity is involved. Viscosity is therefore a key to functional properties of β-glucans. In the intestine, due to the gel-forming property, β-glucans absorb fluids and add the viscosity during the digestion period. In addition, resistance of starch to pancreatic hydrolysis may result from the presence of intact cell walls, which survive processing and cooking and insulate starch in a manner that partially obstructs digestion and absorption (Makelainen *et al.*, 2007).

The delay in glucose absorption and the lower postprandial but sustained insulin secretion after β-glucans ingestion result in a prolonged inhibitory effect on endogenous glucose production and in longer inhibition of lipolysis and delivery suppression of counterregulatory hormones that occur with high blood glucose swings. The reduction in free fatty acid levels improves insulin receptor sensitivity and hence glucose is withdrawn from the circulation at a greater rate. Consequently, blood glucose levels remain close to baseline, despite continued glucose absorption from the small intestine. The peak postprandial blood glucose rise is therefore reduced together with the incremental blood glucose area above baseline—effects that are significant in individuals with insulin resistance (e.g., in obese, sedentary subjects) and diabetes (Kendall *et al.*, 2010; Nazare *et al.*, 2009).

Effects on gut hormones

Plasma insulin responses are closely associated with GIP and GLP-1 responses in healthy subjects. These two incretin hormones have been shown to be potent determinants of the

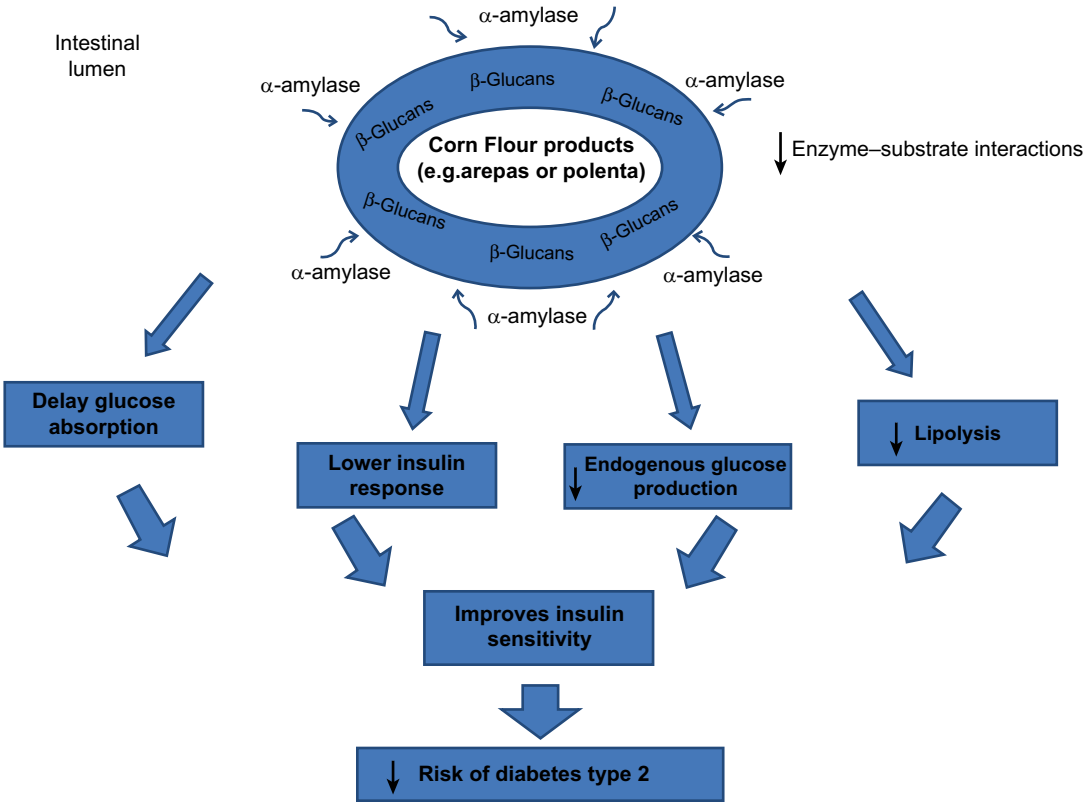

FIGURE 41.2

β-Glucans absorb fluids and add viscosity during the digestion period. The particulate viscous nature of fiber has been suggested to impede enzyme—substrate interactions. Also, the presence of fiber in a form that restricts starch gelatinization or the access of the hydrolytic enzymes to starch can slow the rate of its digestion, which results in favorable metabolic responses and improved insulin sensitivity.

postprandial insulin release that occurs after the increase in blood glucose; therefore, they are also essential in the regulation of postprandial glycemia. Because GIP was reduced after consumption of diets containing soluble fiber (e.g., guar gum) and grain products, it has been hypothesized that the potential mechanism mediating a significant reduction in insulin response after β-glucan consumption may be related to changes in gut hormones. A few studies have investigated the effects of DF on incretin responses; however, controlled studies in humans are few and contradictory. Most of them established that DFs such as β-glucan seem to reduce GIP and to augment GLP-1 responses, probably because of delayed or decreased carbohydrate absorption; nonetheless, the high postprandial response of GLP-1 to β-glucan remains unexplained (Juntunen *et al.*, 2002; Kim *et al.*, 2009).

SUMMARY POINTS

- The addition of β-glucans to degermed precooked corn maize flour produced favorably low metabolic responses in healthy young subjects. The increase in viscosity in the small intestine during the digestion period is the proposed mechanism for this effect.
- The addition of β-glucans to corn maize products is relevant for its therapeutic potential.
- β-Glucans can be incorporated into a wide variety of innovative food products, thus improving the diet of the general population.
- The prolonged inhibitory effect on endogenous glucose production and a longer inhibition of lipolysis after ingestion of β-glucans are important for the treatment of individuals with insulin resistance and diabetes.

- The delay of carbohydrate absorption may be related to the reduced levels observed in glucose-dependent insulinotropic polypeptide, a potent determinant of postprandial insulin release.

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CHAPTER 42

Lupine Kernel Fiber: Metabolic Effects in Human Intervention Studies and Use as a Supplement in Wheat Bread

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

BA Bile acid
CHD Coronary heart disease
CVD Cardiovascular disease

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DF Dietary fiber
HDL High-density lipoprotein
LDL Low-density lipoprotein
LKFiber Lupine kernel fiber
SCFA Short-chain fatty acid

INTRODUCTION

The fortification of bread with different kinds of nutrients is a common trend that is expected to increase in the future. It has been proposed that high organoleptic acceptability as well as beneficial physiological effects need to be demonstrated if nutritionally innovative functional ingredients are to be readily accepted by both the food industry and consumers (Clark and Johnson, 2002). In many developed countries, supplementation with dietary fiber (DF) is of particular importance because far too little DF is consumed, despite the increasing knowledge about its value. In recent years, the importance of DF in the human diet has gained increasing recognition because a high intake of DF has been associated with beneficial effects on human health. There is clear evidence that a diet rich in DF provides protection against diet-related disorders such as obesity, diabetes type 2, cardiovascular disease (CVD), and colon cancer (Bingham *et al.*, 2003; Burkitt, 1969). Current recommendations from the European Food Safety Authority suggest that adults should consume 25 g of DF per day, but the average daily intake of DF in Western society is only 12–18 g. Fiber can be isolated from a wide range of plant sources. Therefore, DF ingredients can have different chemical compositions, physical structures, and, in turn, varying nutritional and technological properties.

TECHNOLOGICAL ISSUES

The consumption of DF could easily be increased by consuming wholemeal and multigrain products, but many people do not like their rough mouthfeel. White bread is perceived as tastier than full-grain brown bread. The fortification of staple foods such as bread with DF seems to be a sound and promising approach for compensating fiber deficiency. However, the enrichment of food with sufficient amounts of DF to induce the desired physiological effects is a challenging task. On the one hand, the addition of DF changes the texture, flavor, and taste of the final products; on the other hand, the processing properties of the food are altered. One particular attribute of insoluble DF such as cereal fiber is the rough mouthfeel that impairs the taste of the respective food and gives the consumer the impression of eating a wholemeal product. A characteristic property of soluble fiber is its high water binding capacity. In low concentrations, this property can be helpful, for example, to enhance the juiciness and to avoid syneresis in various products such as sausages. However, the higher the amounts of fiber, the more water is required during processing. This is often not feasible or desired for bakery products because rheological alterations due to fiber and water addition hinder the processing and some water has to be removed at the end in order to obtain a tasty and nonperishable product. The thermal treatments usually have to be considerably prolonged, and often the desired residual water content cannot be achieved due to the high water retention capacity of the fiber. In addition, fiber supplementation in bakery products usually weakens the structure and baking quality of wheat dough and decreases the volume of the product. Furthermore, many types of DF, such as cereal, fruit, and vegetable fiber, still have the characteristic flavor of their origin and thus limit possible applications to selected products and limit the amounts that can be added. For this reason, DF with a smooth mouthfeel, a neutral flavor, and beneficial physiological effects is required for developing fiber-enriched food products.

LUPINE KERNEL FIBER AS AN ALTERNATIVE INGREDIENT

Besides well-known fibers such as cereal fibers and pectin, a fiber source that has received little attention but that should be considered in the future is lupine fiber. Like soy, peas, and beans,

lupines belong to the family Fabacea, commonly known as legumes. In addition to lupine varieties cultivated as flowering plants, other varieties are suitable as food and feed due to their high nutritional value (Figure 42.1). Important varieties are *Lupinus albus* L., mainly grown in South America and southern Europe; *Lupinus luteus* L., mainly cultivated in the Mediterranean region; and *Lupinus angustifolius* L., which is cultivated in central and eastern Europe, Australia, and New Zealand. Due to the high alkaloid content, old cultivars can only be used for food after a few days' soaking. At the beginning of the twentieth century, the breeding of cultivars that were low in alkaloids and could be used for food without debittering became possible. Lupines contain 5–15% oil, high contents of valuable proteins (30–50%) and DF (25–40%), and very low levels of antinutritional factors such as phytates, protease inhibitors, and lectins (Cowling *et al.*, 1998).

The direct use of lupine flour for bread fortification is limited due to its characteristic flavor, which is caused by accompanying constituents and fatty acid oxidation products. Isolated lupine kernel fiber (LKFiber), however, has a neutral flavor and can therefore be incorporated in high amounts without negative effects on the sensory profile. Compared to cereal fiber, LKFiber products have a smooth texture and a white color and are therefore ideal materials for the enrichment of wheat breads without generating the impression of wholemeal breads. The composition of an LKFiber product that could be used as a food ingredient is shown in Table 42.1. Note that the composition could vary within small ranges depending on the variety and processing conditions.

With regard to its technological properties, LKFiber possesses an excellent water binding capacity (Schweiggert *et al.*, 2009, 2011; Turnbull *et al.*, 2005). This is an important attribute from both a physiological and technological standpoint. The high water binding capacity indicates that this fiber has the potential to bulk and dilute the contents of the upper gastrointestinal tract, delay gastric emptying, and slow orofecal transit, thus prolonging the intestinal phase of nutrient processing and absorption. Apart from the physiological effects, the high water binding capacity results in substantial changes to dough processing and bread making.

FIGURE 42.1
Flowering lupines and lupine seeds. Due to their composition, the seeds of the lupine plant are valuable for animal and human nutrition.

TABLE 42.1 Composition of a Lupine Kernel Fiber Preparation[a]

	Amount (g/100 g)
Total dietary fiber	87
Soluble dietary fiber	53
Insoluble dietary fiber	34
Hemicellulose	11
Cellulose	23
Protein	10
Fat	<0.1

[a]*Lupine kernel fiber preparations can be produced with a total dietary fiber content of 87 g/100 g fiber product. The dietary fiber of lupine contains both soluble and insoluble fiber fractions, whereas the insoluble fiber consists of hemicellulose and cellulose.*

PRODUCTION OF LUPINE KERNEL FIBER

Similar to the production of other fiber preparations, LKFiber can be obtained by extracting proteins and other water-soluble substances from the dehulled and deoiled kernels as outlined in Figure 42.2. In a first step, the hulls of the raw lupine seeds are removed by dehulling and sifting. To enhance the extraction, the seeds are flaked or milled in order to increase the surface and destroy the cells. The oil is removed by extraction with, for example, hexane. In a multistage process, the deoiled and hence white flakes or meal are extracted with water at different pH values and temperatures to remove the sugars, proteins, and

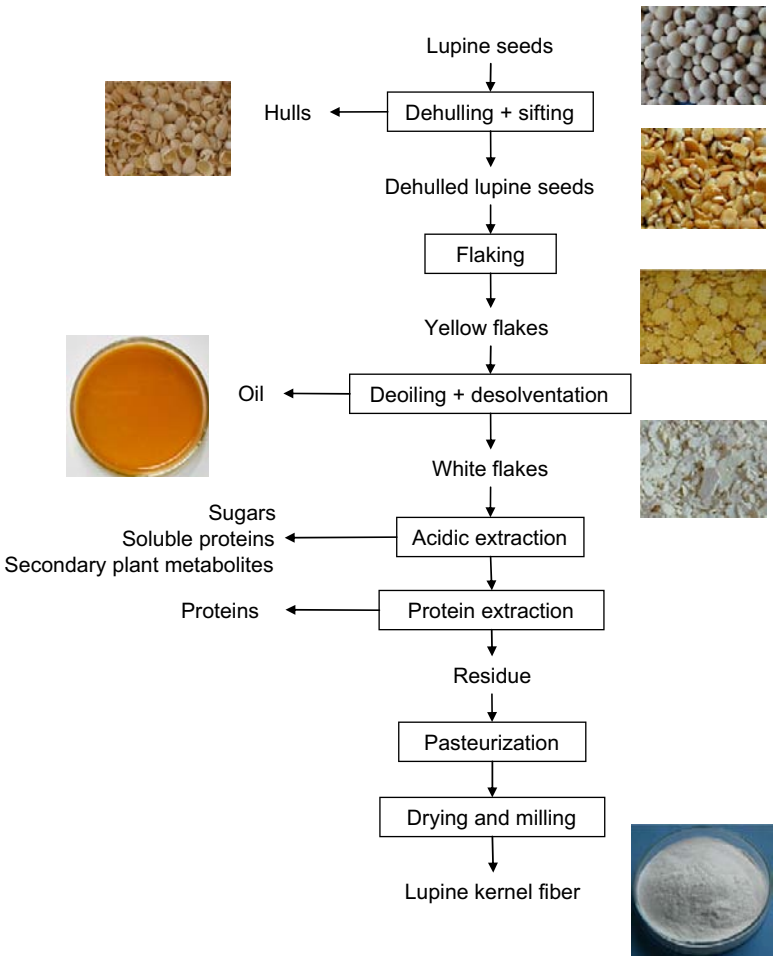

FIGURE 42.2
Multistage process for producing lupine dietary fiber products. Lupine kernel fiber isolation involves pretreatment of the lupine seeds (dehulling, flaking, and deoiling) and the extraction of concomitant constituents such as low-molecular-weight substances and proteins. Several other valuable fractions, such as lupine oil and protein, can be isolated during processing.

secondary plant metabolites. To obtain a smooth and fine LKFiber, the residue must be dried (e.g., by freeze-drying) and milled to the desired particle size.

METABOLIC EFFECTS OF LUPINE KERNEL FIBER

Important metabolic effects of DF for reducing the risk of CVD, diabetes, and cancer were originally suggested by Burkitt (1969) in his DF-hypothesis. A number of reviewers have examined studies concerning the relationship between DF consumption and the incidence of CVD and gastrointestinal disease, and most of them have found protective effects for one or both classes of disease.

Lupine kernel fiber and the risk of cardiovascular disease

The first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study on 9632 men and women who were free of CVD at their baseline examination indicated that legume consumption is significantly and inversely associated with the risk of CVD. Legume consumption four times or more per week, compared with less than once a week, was associated with a 22% lower risk of coronary heart disease (CHD) and an 11% lower risk of CVD (Bazzano *et al.*, 2001). A meta-analysis of 11 intervention trials involving legumes found overall a 6.2% lowering of low-density lipoprotein (LDL) cholesterol and a 22% lowering of triacylglyceroles.

In two randomized crossover studies, the lipid-lowering effects of LKFiber (*Lupinus angustifolius* Boregine) were compared in normo- and hypercholesterolemic humans. In the first study, healthy subjects consumed 25 g of pure LKFiber daily. The moderate hypercholesterolemic subjects of the second trial consumed a high-fiber diet containing 25 g LKFiber per day over 4 weeks (Table 42.2) (Fechner and Jahreis, 2011).

The blood lipids did not change in normocholesterolemic subjects. In contrast, the 4-week intervention with LKFiber-enriched food, studied in hypercholesterolemic subjects, led to a decrease of total plasma cholesterol by 12% ($p < 0.001$). The LDL concentration was lowered by 15% ($p < 0.001$), but high-density lipoprotein (HDL) cholesterol remained unchanged, which resulted in a 12% decline in the LDL:HDL ratio (Fechner and Jahreis, 2011). The reduction of total cholesterol and LDL was similar to the effects of soluble viscous fiber such as psyllium (Anderson *et al.*, 2000). The work group of Johnson also showed only a cholesterol-lowering effect of LKFiber in hypercholesterolemic patients (Hall *et al.*, 2005).

The hypocholesterolemic effect of legumes is primarily attributed to the soluble DF fraction and appears related to its ability to bind bile acids (BAs). The BA binding could lead to a higher BA excretion and thereby a lowering of plasma cholesterol by interrupting the enterohepatic circulation (Eastwood, 1992). The supplementation of LKFiber increased the daily excretion of

TABLE 42.2 Parameters for Two Human Intervention Studies with Lupine Kernel Fiber (LKFiber)[a]

	Study 1	Study 2
Intervention	Pure LKFiber product	LKFiber-enriched food
LKFiber (g/day)	25	25
Duration (weeks)	2	4
No. of subjects	26	54
Age (years)	20–30 ($\overline{X} = 24$)	>20 ($\overline{X} = 47$)
Cholesterol concentration (mmol/l)	Normocholesterolemia: $\overline{X} = 4.9$	Hypercholesterolemia: $\overline{X} = 6.6$
Standardized diet	No	Yes

[a]*Two randomized, crossover studies were conducted to investigate the lipid-lowering effects of LKFiber in normo- and hypercholesterolemic subjects.*

the primary BAs significantly, but the secondary BA excretion was slightly decreased. Presumably, the observed reduction in the pH value (Fechner *et al.*, 2009) is responsible for the rise in the excretion of primary BAs due to LKFiber. A reduction in pH inhibits the activity of the bacterial enzymes that are involved in the conversion of primary to secondary BAs (Bingham *et al.*, 2003). Another possible mechanism for the beneficial effect of LKFiber on blood lipids may be via inhibition of dietary cholesterol absorption. The cholesterol excretion was increased significantly in both studies. Furthermore, a significantly higher output of short-chain fatty acids (SCFAs) was observed during the LKFiber intervention (Fechner *et al.*, 2009). Therefore, the inhibition of hepatic cholesterol production via propionate-mediated effects can also help to explain the modified blood lipid profile (Anderson and Chen, 1979).

In addition to the previously mentioned lipoproteins, a relationship between CVD and other risk factors such as triacylglyceroles and C-reactive protein is described. The C-reactive protein is a marker of vascular diseases but is also believed to play an active role in atherogenesis. The LKFiber-enriched diet led to a significant decrease in the triacylglyceroles by 12% ($p = 0.03$) and in the high-sensitivity C-reactive protein by 19% ($p = 0.02$) (Fechner and Jahreis, 2010). Currently, the underlying mechanisms are unknown.

Furthermore, the intake of legumes can also reduce the risk of CVD due to favorable effects on blood pressure, glycemia, and the risk of diabetes (Anderson and Major, 2002). Consuming a high amount of fiber-enriched food has been reported to increase the perception of satiety and to modify nutritional behavior (lower intake of energy, fat, protein, and cholesterol), which supports long-term weight loss and protects against diet-induced obesity (Blundell *et al.*, 1994).

Lupine kernel fiber and colonic health

DF from different plant sources often possesses unique physicochemical properties, which results in various changes to bowel function. Some epidemiological studies have supported the hypothesis that increased DF intake leads to a reduced risk of colon cancer (Burkitt, 1969). Insoluble DF is considered to reduce the risk because the exposure of colonocytes to potential carcinogens is decreased by enhancing the fecal bulk and reducing the transit time. The fermentation of soluble DF produces SCFAs, of which butyrate has been linked to a reduction in the risk of colon cancer.

LKFiber is a novel food ingredient containing both soluble and insoluble fiber fractions. The objective of two human intervention studies was to examine the broader physiological effects of LKFiber (*L. angustifolius* Boregine) on different stool and digestive parameters in humans. The administration of the tested LKFiber at a dose of 25 g DF/day over 2 weeks (study 1, pure LKFiber) or 4 weeks (study 2, LKFiber-enriched food) was well tolerated by the subjects. The LKFiber interventions resulted in a significantly higher daily fecal mass and daily fecal dry matter. Consequently, the frequency of defecations was increased, the consistency of the stool was improved, and the orofecal transit time was shortened after LKFiber intervention (Fechner *et al.*, 2009). Similar results were obtained by the research group of Johnson. The consumption of a high-fiber diet containing 17−30 g/day LKFiber over 4 weeks increased the frequency of defecation by 0.13 events/day and fecal output by 21% while decreasing transit time by 17% (Johnson *et al.*, 2006). The increased stool weight is caused by the ability to bind water, the promotion of bacterial growth, and the stimulation of intestinal peristalsis. Thus, the contact of harmful substances, especially carcinogens, with the enterocytes is reduced (Cummings *et al.*, 1992).

It is suggested that DF, depending on its structure, is of benefit for the formation of SCFAs in the colon and therefore lowers the pH value, which decreases the formation of carcinogenic substances. The consumption of LKFiber led to enhanced formation of SCFAs whereby the total SCFA excretion as well as the excretion of the main SCFAs (acetate, propionate, and *n*-butyrate) increased significantly. Due to the enhanced formation, the SCFA concentration in

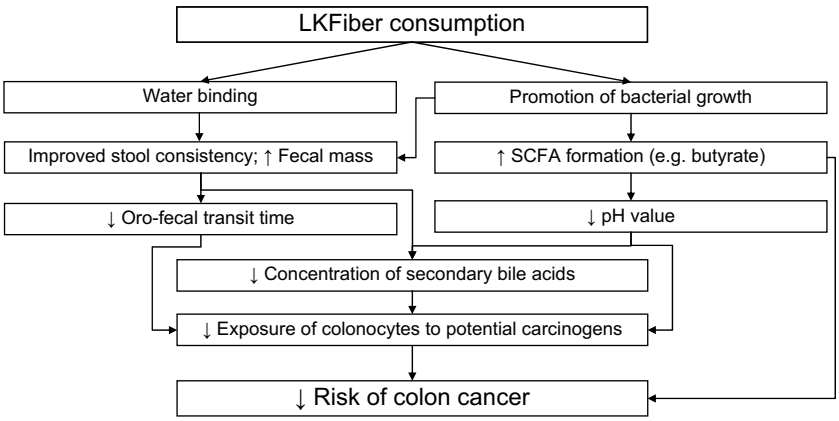

FIGURE 42.3
Schematic summary of the physiological effects of lupine kernel fiber (LKFiber) in the colon. Lupine kernel fiber has a positive impact on the putative risk factors of colon cancer, such as transit time, short-chain fatty acid (SCFA) production, pH value, and the concentration of secondary bile acids.

the feces was elevated and the pH value decreased (Fechner *et al.*, 2009). SCFAs, especially butyrate, are an important energy source for colonocytes. In addition, butyrate is able to reduce the risk of malignant changes through regulation of colonocyte differentiation (Johnson *et al.*, 2006; Topping and Clifton, 2001).

Moreover, the LKFiber intake lowered the fecal concentration of total BAs, especially the secondary BAs (Fechner *et al.*, 2009). The decrease in BA concentration is due to the increase in the daily fecal mass and fecal dry matter. Acid steroids, especially the secondary BAs, are potential risk factors for colorectal cancer (Reddy *et al.*, 1989).

In summary, the results of the studies show that LKFiber has a positive impact on the putative risk factors of colon cancer, such as transit time (daily fecal mass), SCFA production, pH value, and concentration of secondary BAs (Figure 42.3). LKFiber has a positive impact on the function of the colon and on health in general.

BREAD ENRICHED WITH LUPINE DIETARY FIBER

As mentioned previously, the technofunctional, sensory, and physiological properties indicate that lupine fiber is a highly valuable ingredient for food fortification. It has been identified as having potential as a nonintrusive ("invisible") ingredient in foods such as baked goods to form highly palatable fiber-enriched products (Clark and Johnson, 2002; Schweiggert *et al.*, 2009, 2011). However, the incorporation of fiber in general affects the processing conditions as well as the flavor and textural properties of the final products. In particular, the unusually high water binding capacity of LKFiber compared with other fiber products causes several technological changes (Turnbull *et al.*, 2005). Therefore, the application of LKFiber in white bread, one of the largest bread groups produced by the bakery industry today, is a great challenge.

Influence of lupine kernel fiber on the physical properties of dough

In general, the addition of fiber increases the water absorption of dough. This depends on the type of fiber and, in particular, on the ratio of insoluble and soluble fiber (Campos and El-Dash, 1978; Dervas *et al.*, 1999; Wang *et al.*, 2002). The water absorption—the amount of distilled water required to obtain a bread dough containing all ingredients with a consistency of 500 farinograph units—is increased by 2–26% on substituting 3–15% wheat flour by LKFiber (Dalgetty and Baik, 2006; Schweiggert *et al.*, 2011). The standard value for water absorption recommended by Klingler (1995) was easily reached on application of 5% LKFiber, corroborating the fact that the enrichment of bread with LKFiber caused higher dough yield and products with prolonged freshness during storage (Flander *et al.*, 2007; Thebaudin *et al.*, 1997).

The considerably higher water binding properties of LKFiber also have a strong impact on other characteristic dough properties such as the mixing time, the stability of the dough, the dough development time, and the degree of softening. The stability of the dough, which characterizes the resistance of the dough during mixing, is an attribute of the dough strength. It increased in proportion to the amount of LKFiber added, and concomitantly the dough development time was longer. The degree of softening—a feature for overmixing—decreased compared to the degree of softening of the reference material (wheat flour without LKFiber addition). The results support the conclusion that mixing LKFiber with wheat flour leads to a stronger dough that requires significantly higher energy input. Moreover, in order to develop an elastic gluten sheet, care is necessary to maintain an optimum mixing time and avoid overmixing because high-fiber dough has limited tolerance to overmixing. High-LKFiber dough is usually sticky and relatively stiff. Regarding the viscoelastic behavior of fiber-enriched dough, the dough extensibility and resistance to extension are important quality parameters. A combination of good resistance and good extensibility results in desirable dough properties. Data regarding the extension properties of LKFiber are scant. The research work that has been carried out employed lupine flour that still contained the lupine proteins, and this had an apparent impact on the dough quality (Campos and El-Dash, 1978; Dervas *et al.*, 1999). One study investigated the influence of LKFiber on extensibility and resistance to elongation (Schweiggert *et al.*, 2011). The reference dough exhibited elastic and extendible properties, which indicates excellent processing behavior and suitability for long fermentation processes and high proving tolerance. On addition of LKFiber, the extensibility of the dough was decreased, and a pronounced resistance to elongation with increasing fiber concentration could be observed. This means that the dough structure becomes more rigid and tough with poor extensibility. The dough hardly rises during proving, resulting in small pieces with poor spring and small baking volume. Campos and El-Dash (1978) found similar results with addition of sweet lupine flour. However, the impact on extension properties was not as distinctive as that for LKFiber, corroborating the positive influence of lupine proteins on the formation of a gluten network capable of retaining gas during the fermentation process.

Influence of lupine kernel fiber on the quality of baked white bread

Obtaining acceptable loaf volume for breads enriched with LKFiber becomes difficult to accomplish when the fiber content is increased. Fiber supplementation usually weakens the structure and baking quality of wheat dough and decreases bread volume.

On adding LKFiber, it was found that the dough or product yield increased but the whole product volume decreased by approximately 12−20% with ascending LKFiber concentration, which is also described for other fiber products (Dalgetty and Baik, 2006; Wang *et al.*, 2002). Breads made with 0, 10, and 15% LKFiber are shown in Figure 42.4 (Schweiggert *et al.*, 2009).

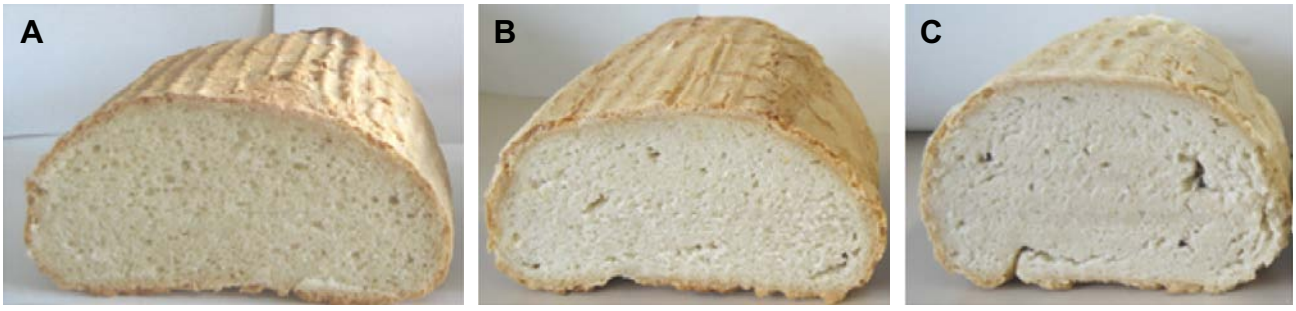

FIGURE 42.4
Cross-sections of the white breads. The addition of lupine kernel fiber (0% (A), 10% (B), and 15% (C) LKFiber) slightly alters the crumb structure, and the bread becomes more firm and moist.

For a pleasant crust color and crumb flavor, baking conditions such as temperature and baking time are quite important (Flander *et al.*, 2007) and have to be adapted for fiber-enriched breads due to the pronounced water binding and retention capacity of the fiber. Due to the different baking times for the reference bread (220°C for 60 min) and lupine-enriched breads (220°C for 80 min), a comparison of the crust color is not possible. The color characteristics of the crumb do not indicate any negative impact of lupine supplementation, supporting the observation that LKFiber may be used as a nonintrusive (invisible) food ingredient (Clark and Johnson, 2002).

In contrast to the color measurements, sensory tests revealed that the fiber addition was noticed by all trained persons, but it was evaluated as acceptable even at higher fiber concentration (Clark and Johnson, 2002; Schweiggert *et al.*, 2009, 2011; Wang *et al.*, 2002). After 3 days of storage, the bread enriched with fiber was preferred compared to the reference bread without fiber addition. Again, the excellent water binding properties, and primarily the moisture retention behavior, are responsible for the perceptible improvement in freshness of the breads. Similar impressions were described generally for fiber-enriched breads (Flander *et al.*, 2007; Thebaudin *et al.*, 1997).

CONCLUSIONS

Lupine seeds are a valuable source of DF. In addition to desirable technofunctional properties such as the water binding capacity and moisture retention behavior, LKFiber also provides several physiological benefits. Based on our own and other human intervention studies, it can be concluded that LKFiber has a positive impact on the putative risk factors of CHD, such as atherosclerosis. In addition, LKFiber is known to provide a health-promoting effect, particularly on the function of colon and on health in general. The inclusion of this palatable lupine fiber in the diet can help predisposed people in the prevention of colon cancer and CHD; also, the fiber consumption can support medical therapies. Due to its neutral taste, mouthfeel, and light color, LKFiber can be easily incorporated into staple foods such as white bread taking into account that an optimum amount of approximately 10% is recommended. In addition to the high consumer acceptance of such breads, the freshness of the breads is considerably prolonged with almost consistent moisture content. Regarding the intention of bread producers, the LKFiber could be used as a substitute for wheat, resulting in reduced carbohydrate content and caloric density and simultaneously increased satiety.

SUMMARY POINTS

- Due to its neutral taste, mouthfeel, and white color, lupine kernel fiber can be incorporated in high amounts into foods without impairing the sensory profile.
- Lupine kernel fiber can be obtained by extracting proteins and other water-soluble substances from the dehulled and deoiled kernels.
- Lupine kernel fiber decreases plasma cholesterol, triacylglyceroles, and C-reactive protein in moderate hypercholesterolemic subjects, but the mechanisms are not fully understood.
- Legumes affect hypertension, diabetes, and obesity. These factors can independently contribute to the cardiovascular protective effects of foods enriched with lupine fiber.
- Lupine kernel fiber has a beneficial impact on the function of the colon and on general health.
- Lupine kernel fiber has a positive impact on the putative risk factors of colon cancer.
- Lupine kernel fiber addition results in increased water absorption of the dough.
- The stability of the dough and dough development time increase with lupine kernel fiber addition.
- Dough extensibility decreases and resistance to elongation increases with increasing lupine kernel fiber addition.
- Dough yields increase considerably, whereas the volume of the bread decreases.

- Bread fortified with lupine kernel fiber at an optimum 10% level was preferred by consumers due to the prolonged freshness.
- Lupine kernel fiber can be used as a substitute for wheat, resulting in reduced carbohydrate content and caloric density.

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CHAPTER 43

Metabolic Effects of Propionic Acid-Enriched Breads

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

GI Glycemic index
GRAS Generally recognized as safe
SCFA Short-chain fatty acids

INTRODUCTION

An increasing body of evidence suggests that a low glycemic index (GI) diet has a therapeutic as well as a preventive potential in relation to the insulin resistance syndrome (Del Prato *et al.*, 1994; Järvi *et al.*, 1999). The tailoring of low-GI bread products offers a particular challenge due to their generally high GI and abundance in the diet. One of the approaches that can be used to lower the GI of bread products is the addition of organic acids or corresponding salts. For instance, sodium propionate has been widely used in the baking industry in low concentrations since 1930 to inhibit mold and bacteria growth. The literature is relatively

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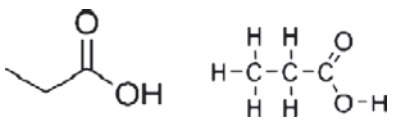
consistent in showing that oral supplementation with sodium propionate in humans enhances satiety and reduces the postprandial glycemic and insulinemic responses (Darwiche *et al.*, 2001; Liljeberg and Björck 1996; Liljeberg *et al.*, 1995; Todesco *et al.*, 1991). These metabolic effects suggest a potential therapeutic role for sodium propionate in the treatment of diseases related to insulin resistance. Bread products with added sodium propionate could be of particular interest in the coming years due to their capacity to optimize dietary treatments/interventions in insulin-related metabolic diseases by provoking low glycemic response, thereby producing fewer fluctuations in blood glucose concentrations. This chapter discusses the metabolic effects of propionate-enriched breads and highlights some promising research avenues.

CHARACTERISTICS OF PROPIONIC ACID

The chemical compound propionic acid (systematically named propanoic acid) is a naturally occurring carboxylic acid with chemical formula CH_3CH_2COOH (structural formula shown in Figure 43.1). In its pure state, propionic acid is a colorless, corrosive liquid with a sharp, somewhat unpleasant, odor. The anion $CH_3CH_2COO^-$ as well as the salts and esters of propionic acid are known as propionates (or propanoates). The physical properties of propionic acid are intermediate between those of the smaller carboxylic acids, formic and acetic acids, and those of the larger fatty acids. It is miscible with water, but it can be extracted from water with the addition of salt. Like acetic and formic acids, its gas grossly violates the ideal gas law because it does not consist of individual propionic acid molecules but instead of hydrogen-bonded pairs of molecules. It also undergoes such hydrogen pairing when solubilized in aqueous solution.

Propionic acid was first described in 1844 by Johann Gottlieb, who found it among the degradation products of sugar. During the following few years, other chemists produced propionic acid in various other ways, but none of them realized they were reproducing the same substance. In 1847, the French chemist Jean-Baptiste Dumas established that all these acids were the same compound that he called propionic acid. Propionic acid originates from the Greek words *protos* (first) and *pion* (fat) because it was the smallest acid molecule that exhibited the properties of other fatty acids, such as producing an oily layer when salted out from water and showing a soapy potassium salt.

In industry, propionic acid is mainly produced by the hydrocarboxylation of ethylene using nickel carbonyl as the catalyst. It is also produced by the aerobic oxidation of propionaldehyde. In the presence of cobalt or manganese ions, this reaction proceeds rapidly even at mild temperatures. Large amounts of propionic acid were once produced as a by-product of acetic acid manufacture, but changes in the manufacturing of acetic acid have made this procedure a very minor source of propionic acid nowadays. Propionic acid is produced biologically and synthesized as its coenzyme A ester, propionyl-CoA, from the metabolic breakdown of fatty acids that contain odd numbers of carbon atoms and from the breakdown of certain amino acids. Bacteria of the genus *Propionibacterium* produce propionic acid as the end product of their anaerobic metabolism. This class of bacteria is commonly found in the stomachs of



Skeletal structure Flat structure

FIGURE 43.1
Structural formula of propionic acid.

ruminants and the sweat glands of humans, and their activity is partially responsible for the odor of both Swiss cheese and sweat.

Propionic acid inhibits the growth of mold and some bacteria at concentrations varying between 0.1 and 1% by weight (Boyaval and Corre, 1995). As a result, most of the propionic acid produced is used as a preservative in both the animal feeding manufacturing and the human food industries. In animal feed, it is used either directly or as its ammonium salt. Its use in ruminant feeding has led to the development of an antibiotic, Monensin, that is added to cattle feed to promote propionibacteria in the rumen over the bacteria population that produces acetic acid. Propionibacteria metabolism produces less carbon dioxide and less methane in the rumen of ruminants, and the promotion of this bacteria population in rumen increases the conversion of feed into body weight gain, which contributes to the profitability of the industry. This application of the use of propionic acid in the animal feeding industry accounts for approximately half of the world production of propionic acid. In human foods, especially bread and other baked goods, it is used as its sodium or calcium salt. Propionic acid is also useful as a chemical intermediate. It can be used to modify synthetic cellulose fibers. It is also used to make pesticides and pharmaceuticals. The esters of propionic acid have fruitlike odors and are sometimes used as solvents or artificial flavorings.

The main hazard associated with propionic acid use is chemical burns that result from direct contact with the concentrated acid. Studies using laboratory animals showed that the only adverse health effect associated with long-term exposure to small oral amounts of propionic acid has been ulceration of the esophagus and the stomach. No toxic, mutagenic, carcinogenic, or reproductive effects have been reported. In the body, propionic acid has an elevated turnover rate and is readily oxidized as a precursor of the Krebs cycle intermediate succinyl-CoA. Its metabolic waste is eliminated from the body as carbon dioxide, which does not really represent a metabolic waste but, rather, a biological combustion by-product. The strategic metabolic role of propionic acid as carbon donor in the synthesis of succinyl-CoA to sustain cellular oxidative metabolism avoids any bioaccumulation. Its calcium salt and, to a lesser extent, its sodium salt have been used for more than 50 years as an inhibitor of mold growth in bread. The propionates have GRAS (generally recognized as safe) status for use in foods and have no upper limits imposed from regulatory organizations with the exception for breads, rolls, and cheese that are regulated under the Standards of Identity. Propionate salts can be used up to 0.3% in cheese products and to 0.32% by weight in flour used in white bread and rolls (Boyaval and Corre, 1995).

EFFECTS OF PROPIONATE ON APPETITE AND METABOLISM

Animal studies

Short-chain fatty acids (SCFAs)—that is, acetic, propionic, and butyric acids—are the main energy source for ruminants, generally accounting for 50–75% of digested energy. They are produced from the microbial fermentation of food in the rumen and are known to control feed intake. Propionic acid, which is a major SCFA produced in the rumen when the ration contains more cereals than forage, is responsible for a feeding-induced regulation behavior in ruminants and its absorption results in a downregulated energy intake in these animals. Indeed, many experiments have shown that intraruminal infusions of a SCFA mixture (acetic, propionic, and butyric acids) with a composition similar to that frequently found in the rumen cause a decrease in feed intake during the course of a meal (Baile, 1971; Bhattacharya and Warner, 1967). These effects on feed intake appear inversely proportional to the amount of SCFA infused, and the extent of the responses varies according to the amount of individual SCFA in the mixture. Among the three SCFAs, propionate likely plays the major and the special role in this regulation. Local administration of anesthetics into the rumen eliminates the anorectic effects of acetate and butyrate infusions but not the regulatory effects of propionate

infusions (Martin and Baile, 1972). Although reductions in stomach or rumen pH are known to decrease feed intake (Baile, 1971; Bhattacharya and Warner, 1967), the administration of these organic acids (propionate) as a salt component (sodium propionate) that prevented pH drop induced similar regulatory responses. Likewise, an infusion of sodium propionate into the hepatic portal vein depresses feed intake in sheep, whereas a similar infusion into a jugular vein exerts no regulatory effect on intake (Anil and Forbes, 1980). In order to clarify the nervous regulation of feed intake by propionate, lesions of the nerves of the hepatic plexus have been shown to eliminate the downregulatory effect of propionate on feed intake in the portal vein (Anil and Forbes, 1980). This control of feed intake also involves neural receptors in liver sensitive to the availability of oxidizable substrates, including propionate. Thermo- and osmoreceptors in liver also appear to be involved in this satiety process (Andrews, 1986).

Although the role of propionate in feed intake control is established in ruminants, there is little such evidence in single-stomached animals. The majority of the studies on this topic had as a goal to better understand feed intake control of farm animals in order to improve growth performance and economic productivity. Accordingly, similar evidence on anorectic effects of propionate in monogastric animals comes from studies using broiler chicks or turkeys. Pinchasov and co-workers (1993) showed that the incorporation of propionic acid into a low-energy diet significantly inhibited voluntary feed intake. However, after 10 weeks of *ad libitum* consumption, dietary propionate supplementation was proven less effective in controlling pullet growth than conventional feed restriction. The depression in feed intake and body weight with propionate fed as sodium propionate is induced no matter the route of administration, such as in drinking water or through the feeds in hatched turkeys (Donaldson *et al.*, 1994). Similarly, voluntary feed and energy intakes, as well as body weight gain, decreased significantly with the inclusion of propionic acid in the diet of female broiler chicks (Pinchasov and Elmaliah, 1995). Moreover, the relative weight of the abdominal adipose tissue was significantly decreased with the dietary inclusion of propionate. Giesting and Easter (1985) assessed the effects of organic acid supplementation on performance of starter and finisher pigs. The pig has many similarities with humans, making it an excellent research model to study metabolism on several aspects. As shown in Table 43.1, the results indicated that the addition of each acid (fumaric acid and citric acid) improved the efficiency of gain, whereas propionate depressed feed intake (Giesting and Easter, 1985). Similar results were found by Castell and co-workers (1994), who observed reduced average daily weight gains and feed intake when propionate was present in pig starter and grower diets, again emphasizing the anorectic effect of propionate.

The hypocholesterolemic effect of dietary propionate was observed in a study indicating that 0.5% sodium propionate-supplemented diets significantly reduced cholesterol accumulation in both serum and liver of cholesterol-fed rats (Chen *et al.*, 1984). In a second study involving

TABLE 43.1 Effect of 2% Organic Acid Supplementation on Performance of Starter Pigs[a]

Item	Control	Propionic Acid	Fumaric Acid	Citric Acid	Pooled Standard Error
Diet pH	5.78	4.71	4.18*	4.06*	—
Daily gain (g)	252	241	264	260	11.14
Daily feed intake (g)	492	438*	480	473	15.89
Gain/feed	0.51	0.55*	0.55*	0.55*	0.014

Source: Adapted from Giesting and Easter (1985).

[a]*The addition of each organic acid significantly improved the efficiency of feed utilization of starter pigs relative to the controls, whereas propionate supplementation caused a significant reduction in feed intake. Values are means with pooled standard errors. Pigs' average initial and final weights were 7.5 and 14.6 kg, respectively.*

**Significantly different from control ($p < 0.05$).*

adult male rats fed a nonpurified diet supplemented with 5% sodium propionate, plasma cholesterol concentrations were significantly depressed (Illman *et al.*, 1988). However, the authors observed that the mechanism responsible for the hypocholesterolemic effects of dietary propionate involved a redistribution of cholesterol from the plasma to the liver rather than inhibition of hepatic and intestinal cholesterol synthesis. Berggren and co-workers (1996) studied obese hyperinsulinemic (fa/fa) rats during a 19-day test period with sodium propionate fed either orally through the diet (1 g/day) or infused rectally (0.15 g/day) to animals given diets high in cholesterol (20 g/kg) and saturated fat (130 g/kg). At the end of the test period, total liver cholesterol pools were 20% lower in rats given dietary or rectally infused propionate (481 and 484 mg, respectively) compared to that of the control group (614 mg). In addition, fasting plasma glucose concentrations decreased significantly during the test period. These results are concordant with those of Boillot *et al.* (1995), who showed that dietary propionate chronically ingested by normal rats decreased fasting glycemia, but no effect on feed intake, body weight, hepatic glucose production, or whole-body glucose utilization was observed. In pigs, orally administered propionate appeared to depress cholesterol synthesis only when tallow was included in the diet (Boila *et al.*, 1981). In contrast, when propionate was infused into the cecum of pigs, increased total cholesterol levels were observed (Beaulieu and McBurney, 1992), whereas no impact was noted by others (Bach Knudsen and Canibe, 1993). In conclusion, the majority of evidence recorded in animals indicates a role for propionate in decreasing food intake; however, more studies are needed to shed light on the role of propionate on cholesterol and glucose metabolism.

Human studies

The available literature regarding the effects of propionate on appetite control and metabolism in humans is scarce. However, Venter *et al.* (1990) observed an increased serum high-density lipoprotein cholesterol concentration in healthy subjects after dietary supplementation with propionate, with no effect on total cholesterol concentration. Furthermore, propionate supplementation significantly decreased fasting serum glucose levels and maximum insulin increments during glucose tolerance tests. In that double-blind, paired-comparison, placebo-controlled study, the diet of 10 healthy female volunteers was supplemented for 7 weeks with 7.5 g sodium propionate daily in a capsule form, whereas the diet of the 10 control group members was supplemented with dibasic calcium phosphate in identical capsules as placebo. Likewise, Todesco *et al.* (1991) observed that postprandial blood glucose and insulin responses were significantly reduced in healthy subjects when propionate was incorporated into bread (3.3 g/50 g carbohydrate). The authors concluded that reduced postprandial glucose response when propionate is added to bread is due to decreased digestibility by inhibiting amylase activity, and the longer term effects on carbohydrate metabolism could be the result of adaptation in the absorptive capacity of the small intestine. Furthermore, the significant increase in fecal weight and increased anaerobic:aerobic bacteria ratio without changes in the breath-hydrogen response might suggest that propionate consumption results in increased starch losses to the colon (Table 43.2). In such an eventuality, the rate and type of fermentation of the starch may be reduced by the inhibitory effect of the increased amount of propionate.

Liljeberg *et al.* (1995) assessed glycemic and insulinemic responses in addition to satiety scores after ingestion of barley bread enriched with different additives in healthy human subjects. The results showed that consumption of bread baked with sodium propionate significantly lowered the postprandial blood glucose and insulin responses, and it significantly prolonged the duration of satiety compared with all other breads. As shown in Table 43.3, when calculating the areas under the satiety curve (0–180 min), a significantly higher value was found for the bread baked with the high concentration of sodium propionate than for wholemeal bread (reference product). However, the propionate-enriched bread at the lower concentration was judged more palatable by the participants. In that study, the rate of *in vitro* amylosis was not reduced by the ingestion of the bread containing propionate, suggesting that the beneficial

TABLE 43.2 Effect of Supplementing the Diet with Propionate for 1 Week on Fecal Weight and Colonic Micloflora[a]

	Day 0	White Bread	Propionate Bread
Fecal wet weight (g/day)	97.6 ± 30.5	117.4 ± 34.6	149.0 ± 43.2*
Fecal dry weight (g/day)	37.2 ± 22.6	41.3 ± 11.0	53.3 ± 14.9**
Anaerobic bacteria (log_{10} CFU fecal output/day)	10.5 ± 0.1	10.5 ± 1.2	11.1 ± 0.2*
Aerobic bacteria (log_{10} CFU fecal output/day)	9.1 ± 0.4	9.7 ± 0.5	9.3 ± 0.6
Anaerobic:aerobic bacteria	1.4 ± 0.5	0.8 ± 1.2	1.8 ± 0.6*
Bifido bacteria (log_{10} CFU fecal output/day)	9.0 ± 0.4	9.0 ± 0.4	9.6 ± 0.4*

Source: Adapted from Todesco *et al.* (1991).
[a]*One week of dietary supplementation with sodium propionate in bread significantly increased fecal bulk and anaerobic microflora, specifically as bifidobacteria. Values are expressed as mean ± SE,* n = *6.*
**Significantly different from Day 0 (*$p < 0.05$*)*
***Significantly different from Day 0 (*$p < 0.01$*).*

impact of this salt on the metabolic responses and satiety was related to effects other than the reduced rate of starch hydrolysis, as proposed by Todesco and co-workers (1991). Indeed, in a similar subsequent study in which the authors confirmed their previous observations that bread products with added sodium propionate flattened the postprandial blood glucose and insulin rise and prolonged satiety (Table 43.4), Liljeberg and Björck (1996) showed that a delayed gastric emptying rate could better explain the reduced postmeal glucose and insulin responses seen with bread products containing organic acids such as propionate. The authors used paracetamol in bread products as an indirect marker of the gastric emptying rate. However, one obvious disadvantage of this technique is that the food structure, and hence the gastric release of nutrients, may be affected as well as that of the marker due to differential enclosure time within the stomach. An alternative method for measuring the gastric emptying rate is ultrasonography. The advantages of using this method are that it is a noninvasive approach that does not affect the studied product and the results obtained are not confounded by other gastrointestinal events. In an attempt to avoid the disadvantages of the use of paracetamol, Darwiche and co-workers (2001) conducted a study aimed at evaluating, with the use

TABLE 43.3 Areas under the Satiety Curve (AUC) for Bread Products Added with Different Components[a]

	AUC
Wholemeal bread	316.2 ± 76.7[a]
Plus sourdough	317.2 ± 42.5[a,b]
Plus lactic acid	393.9 ± 62.5[a,b]
Plus Ca-lactate	425.9 ± 75.4[a,b]
Plus Na-propionate	409.7 ± 72.9[a,b]
Plus Na-propionate, high concentration	510.7 ± 89.8[b]

Source: Adapted from Liljeberg *et al.* (1995).
[a]*Consumption of bread baked with sodium propionate significantly prolonged the duration of satiety compared with all other breads. Values are expressed as mean ± SEM,* n = *11. Satiety was measured with a unitless scale. Values not sharing the same letters are significantly different (*$p < 0.05$*).*

TABLE 43.4 Postprandial Glucose and Insulin Areas under the Curve in Healthy Subjects after Two Different Bread Meals[a]

	Wholemeal Barley Reference Bread	Wholemeal Bread + Sodium Propionate
Glucose		
0–45 min AUC (mmol min/l)	67.5 ± 8.8	31.9 ± 6.3*
0–95 min AUC (mmol min/l)	117.8 ± 19.0	74.4 ± 11.5*
0–120 min AUC (mmol min/l)	125.4 ± 20.2	81.0 ± 12.2*
Insulin		
0–45 min AUC (nmol min/l)	10.8 ± 1.8	4.6 ± 0.8*
0–95 min AUC (nmol min/l)	21.3 ± 3.7	12.7 ± 1.8*
0–120 min AUC (nmol min/l)	23.2 ± 4.3	15.4 ± 2.2*
Satiety		
0–180 min AUC	304.2 ± 73.4	438.4 ± 93.1*

AUC, area under the curve.
Source: Adapted from Liljeberg and Björck (1996).
[a]*Bread products with added sodium propionate significantly lowered blood glucose and insulin responses and prolonged satiety. Values are expressed as mean ± SEM,* n = *12. Satiety was measured with a unitless scale.*
**Significantly different from the reference bread* ($p < 0.05$).

of ultrasonography, whether the lowered glycemic and insulinemic responses to bread ingestion after the addition of sodium propionate are explained by a specific effect of propionate on the gastric emptying rate. The authors indeed confirmed their previous hypothesis and observed that the gastric emptying rate of the barley bread decreased markedly after the addition of sodium propionate (Figure 43.2). These changes in the gastric emptying rate were accompanied by lowered glycemic and insulinemic responses and increased feeling of fullness. Prolonged postmeal satiety usually occurs concomitant with low gastric emptying rate because the extension of the stomach is potentially one factor that promotes a feeling of satiety. As discussed by Liljeberg and Björck (1996), different theories have been presented regarding the effects of salts of organic acids on gastric emptying. A nonspecific acid or pH receptor situated in the proximal part of the small intestine is responsible for the inhibition of gastric emptying (Lin *et al.*, 1990). The mechanisms by which sodium propionate affects gastric emptying have not been clarified. However, an influence of sodium propionate on gastric secretion is one potential mechanism (Darwiche *et al.*, 2001). In conclusion, human studies are apparently

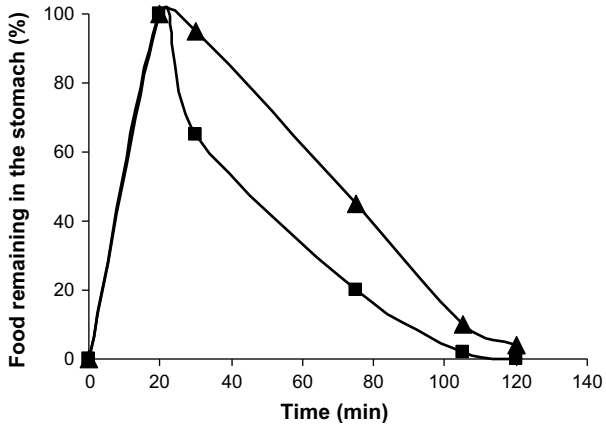


FIGURE 43.2
Median amount of food remaining in the stomach in healthy subjects after ingestion of a wholemeal barley bread ■ and an identical bread with added sodium propionate ▲. The gastric emptying rate of the barley bread decreased significantly after the addition of sodium propionate. Results are expressed as mean values, $n = 9$. *Source: Adapted from Darwiche* et al. *(2001).*

concordant in showing a beneficial effect of bread products with added sodium propionate on postprandial glucose and insulin responses as well as on satiety. Furthermore, the lowered glycemic response to the ingestion of bread with added sodium propionate could be related to a lowered gastric emptying rate. However, more studies are needed to clarify the underlying mechanisms.

THE LOW GLYCEMIC INDEX DIET

Effects on human health

Diseases relating to insulin resistance syndrome are common causes of death in Western societies, and the current increase in type 2 diabetes is being referred to as an epidemic. During approximately the past 15 years, an important number of studies have identified a low-GI diet as beneficial in relation to the insulin resistance syndrome. With a few exceptions, these studies have shown that a low-GI diet not only improves certain metabolic parameters associated with insulin resistance but also reduces insulin resistance *per se* (Del Prato *et al.*, 1994). In addition to improvements in glucose and lipid metabolism, there are indications of improvements in fibrinolytic activity (Järvi *et al.*, 1999), suggesting a beneficial role in diabetes and cardiovascular disease. Based on this evidence, the Food and Agriculture Organization/World Health Organization (1998) expert consultation on dietary carbohydrates strongly advocates the relevance of the GI concept, particularly for individuals with impaired glucose tolerance.

The GI is a system used to classify carbohydrate-containing foods according to how they affect blood glucose concentrations in the postprandial period (Jenkins *et al.*, 1981). GI is determined by measuring the 2-h incremental area under the blood glucose curve after consuming a test food (containing 50 g available carbohydrate) relative to that of a control, either white bread or glucose. Most varieties of bread, rice, breakfast cereals, and potato products have a high GI because the industry processing methods fully hydrate their starch. Hydrated starch is consequently rapidly hydrolyzed into glucose in the human digestive tract. By contrast, nonstarchy vegetables, legumes, nuts, and fruits have a low GI.

The metabolic requirement of the human brain for glucose provides a theoretical basis for understanding the evolving importance of GI in health. Hormonal regulatory systems have evolved to maintain stable concentrations of blood glucose under various conditions, such as fasting or feasting, consumption of foods with varying nutrient composition, varying levels of physical activity, illness, and pregnancy. However, one environmental condition rarely encountered before the modern era is the wide availability of high-GI foods. Before the agricultural revolution, human beings did not often consume grain products and concentrated sugars. With the technological progress of cereal grain processing, the GI of human diets increased substantially. In the past few decades, prevailing diets in the United States and Europe have become even higher in GI because of dual concomitance of daily increase in carbohydrate consumption with processed carbohydrates in food (Ludwig, 2007).

A high-GI diet elicits a sequence of hormonal events that challenge glucose homeostasis. Soon after a high-GI meal, blood insulin level rises higher than that after a low-GI meal with similar nutrients. Conversely, a high-GI meal inhibits glucagon secretion. The strikingly increased insulin:glucagon ratio constitutes a powerful anabolic stimulus, promoting uptake of nutrients in liver, muscle, and fat and suppressing hepatic glucose output. Within 60 min after a high-GI meal, blood glucose begins to fall, often reaching levels below fasting, and release of fatty acids from adipose tissue is suppressed. The body's attempt to restore the metabolic fuel concentrations to normal stimulates hunger and overeating during this metabolic occurrence of rapidly declining blood glucose with low nonesterified fatty acid concentration. In addition, the early postprandial hyperglycemia and hyperinsulinemia and the late postprandial hypoglycemia and counterregulatory hormone response could adversely affect body composition and increase the risk for diabetes, cardiovascular disease, and cancer (Ludwig, 2007).

TECHNOLOGICAL ISSUES

Worldwide, bread products constitute a major source of dietary carbohydrates. Consequently, there is a need for new technologies that can modulate the GI of bread. The addition of organic acids such as propionate is one of the methods that can be used to lower the GI of bread without adverse effect due to its rapid cellular oxidative metabolism. As shown in several studies (Darwiche *et al.*, 2001; Liljeberg and Björck, 1996; Liljeberg *et al.*, 1995; Todesco *et al.*, 1991), bread products with added sodium propionate exert beneficial metabolic responses on glucose and insulin levels as well as on feelings of satiety. In the near future, the food industry will likely add propionate to foods to produce non-acidic low-GI foods. With the increasing incidence of type 2 diabetes and other metabolic diseases, the beneficial potential of propionate as a preventive approach to the insulin resistance syndrome is likely a sound aspect to exploit (Chaput *et al.*, 2006). Experimental studies are needed to further document this topic and to tailor a low-GI bread possessing a good palatability while retaining the array of positive effects on metabolism and appetite control.

SUMMARY POINTS

- Propionic acid and its salts are widely used in industry and especially in the food industry as antifungal agents.
- The majority of evidence in animals indicates a role for propionate in decreasing feed intake.
- Human studies have shown a beneficial effect of sodium propionate-enriched bread products on postprandial glucose and insulin responses as well as on satiety.
- The lowered glycemic response to ingestion of bread with added sodium propionate appears to be related to a lowered gastric emptying rate.
- The use of propionate offers a new avenue to innovate in the production of low glycemic index breads.

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CHAPTER 44

Folic Acid and Colon Cancer: Impact of Wheat Flour Fortification with Folic Acid

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

5-MTHF 5-Methyltetrahydrofolate
CRC Colorectal cancer
FA Folic acid
KIR Killer cell Ig-like inhibitory and activator receptor
MTR Methionine synthase
NK Natural killer
NTDs Neural tube defects
SAH *S*-adenosylhomocysteine
SAM *S*-adenosylmethionine
SHMT Serine hydroxymethyltransferase

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common cancer in men and the third most common cancer in women worldwide. In the 2007 World Cancer Research Fund/American Institute for Cancer Research report, *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective,* it was concluded that there was a limited suggestive association of increased risk for colorectal cancer with intake of foods containing low folate concentration.

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Folate is a water-soluble B vitamin that participates in one-carbon metabolism, which has a critical function in methylation reactions and in DNA synthesis and repair. Thus, folate could play an important role in the pathogenesis of several disorders in humans, including anemia, cancer, cardiovascular disease, thromboembolic processes, neural tube defects (NTDs) and other congenital defects, neuropsychiatric disorders, and also in adverse pregnancy outcomes.

The association between dietary folate intake including natural and synthetic folic acid (FA) and risk of CRC has been evaluated in numerous epidemiologic studies. Results from these analytic investigations have generally been mixed. Whereas some studies have reported inverse associations, other studies have observed null and positive associations. Based on the negative association between low folate intake and highest risk of CRC, the total folate intake associated with colorectal adenoma or cancer risk reduction was estimated to be 600 μg/day (Bailey, 2003).

Although it is plausible that mandatory FA fortification could be associated with a decline in CRC incidence, a temporal association between FA fortification of enriched cereal grains or wheat flour and an increase in the incidence of CRC in the United States, Canada (Mason *et al.*, 2007), and Chile has been reported (Hirsch *et al.*, 2009). Moreover, in a meta-analysis aimed to determine the effect of FA supplementation on 3-year CRC risk, evaluated with a colonoscopic follow-up, FA supplementation did not have an effect on the overall adenoma recurrence. However, colonic surveillance beyond 3 years revealed an increased risk of colorectal adenoma, especially advanced adenomas, among those participants randomized to the FA-supplemented group (Fife *et al.*, 2009).

FOLATE METABOLISM AND FUNCTION

Folate is a water-soluble B vitamin that is present naturally in foods such as green leafy vegetables, asparagus, broccoli, Brussels sprouts, citrus fruit, legumes, dry cereals, whole grain, yeast, lima beans, liver, and other organ meats. Folates are the major donor and acceptor of one-carbon units in metabolic reactions in the tissues, known as one-carbon metabolism (Smith *et al.*, 2008). These one-carbon units can be at the oxidation level of methanol (5-methyltetrahydrofolate (5-MTHF)), formaldehyde (5,10-methyltetrahydrofolate), or formate (5- or 10-formyltetrahydrofolate or 5,-10-methenyltetrahydrofolate). Basically, all the folate forms in the tissue are polyglutamate in which the glutamate tail is extended via the γ-carboxyl of glutamate. The reaction of folate to polyglutamate forms is required for their biological activity because the polyglutamate forms are more effective substrates for folate-dependent enzymes than are monoglutamates.

The synthetic form of folate used in food fortification is FA (pteroylmonoglutamate), which differs from the natural compound because it is fully oxidized and contains only one conjugated glutamate residue (monoglutamyl form) and is not an active form of the coenzyme. In bread fortification, FA has higher stability and bioavailability (100% with empty stomach and 85% with food) than the natural forms (50%) because it is rapidly absorbed across the intestine. On the other hand, food folate must be cut to the monoglutamyl forms by a brush border glutamyl hydrolase before absorption. The process of absorption occurs in the jejunum by a saturable pH-sensitive transporter that transports oxidized and reduced folates. Most absorbed folates and FA are metabolized to 5-MTHF during their passage across the intestinal mucosa. However, when high amounts of FA are consumed, as with bread fortification, a percentage of unmetabolized folate appears in the peripheral circulation.

Folate is excreted by urine; however, the renal excretion capacity can be exceeded and, thus, plasma or serum levels can increase. Tissue folate levels increase less than those of plasma due to the limited capability of tissues to metabolize large doses to the polyglutamate form required for cellular inclusion.

Folate monoglutamate in plasma is transported to the tissues via the reduced folate universal carrier that has poor affinity for FA. Another high-affinity folate transporter, known as folate-binding protein, is expressed at a high level in specific tissues such as choroid plexus, kidney proximal tubes, and placenta and in different human tumors. Low levels of this receptor are expressed in a variety of other tissues. A third cellular transporter has been identified that transports reduced folate monoglutamates into the mitochondria.

When the metabolization of folate is saturated through the methionine synthetase reaction in the cell, much of the new folate absorbed is not retained by tissues and appears in the circulation, predominantly as 5-MTHF.

The biochemical function of folate is mediating the transfer of one-carbon units involved in nucleotide biosynthesis, the methionine cycle, and biological methylation reactions (Figure 44.1). In the methionine cycle, 5-MlTHF transfers one methyl group to homocysteine to synthesize methionine, guaranteeing the provision of *S*-adenosylmethionine (SAM), the primary methyl group donor for most biological methylation reactions, including that of DNA.

The remethylation of homocysteine to methionine is catalyzed by methionine synthase (MTR), a vitamin B_{12} (cobalamin)-dependent enzyme. The reductive methylation of the cobalamin cofactor of MTR to its active state is catalyzed by methionine synthase reductase (MTRR). After donating the methyl group, 5-MTHF is converted to THF and is subsequently transformed to 5,10-methylene THF by serine hydroxymethyltransferase (SHMT). SHMT catalyzes the reversible interconversion of serine and THF to glycine and 5,10-methylene THF and serves as a major entry point for one-carbon units into the folate pathway. 5,10-Methylene THF is a key substrate in folate metabolism, which can be directed toward nucleotide (thymidylate and purines) biosynthesis or toward methionine regeneration. The cellular concentration of 5,10-methylene THF appears to regulate the flux of this substrate into these different pathways.

Several lines of evidence indicate that folate metabolism is regulated by SAM synthesis because the end product and inhibitor of these methylation reactions is *S*-adenosylhomocysteine

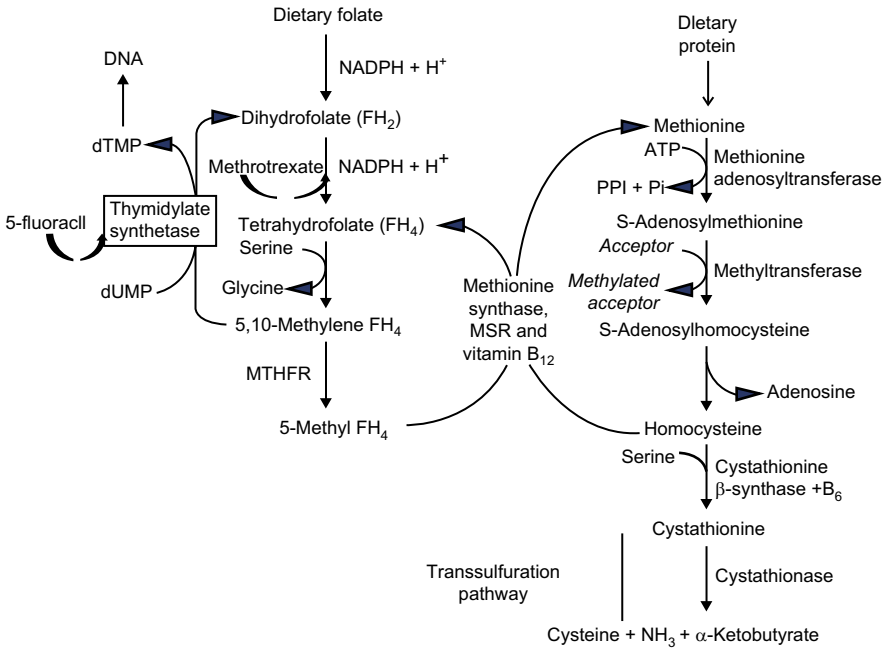

FIGURE 44.1
The methylation and folate cycle.

(SAH)—therefore, the SAM:SAH ratio has been termed the methylation index—and because SAM synthesis has a metabolic priority over thymidylate biosynthesis. Probably a limited methyl group availability, caused by either folate or methionine deficiency, shifts the flux of one-carbon units among folate-dependent pathways preferentially shuttling folate cofactors to the methionine cycle to protect methylation reactions and thereby suppress DNA synthesis.

Thus, deficient or excess intake of folate due to supplementation or bread fortification is associated with imbalances in one-carbon metabolism that could facilitate the development of several chronic diseases in animals and humans such as cancer.

FOLATE AND CANCER

Because folate is essential for methylation processes, and any disruption of these metabolic pathways could induce carcinogenesis, normal folate levels are critical for cancer prevention, as explained here (Kim, 2007):

1. Cellular replication: As cofactor for the synthesis of purines and thymidylate, folate plays a crucial role in DNA synthesis and replication. Therefore, in cancer cells that have an accelerated DNA replication and cell division rate, folate requirements are high. This effect was best demonstrated in the 1940s when Farber observed that large doses of FA given to individuals with acute leukemia significantly accelerated the rate of expansion of the leukemic clone. Therefore, bread fortification with high concentrations of FA could accelerate preexistent cancer cell replication. On the contrary, the absence of folate or the interruption of folate metabolism produces inhibition of tumor growth due to ineffective DNA synthesis. This is the basis of the antifolate agents (methotrexate and 5-fluorouracil) used for cancer chemotherapy in clinical practice.
2. Global and gene-specific methylation status: A consequence of folate deficiency is an increase in intracellular SAH and DNA hypomethylation, which is associated with genome instability, alterations in the expression of specific genes, induction of cellular differentiation, alterations in chromatin conformation, and cell phenotypic changes that contribute to carcinogenesis. In the case of tumor suppressors, for example, promoter hypermethylation can give support to tumorigenesis. Thus, dietary methyl group supplementation or bread fortification (FA) could increase genomic and promoter DNA methylation when the enzymatic system is saturated.
3. Protein methylation: SAM is the methyl donor for the methylation of carboxy, histidine, lysine, and arginine residues in proteins, which have a major effect on protein repair, targeting, signal transduction and modulation of enzyme activity, RNA metabolism, and transcription regulation. Methylation of lysine and arginine residues in histones participates in the regulation of gene expression and in epigenetic silencing, thus promoting the formation of heterochromatin. Folate concentration can influence demethylation and therefore promote epigenetic mechanisms.
4. Natural killer (NK) activity: NK cells are part of the nonspecific immune response and can kill tumor cells (epithelial tumor cells). NK cells express killer cell Ig-like inhibitory and activator receptor (KIR) 3. Experimentally, demethylation of *KIR* genes by 5-aza-2-deoxycytidine leads to rapid induction of *KIR* expression, whereas *in vitro* DNA methylation of the CpG cluster leads to inhibition of *KIR* promoter activity. Thus, folate levels could regulate NK activity, the first barrier to preventing endothelial cancer cell growth.

Consequently, low intake of folate equivalent or high intake associated with flour fortification may alter the methylation reactions and lead to carcinogenesis. In particular, colorectal neoplasms, both carcinomas and adenomas, show a decreased global DNA methylation level compared to normal tissue. Conversely, studies have shown methylation of the promoter region of specific tumor suppressor genes in colorectal tumors that are increasingly recognized to play an important role in cancer development through silencing of gene transcription.

Evidence suggests that DNA hypomethylation and hypermethylation are independent processes and contribute separately to the process of carcinogenesis (Mathers, 2009).

EPIDEMIOLOGICAL STUDIES

Before mandatory FA fortification was begun in many countries, the inverse association with folate intake or serum levels with CRC was shown whether folate was assessed in the diet or in blood.

More than 30 retrospective epidemiologic studies have explored the link between dietary folate or total folate intake (dietary and/or total folate intake including supplemental FA) and the risk of CRC or adenoma. Most of them reported a significant or ambiguous inverse association. Together, these retrospective studies suggest a 140% reduction in the odds ratio of CRC in subjects with the highest folate intake compared with those with the lowest intake, without clinical evidence of folate deficiency. Moreover, the relationship between blood levels of folate and the risk of CRC and adenoma is less well-defined than that with folate intake.

In 1996, Tseng *et al.* observed in women that only folate was inversely associated with adenoma risk, even after adjusting for other individual micronutrients. In the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS), colorectal adenoma risk was 30–40% lower in individuals whose median folate intakes were 711 mg/day in women and 847 mg/day in men compared to the risk associated with the lowest folate intakes (166 mg/day for women and 241 mg/day for men). In the NHS, women whose total folate intake (i.e., diet plus supplements) was $\geq$400 mg/day had a 31% decreased risk of colon cancer compared to women in the lowest folate intake group ($<$200 mg/day). Eighty-six percent of the high-folate intake (400 mg/day) group consumed daily multivitamins containing FA. In another study, Ma *et al.* (1997) found that serum folate levels below 6 nmol/l were associated with higher risk of CRC. Similar inverse associations between folate intake or status and colorectal adenoma risk were found in other studies. In a study of the epidemiology of CRC in Asia, Yee *et al.* (2009) demonstrated that folate supplementation is not effective in preventing CRC.

A meta-analysis published in 2005 of seven cohort and nine case–control studies that examined the association between folate consumption and colorectal cancer risk showed that among cohort studies, there was a significantly (25%) lower risk of CRC among those in the highest category of dietary folate intake compared to those in the lowest category, with no evidence of heterogeneity between the study estimates. However, the association between food folate consumption and low CRC (relative risk (RR) for high vs. low intake, 0.75; 95% confidence interval (CI), 0.64–0.89) disappears with total folate intake (folate from foods and supplements; RR for high vs. low intake, 0.95; 95% CI, 0.81–1.11) (Sanjoaquin *et al.*, 2005). In a nested case–control study of the Japanese population (the Japan Public Health Center-based prospective study), a rich plasma folate status did not prevent CRC (Otani *et al.*, 2008).

In contrast, opposite results established a positive association between folate intake and CRC risk. In the Colorectal Cancer Study conducted in Melbourne, Australia—a case–control study designed to identify dietary factors associated with CRC risk in 715 incident cases compared with 727 age/sex frequency-matched randomly chosen community controls—a quantitative assessment of all foods eaten was performed, and folate showed an increased risk at the highest level of consumption (Kune and Watson, 2006). In a Dutch case–control study comparing cases with at least one histologically confirmed colorectal adenoma ($n = 768$) and controls with no history of any type of colorectal polyp ($n = 709$), folate seemed to be a risk factor for colorectal adenomas, especially when vitamin B_2 intake was low (van den Donk *et al.*, 2005).

Consequently, epidemiological data show that folate intake has a dual effect on CRC risk. Apparently, low and excessive folate intakes as a result of bread fortification or supplementation are associated with high risk of this cancer.

CLINICAL TRIALS AND FOLIC ACID

A few placebo-controlled studies have demonstrated an increase in cancer outcomes with folate supplementation. The Aspirin/Folate Polyp Prevention Trial found that aspirin, but not FA, reduced recurrence of colorectal adenomas. The trial had a factorial design with three aspirin (placebo and 81 and 325 mg/day) and two FA (placebo and 1 mg/day) groups studied for 6 years. There were 884 subjects who had colonoscopic evaluation for adenomas during the trial. Among individuals who received aspirin plus FA, adenoma recurrence increased significantly, in contrast to those who received aspirin alone, which had a chemoprotective effect on colorectal adenoma. Moreover, a significant excess of prostate cancers was observed in the folate group (Cole *et al.*, 2007).

Other double-blind, randomized trials among participants of two large prospective cohorts, the HPFS and the NHS, evaluated the effect of FA supplementation on recurrent colorectal adenoma. Participants were randomly assigned to receive FA (1 mg/day; $n = 338$) or placebo ($n = 334$) for 3 to 6½ years. Among subjects with plasma folate levels at baseline below 17 nmol/l, FA supplementation was associated with a significant decrease in adenoma recurrence (RR, 0.61; 95% CI, 0.42−0.90; $p = 0.01$), whereas for subjects with high folate concentrations at baseline (>17 nmol/l) supplemental FA had no significant effect (Wu *et al.*, 2009).

Two randomized, double-blind, placebo-controlled clinical trials (the Norwegian Vitamin Trial and the Western Norway B Vitamin Intervention Trial) were performed between 1998 and 2005. A total of 6837 patients with ischemic heart disease were treated with oral B vitamins (FA, 0.8 mg/day) plus vitamin B_{12} (0.4 mg/day) and vitamin B_6 (40 mg/day) ($n = 1708$), FA (0.8 mg/day) plus vitamin B_{12} (0.4 mg/day) ($n = 1703$), vitamin B_6 alone (40 mg/day) ($n = 1705$), or placebo ($n = 1721$). Treatment lasted from 1998 to 2005, and subjects were followed-up until December 31, 2007. Serum folate concentration increased sixfold, and cancer incidence and cancer mortality were greater in the group that received FA and vitamin B_{12} (Ebbing *et al.*, 2009).

EXPERIMENTAL STUDIES AND FOLIC ACID

In animal models, there is evidence that supplementation with FA has a promoting effect on carcinogenesis, and that folate deficiency reduces the development of CRC and ileal polyps. In neoplastic cell cultures, interruption of folate metabolism generates an inhibition of tumoral cell replication as a result of ineffective DNA synthesis (Bashir *et al.*, 2004).

In old mice, the increase in genomic and p16 promoter DNA methylation, a phenomenon observed in the earliest stages of carcinogenesis, was directly related to dietary folate. Age-related enhancement of p16 expression occurred in folate-repleted ($p = 0.001$) and folate-supplemented groups ($p = 0.041$) but not in the folate-depleted groups. This possibly indicates that adequacy of dietary folate is important to maintain an adequate expression of p16 in the aged colon (Keyes *et al.*, 2007).

In summary, the lines of evidence indicate that intracellular folate depletion suppresses the progression of existing neoplasms. In neoplastic cells, in which DNA replication and cell division occur at an accelerated rate, folate depletion or interruption of folate metabolism causes ineffective DNA synthesis, resulting in inhibition of tumor growth.

FOLIC ACID FORTIFICATION AND COLORECTAL CANCER

Many countries have implemented mandatory or voluntary FA fortification of flour and uncooked cereal grain products to reduce the risk of NTDs. The United States and Canada have mandated fortification since 1998. Chile started in 2000, and the rest of the American countries started later. As a consequence, the incidence of births complicated by NTDs has declined 20−50% in the United States, Canada, Chile, and Costa Rica (Dary, 2009).

In the United States and Canada, population-based studies showed an approximately twofold increase in plasma folate levels in the adult population. Simultaneously, an increase in CRC rates occurred probably as a result of FA fortification in North America in 1996 and 2000, based on population-based observations from two representative data sets from the United States and Canada (the nationwide Surveillance Research Program and Canadian Cancer Statistics 2006, respectively). Apparently, this change in CRC rate is not a consequence of changes in the rate of colorectal endoscopic procedures, as the authors discussed. These observations alone do not prove causality but are consistent with the known effects of folate on existing neoplasms, as shown in both preclinical and clinical studies (Mason *et al.*, 2007).

In Chile, the fortification program showed a 40% reduction in the rate of NTD in 1 year. In women of reproductive age, serum folate levels increased from 9.7 to 37 nmol/l (Hertramf and Cortes, 2004). In elderly people, who consumed an average of 220 g of bread/day, equivalent to 185 g of flour and containing 410 mg FA (fortification), serum folate levels increased from 16.2 to 32nmol/l, and homocysteine levels decreased and masked vitamin B_{12} deficiency. Moreover, serum folate levels increased to more than 40 nmol/l in 37% of these individuals (Hirsch *et al.*, 2002). As in the United States and Canada (Mason *et al.*, 2007), a temporal association between folate fortification and an increase in CRC hospital discharge was observed. The rate/ratio between the period before and after the fortification for colon cancer in the group aged 45–64 years was 2.6 (99% CI, 2.93–2.58) and for the group aged 65–79 years it was 2.9 (99% CI, 3.25–2.86), as shown in Figures 44.2 and 44.3 (Hirsch *et al.*, 2009).

One possible explanation for this finding is that this increase is causally related to FA fortification. Other explanations for the increase in the discharge rates for CRC could be an increase in the incidence of risk factors such as obesity, low intake of fiber and calcium, and high intake in fat and red meat. The prevalence of obesity increased from 19.7% in 1997 to 22% in 2003 (Vio *et al.*, 2008). Unfortunately, there are no data on other attributable risks of these factors, but according to Food and Agricultural Organization data, the supply of calories and protein has not changed significantly.

FA fortification program had an effect on homocysteine levels (Hirsch *et al.*, 2002). We therefore expected a decline in the rates of cardiovascular disease because hyperhomocysteinemia is considered a cardiovascular risk factor (Bostom *et al.*,1999; Clarke *et al.*, 1991). However, the discharge rates for cardiovascular diseases did not change in the two study periods. This is consistent with FA supplementation trials, which have not reported a reduction in the incidence of cardiovascular events (Wierzbicki, 2007).

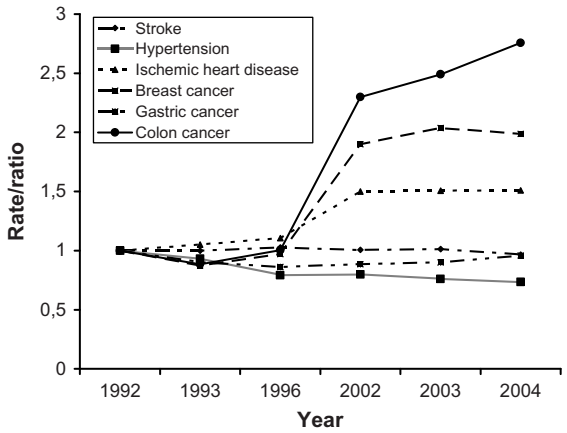

FIGURE 44.2
Rate/ratio of hospital discharge because of colon cancer in adults aged 45–64 years, before and after the start of the mandatory flour fortification program with 220 mg of synthetic folic acid/100 g of wheat flour. Rate/ratios are expressed as the rate for each year/the rate for 1992.

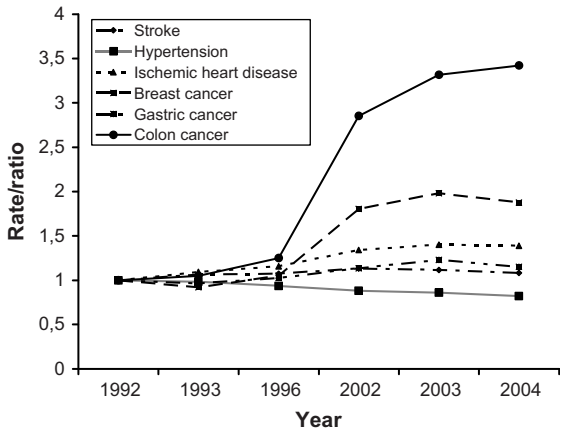

FIGURE 44.3
Rate/ratio of hospital discharge because of colon cancer in adults aged 65—79 years, before and after the start of the mandatory flour fortification program with 220 mg of synthetic folic acid/100 g of wheat flour. Rate/ratios are expressed as the rate for each year/the rate for 1992.

There is no cancer registry in Chile. Therefore, the only means to study the impact of fortification on CRC incidence is to use indirect data. Because a form indicating the diagnosis and other variables must be completed for every discharge from every hospital in Chile, this information, which is complete and reliable, can be used as a proxy for disease incidence. Thus, we used it to study the trends in the incidence of CRC and compare it with that of other diseases as a control. The changes in disease frequency detected using hospital discharge data coincided with mortality trends for breast, colorectal, and gastric cancer. This gives further support to the validity of hospital discharge data as a proxy for disease incidence.

This agreement in the observational studies in three countries after 10 years of the fortification program does not prove causality, but it is consistent with the known effects of folate on existing neoplasms (adenomas), as demonstrated in experimental and clinical studies. Another plausible explanation for the increase in CRC associated with FA fortification is that supraphysiologic fortification of bread or supplementation increases circulating unmetabolized FA, and the real consequence of this is unknown.

There is evidence that daily ingestion of 400 μg or plasma levels higher than 40 nmol/l produces a sustained appearance of unmetabolized FA in the blood. Troen *et al.* (2006) observed that increasing concentrations of plasma FA among postmenopausal women who took FA supplements were inversely associated with decreases in the cytotoxicity of circulating NK cells, which play a role in the destruction of arising clones of endothelial cancer cells.

Global methylation status may also be altered with high folate plasma levels. We observed that healthy male subjects in the fortification era, without vitamin supplement, with plasma folate levels higher than 45 nmol/l had higher SAM and SAH concentrations than those of subjects with normal folate levels (Hirsch *et al.*, 2008).

In summary, there is evidence that an FA fortification program with 150 or 220 μg of synthetic FA/100 g of wheat flour may be associated with an additional risk of CRC. Thus, it is crucial to evaluate this finding to determine the safe upper limit for folate intake as well as the safe upper folate concentration and the amount of FA necessary to prevent NTDs and to minimize possible new adverse effects.

SUMMARY POINTS

- Folate has a critical function in methylation reactions and in DNA synthesis and repair.
- Folate may accelerate tumor cell growth.

- In the mandatory era of FA fortification, approximately 40% of the population has supraphysiologic levels of serum or plasma folate.
- Low and high folate intake or plasma concentration are related with the risk of CRC.
- The upper limit for FA intake as well as the safe upper folate concentration need to be determined to prevent adverse effects.

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CHAPTER 45

Effects of the Soybean Flour Diet on Insulin Secretion and Action

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

ACC Acetyl-coenzyme A carboxylase
Akt Protein kinase B
AMPK Adenosine monophosphate-activated protein kinase S6K1
ARC Arcuate nucleus
CLY ATP citrate lyase
FAS Fatty acid synthase
GLUT-2 Glucose transporter-2
IRS Insulin receptor substrate
ME Malic enzyme
NPY Neuropeptide Y
PI3-K Phosphatidylinositol 3-kinase
PPAR Peroxisome proliferator-activated receptor
PVN Paraventricular nucleus
SREBP-1c Sterol regulatory element-binding protein-1c
UCP-1- Uncoupling protein-1

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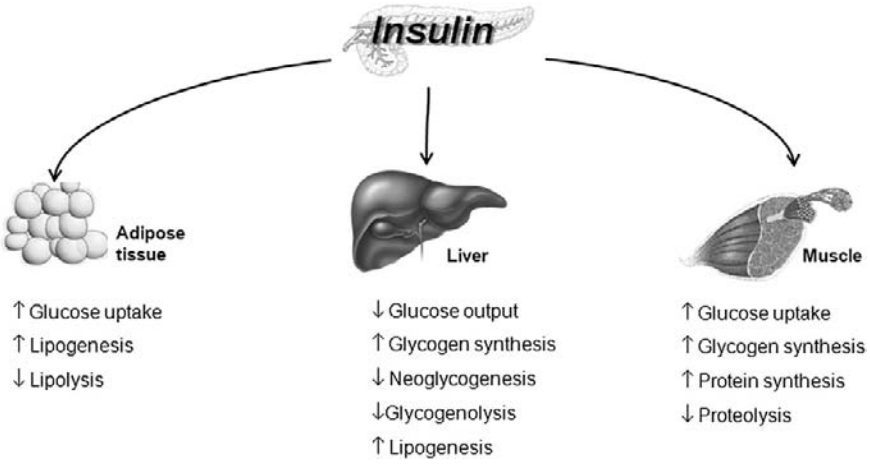

FIGURE 45.1
Insulin actions on target tissues. Insulin exerts anabolic effects on tissues handling key fuels. Primarily targets are white adipose tissue (left), liver (center), and skeletal muscles (right).

INTRODUCTION

Soybean (*Glycine max*) is an important plant for human and animal nutrition due to its large amounts of protein, lipid, carbohydrates, minerals, vitamins, and fiber. In addition to its high nutritional value, soybean protein is unique among the plant-based proteins in that it contains the largest concentrations of isoflavones (biologically active plant substances with structures similar to that of estradiol), which possess the ability to bind to estrogen receptors in various cells (Bhathena and Velasquez, 2002).

Because of its reduced cost and elevated nutritional value, soybean flour has been used as alternative feed in the recovery of nutritional status. In addition, soy-based ingredients possess properties that lead to metabolic and/or physiological effects, preventing and/or inducing therapeutic activities on a series of chronic disease, such as diabetes mellitus, obesity, and dyslipidemias.

Part of the beneficial effects of soybean and by-products has been associated with their effects on insulin secretion and action. Insulin is an anabolic, polypeptide hormone synthesized by pancreatic β-cells, whose synthesis is activated by an increase of nutrients, especially glucose. Insulin acts on several periphery tissues, including liver, muscle, and adipose tissue (Figure 45.1).

HUMAN CLINICAL TRIALS

Many studies have reported beneficial effects of soy consumption on human health, but most of these investigations evaluated its effects in serum lipids. Human clinical trials have shown antiobesity and antidiabetic effects of soy protein and isoflavones (Table 45.1).

Current information regarding the effect of soy on body composition and body fat distribution is very limited. In a randomized, double-blind, placebo-controlled 3-month trial and a cross-sectional study of postmenopausal women, a diet high in soy decreased body mass index and waist circumference (Sites *et al.*, 2007). By contrast, one randomized, placebo-controlled trial of soy on body composition performed in perimenopausal or postmenopausal women reported that soy did not affect body mass index (Kim *et al.*, 2005).

Inclusion of isoflavones in the diet of postmenopausal women with type 2 diabetes and metabolic syndrome has been shown to improve glycemic control, insulin resistance, and glycated hemoglobin (HbA1c) (Azadbakht *et al.*, 2007; Jayagopal *et al.*, 2002). In

TABLE 45.1 Effect of Soybean Diet in Human Clinical Trials

Patients	Intervention	Duration	Effect	Reference
15 postmenopausal women	Shake containing 20 g soy protein + 160 mg isoflavones versus isocaloric casein placebo shake	12 weeks	Total and subcutaneous abdominal fat decreased	Sites *et al*. (2007)
42 postmenopausal women with the metabolic syndrome	Soy protein diet, or a soy nut diet versus control diet (Dietary Approaches to Stop Hypertension)	8 weeks	Soy nut diet decreased HOMA-IR index, fasting plasma glucose, and serum C-peptide concentrations	Azadbakht *et al*. (2007)
30 type 2 diabetics	600 mg soybean-derived pinitol or placebo twice daily	13 weeks	Pinitol decreased fasting plasma glucose, insulin, fructosamine, $HbA_{1c,}$ and the HOMA-IR index	Kim *et al*. (2005)
32 postmenopausal women	Diet-controlled type 2 diabetes supplemented with soy protein 30 g/day, isoflavones 132 mg/day, versus placebo	12 weeks	Reduced fasting insulin, insulin resistance, and HbA_{1c}	Jayagopal *et al*. (2002)
208 postmenopausal women	Association between usual dietary isoflavone intake and cardiovascular disease risk factors	Usual frequency of consumption during 1 year for tofu, bean curd, and meat substitutes made from soy	High genistein intake reduced body mass index, waist circumference, and fasting insulin	Goodman-Gruen and Kritz-Sliverstein (2001)

HOMA-IR, homeostasis model assessment for insulin resistance.

cross-sectional (Goodman-Gruen and Kritz-Sliverstein, 2001) and cross-over trials (Duncan *et al.*, 1999) in postmenopausal women on high soy diets, there was a trend toward lower fasting insulin.

The mechanisms by which soybean diet may exert its beneficial effects are not completely clear. Due to the difficulties in working with humans, the literature mostly describes effects of a soybean diet in rodents.

ANIMAL STUDIES

Effects of soybean on insulin secretion

Studies have shown inconsistent effects of soy-containing diets or soy isoflavones on serum insulin concentration. It has been reported that consumption of soybean, soybean isoflavones, soybean protein isolates, and soybean flour reduces hyperinsulinemia and serum insulin levels in the fasting and postprandial states (Ascencio *et al.*,2004; Lavigne *et al.*, 2000; Noriega-López *et al.*, 2007) or increases insulin concentration in the basal and fed states as well as total area under the insulin curves in response to a glucose load (Arruda Oliveira *et al.*, 2008; Kavanagh *et al.*, 2008). These discrepancies are possibly due to differences in the experimental design, the composition of these diets, and the duration of the dietary intervention. It is not clear whether the effect of soybean diet on serum insulin concentration is associated with peripheral insulin sensitivity or changes in the mechanism of insulin secretion.

Insulin secretion by pancreatic β-cells is regulated by plasma glucose concentration, as shown in Figure 45.2.

In vitro studies using clonal pancreatic β-cell line and cultured or fresh islets from different animal models have been carried out in the attempt to verify whether soybean alters insulin

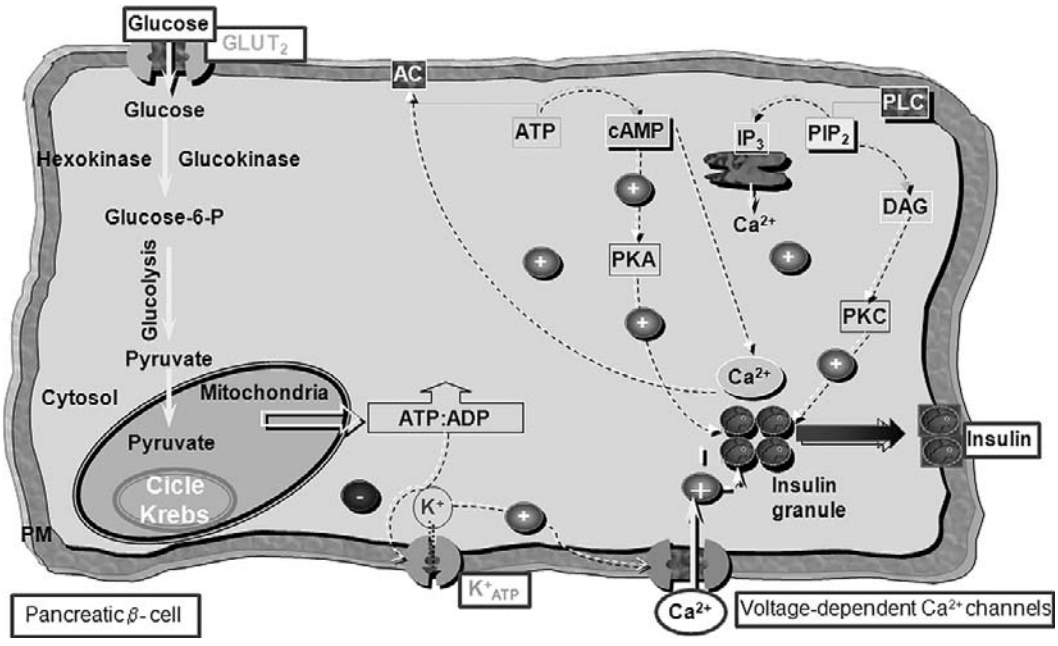

FIGURE 45.2
Coupling mechanism of glucose-induced insulin secretion. Glucose enters the pancreatic β-cell through GLUT-2 transporters and its metabolism increases the ATP:ADP ratio, causing K^+ ATP-sensitive channels to close, which depolarizes the plasma membrane (PM). Ca^{2+} L-type channels then open and allow Ca^{2+} entry, which activates the exocytotic machinery. This primary mechanism is modulated by classic hormone-responsive second messenger pathways such as adenylyl-cyclase (AC)/cAMP/protein kinase A (PKA) and phospholipase C (PLC)/inositol triphosphate (IP_3)/diacylglycerol (DAG)/protein kinase C (PKC).

secretion, to identify which soybean components could modify the insulin secretion, and to elucidate its mechanisms of action. At least two soybean components have been strongly associated with changes in insulin secretion: genistein and the amino acid pattern of soy protein. However, the secretory response of pancreatic β-cells has been considerably variable.

Long-term soy protein consumption, for example, stimulated to a lesser extent insulin secretion, even in the presence of high saturated fat, than did a casein diet fed to animals. This effect was associated with a decrease in pancreatic islet area, insulin, and peroxisome proliferator-activated receptor-γ (PPAR-γ) mRNA expression (which prevented the induction of glucose transporter-2 (GLUT-2), reducing glucose entrance to the β-cell and therefore impairing insulin secretion). The same effects were observed in isolated pancreatic islets cultured with the amino acid concentration resembling those found after soy protein consumption as well as with isoflavones (Noriega-López *et al.*, 2007). Hence, at least in this experimental model, which exhibits hyperinsulinemia, the combined effect of amino acids and isoflavones present in the soy protein reduced the insulin secretion.

In contrast, soybean flour diet increased insulin secretion in response to glucose by islets from control rats and partially restored the poor glucose-induced insulin secretion in islets from adult rats recovered from early malnutrition (Veloso *et al.*, 2008). In this case, the increased insulin secretion did not result from the amino acid composition of soybean proteins because both protein sources contained equivalent concentrations of amino acids, especially arginine (insulinotropic nutrient), and rats maintained with soybean flour or casein diet had similar serum concentrations of this amino acid in the fed state (unpublished data). The effects seemed to be mediated by the cAMP/PKA pathway possibly favored by genistein (Veloso *et al.*, 2008) because this isoflavone increases intracellular cAMP and PKA content in both cell lines and the islets (Liu *et al.*, 2006) (Figure 45.3).

In addition to the mechanisms described previously, the inhibitory or stimulatory effects of genistein on insulin secretion have also been attributed to its inhibitor effect on protein kinase

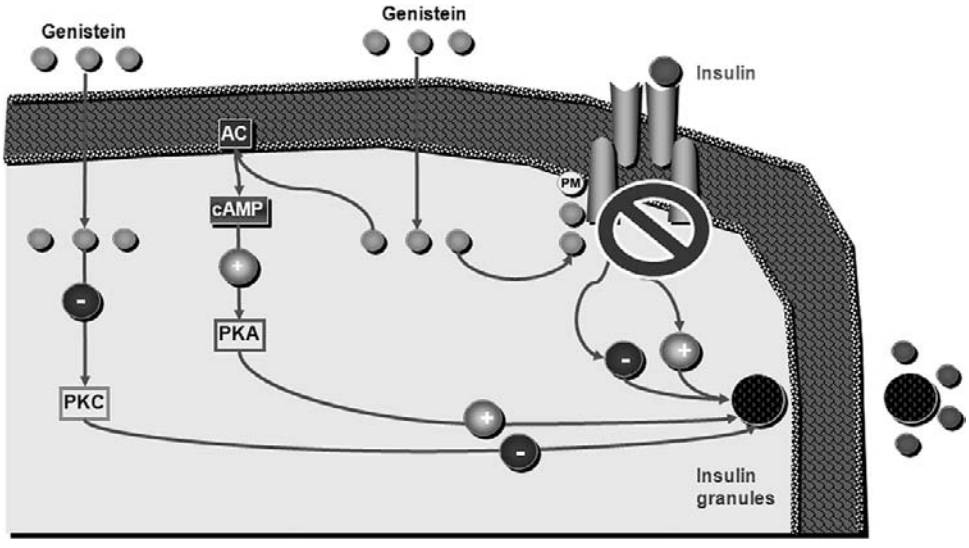

FIGURE 45.3
Inhibitory and stimulatory effects of genistein on insulin secretion mechanism. In pancreatic β-cells, genistein is reported to impair protein kinase C (PKC) potentialization of insulin secretion, to increase adenylyl cyclase (AC) activation, and to impair the insulin receptor signaling. PKA, protein kinase A; PM, plasma membrane.

C and tyrosine kinase proteins (Jones and Persaud, 1994; Persaud *et al.*, 1999; Sorenson *et al.*, 1994) (see Figure 45.3).

Effects of soybean insulin action

Many of the metabolic effects of insulin, including glucose uptake and glycogen synthesis, are mediated by a signaling pathway involving insulin receptor substrate (IRS) proteins, phosphorylation, and activation of phosphatidylinositol 3-kinase (PI3-K) and protein kinase B (Akt) (Figure 45.4).

Another classical insulin action is modulating lipid metabolism mediated by SREBP-1c, whose activation results in the induction of genes for enzymes involved in the biosynthesis of fatty acids such as acetyl-coenzyme A carboxylase (ACC), fatty acid synthase (FAS), malic enzyme (ME), and ATP citrate lyase (CLY). A transcription factor that is a direct target gene of SREBP-1c is PPAR-γ, and both exert cooperative and additive stimulation of the uptake of glucose and fatty acids and their subsequent conversion to triglycerides.

There is evidence that soy-containing diets interfere in various steps of the insulin signaling pathway, in transcription factor expression, and in the content and activity of key enzymes that participate in carbohydrate and lipid metabolism. Moreover, it has been demonstrated that soybean diet reduces body fat gain, which is an effect that may be useful for the prevention and treatment of obesity, diabetes mellitus and fat liver.

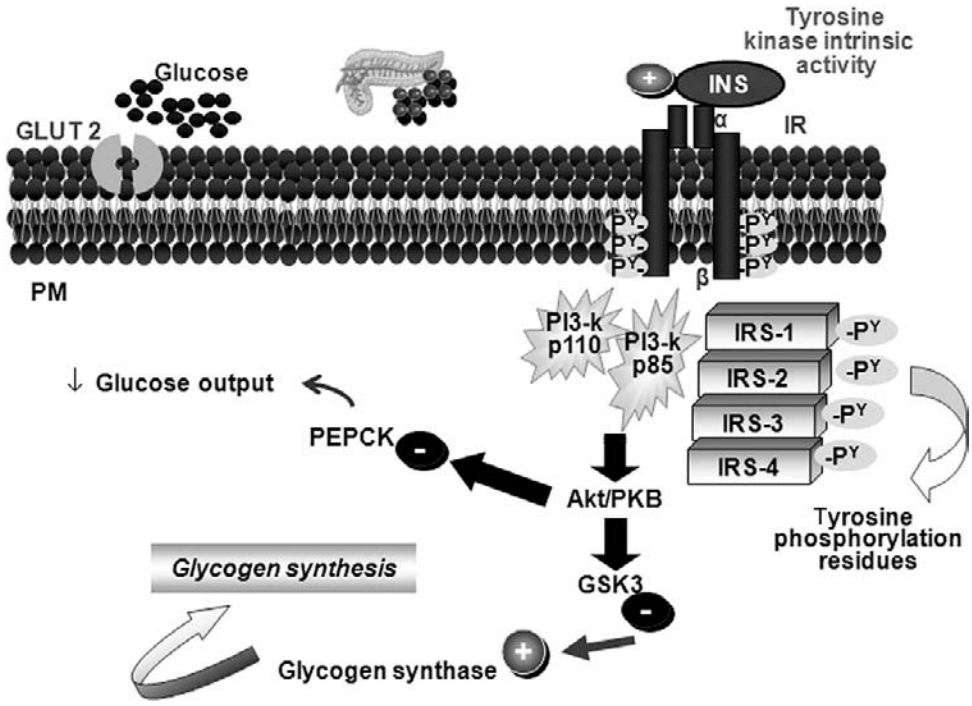

FIGURE 45.4
Insulin signaling mechanism that regulates glycogen synthesis and glucose output in the liver. Glucose uptake and glycogen synthesis are mediated by IRSs, phosphorylation/activation of PI3-K, and Akt (also called protein kinase B). Insulin binds the receptor (IR) and activates its tyrosine kinase activity. The intracellular part autophosphorylates and rapidly phosphorylates IRS-1/2. The signaling cascade activates PI3-K, which activates Akt. Akt phosphorylates glycogen synthase kinase-3 (GSK3), activating glycogen synthase activity and thus glycogen synthesis. Akt phosphorylates a family of transcription factors (Forkhead family), which results in inhibition of the phosphoenolpyruvate carboxykinase (PEPCK) expression and thus gluconeogenesis in liver. PM, plasma membrane.

EFFECT OF SOYBEAN DIET ON BODY COMPOSITION AND ENERGY BALANCE

Body weight tends to remain within a relatively narrow range despite large day-to-day fluctuations in the amount of food consumed. Obesity is thought to be the result of an energy intake–energy expenditure imbalance. Both are regulated by the hypothalamus, which processes central and peripheral signals. Within the hypothalamus, neurons residing in the arcuate nucleus (ARC)–paraventricular nucleus (PVN)–perifornical/lateral hypothalamus axis communicate with each other and are subject to the influence of several peripheral factors, including leptin and insulin.

Soy protein, isoflavones, and/or others components (saponins, tetrapeptides, fiber, etc.) act together or separately to alter several hormonal, metabolic, and neuroendocrine parameters involved in maintaining body homeostatic balance, energy expenditure, and feeding behavior.

It has been reported that isoflavones increase food and water intake and concentrations of neuropeptide Y (NPY) in the ARC and PVN nuclei of the hypothalamus. Such alterations are accompanied by decreased levels of plasma leptin and insulin. It is well established that NPY neurons, whose perikarya reside in the ARC nucleus and project to PVN, comprise an extremely important orexigenic neural pathway. Hence, in this case, at least one factor contributing to the higher food intake was the increased levels of NPY in this system. Interestingly, there is a reciprocal relationship between circulating insulin and leptin titers and NPY concentrations in PVN. Either insulin or leptin is associated with increased pre-proNPY messenger RNA expression in the ARC nucleus, and increased NPY levels in PVN provide an important signal to the NPY system to initiate feeding. Thus, by reducing secretion of insulin and/or leptin, chronic consumption of the isoflavone diet results in upregulation of the orexigenic NPY circuit in the hypothalamus, which in turn stimulates food and water intake. Despite high food intake, these animals exhibited reduced body and adipose tissue weights, which could be associated with high circulating triiodothyronine levels and the uncoupling protein-1 mRNA levels in brown adipose tissue, which alter the energy expenditure or thermogenesis (Lephart *et al.*, 2004). However, with regard to body weight and food intake, other experiments in rats have demonstrated that dietary isoflavones do not affect body weight (Ju *et al.*, 2001) and decrease food intake (Davis *et al.*, 2007; Penza *et al.*, 2006). Moreover, soy isoflavones have been demonstrated to affect adipose tissue without affecting food consumption. Enhanced lipolysis and inhibited lipogenesis in the white adipose tissue determined by phytoestrogens are factors that could help reduce adiposity without affecting food intake.

It has been reported that rats kept on a soybean flour diet showed reduced proportions of fat deposits, even though they ate proportionally the same amount of diet and showed lower energy expenditure than those fed the casein diet. Especially during nutritional recovery, soybean flour diet produced low energy efficiency that was reflected in less energy gain as protein and proportion of carcass protein and low energy gain as lipid. It is possible that low digestibility of soybean flour diet has been critical to accretion of carcass protein in animals that suffered previous nutritional deficit. However, serum albumin and total protein concentrations from rats recovered with soybean flour diet were similar to those of control rats and higher that those of malnourished rats. It became clear that the soybean diet was efficient for nutritional recovery. Serum insulin level was increased and fat mass diminished in animals fed with soybean diet, without alterations of serum leptin levels. This suggests that although these animals possess fewer adipocytes, they release higher amounts of leptin. Also, serum leptin concentration was unrelated to food or energy intake and energy expenditure (Cheim *et al.*, 2009). However, maintenance of normal serum leptin concentrations can be beneficial to prevent leptin resistance and obesity.

EFFECT OF SOYBEAN DIET ON INSULIN SIGNALING IN LIVER

The liver is mainly responsible for maintaining normal concentrations of blood glucose by its ability to store glucose as glycogen and to produce glucose from glycogen breakdown or

through the use of gluconeogenic precursors. These processes are regulated by hormones, mainly insulin and glucagon.

Soybean flour diet consumption by recovered and control rats did not modify serum glucose levels and raised serum insulin levels in the fed state. Despite elevated serum insulin levels, these rats maintained normal glycemia, possibly at the expense of an elevated hepatic glucose output, as suggested by the low hepatic glycogen content in the fed state. These results addressed an enhanced glycogenolysis due to an increase in glucagon levels and/or resistance to insulin because of the inhibitory effect of genistein on the tyrosine kinase activity of the insulin receptor and/or its substrates (Arruda Oliveira *et al.*, 2008). The first hypothesis was rejected, taking into account the unchanged serum glucagon levels in this animal model. Interestingly, in agreement with the latter hypothesis, it was verified that soybean flour diet reduced liver IR and IRS-1 levels, the IRS-1/PI3-K association, and the consequent phosphorylation of Akt. However, soybean diet favored an increase in insulin resistance among molecular mechanisms that did differ as a function of previous nutritional status. In recovered rats, a soybean diet resulted in liver insulin resistance due, at least in part, to an increased expression of p85, which favored the reduction of the IRS-1/PI3-K association. In control rats fed with soybean flour diet, the insulin resistance appeared to have resulted in a reduction of phosphorylation IRS-1 (Feres *et al.*, 2010).

The liver is also a central organ of lipid processing, and many studies describe the ability of soy derivatives to modify liver lipid metabolism. Nonalcoholic fatty liver disease is now recognized as the most common type of liver disease, being frequently associated with insulin resistance and metabolic syndrome.

Several studies have shown that soy protein and its isoflavones favorably affect hepatic lipid metabolism, preventing fat accumulation by its effects on genes involved in fatty acid biosynthesis and oxidation (Figure 45.5). This effect has been attributed to the protective role of soybean on insulin resistance.

Curiously, soybean flour diet consumption by recovered and control rats that exhibit liver insulin resistance signals also shows reduced lipogenesis and liver fat storage, diminished PPAR-γ,, EM, and CLY contents, as well as CLY and ME activities (unpublished data). PPAR-α mRNA abundance was higher in rats fed with soybean flour diet than in those maintained with casein diet, but the protein content was similar in all groups. ACC-α and ACC-β mRNA expression was markedly reduced by soybean flour diet, whereas the ACC content and phospho-[Ser79]-ACC content were reduced only in control rats that received soybean flour diet. Messenger RNA and protein expression of SREBP-1c, adenosine monophosphate-activated protein kinase S6K1 (AMPK), and phospho-[Thr172]-AMPK was not modified by the soybean diet. Hence, at least in these animal models (control and recovered rats), the soybean flour diet reduced liver lipid concentration through down-regulation of the ACC gene and protein expressions rather than by phosphorylation status, which possibly resulted in decreased lipogenesis and increased β-oxidation (Milanski *et al.*, 2009).

Similar results were observed in the soy protein consumption by Zucker obese fa/fa rats. This animal model showed reduction in the accumulation of cholesterol and tryacylglycerol in the liver, preventing the development of fatty livers. The reduction in hepatic cholesterol was associated with a low expression of liver X receptor α and its target genes (e.g., 7α-hydroxylase and ATP binding cassette A1). Moreover, soy protein also decreased lipogenesis through a decrease in the expression of SREBP-1 and of its target enzymes, such as FAS and ME. Furthermore, the reduction of hepatic lipids was also attributed to an increase in fatty acid oxidation because soy protein increases PPAR-α and carnitine palmitoyltransferase-1 expression (Ascencio *et al.*, 2004).

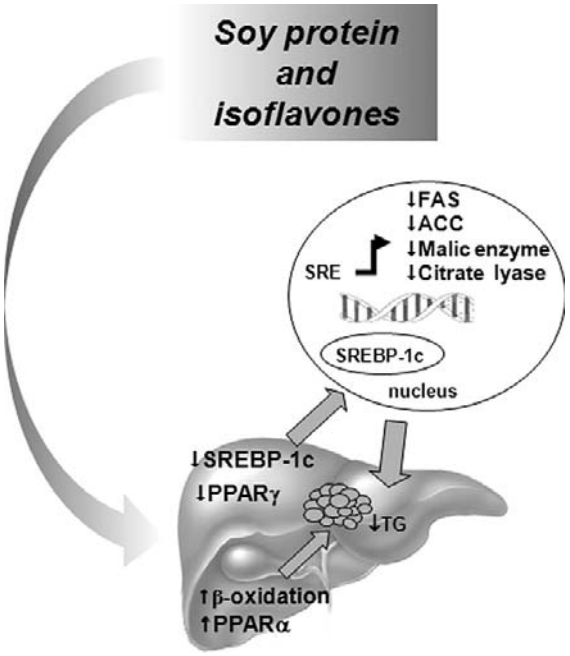

FIGURE 45.5
Effects of soy protein and isoflavones on liver. Fat accumulation is determined by the balance between fat synthesis (lipogenesis) and fat breakdown (lipolysis/fatty acid oxidation). Hepatic lipogenic transcription factors and enzymes crucial in modulating lipid metabolism are SREBP-1c, PPAR-γ, FAS, ME, CLY, ACC-α (favoring lipogenesis), PPAR-α, ACC-β, and AMPK (favoring fatty acid β-oxidation). PPAR-α stimulates fatty acid oxidation by inducing CPT-1 expression, together with other β-oxidation enzymes. Soy protein and its isoflavones favorably prevent fat accumulation by controlling these genes.

EFFECT OF SOYBEAN DIET ON INSULIN SIGNALING IN SKELETAL MUSCLE

The beneficial effects on glucose metabolism and insulin dynamics have been attributed to soy protein or isoflavone components. Such effects appear to be largely explained by improved insulin sensitivity, as shown by increased insulin action in skeletal muscle (Cederroth *et al.*, 2008; Lavigne *et al.*, 2000). However, the molecular and physiological mechanisms underlying these metabolic actions have not been determined.

Mice fed soy (isoflavone content of the diet ~600 ppm) from intrauterine life to approximately 25 weeks of age showed reduced serum insulin levels and pancreatic insulin content and improved insulin sensitivity. Because skeletal muscles account for approximately 42% of the total body mass in male mice, Um *et al.* (2004) suggested that the increased whole-body insulin sensitivity of high-phytoestrogen-fed mice is, to a large extent, a consequence of increased insulin sensitivity in skeletal muscles. These high-phytoestrogen-fed mice showed significantly lower IRS-1, mammalian target of rapamicyn (mTOR), and ribosomal protein S6 kinase beta-1 (S6K1 or $p70^{S6K}$) levels. In response to insulin, S6K1 phosphorylates IRS-1 on two inhibitory serine residues (S636/S639), preventing further activation of the PI3-K/Akt signaling pathway (Um *et al.*, 2004). Thus, lower S6K1 levels inducing decreased phosphorylation of the IRS-1 inhibitory serines (S636/S639) could be one of the mechanisms involved in improving insulin sensitivity in skeletal muscles of mice fed a high-phytoestrogen diet. Also consistent with a direct effect of phytoestrogens on muscle tissues, Cederroth *et al.* (2008) related the increased AMPK activation with reduced repression of the insulin signaling exercised by AMPK-dependent inhibition of the mTOR/S6K1 axis.

Specific amino acids of soy protein could regulate skeletal muscle insulin sensitivity for glucose disposal by directly modulating the insulin signaling pathway (Lavigne *et al.*, 2000). It has been proposed to explain decreased insulin-stimulated tyrosine phosphorylation of IRS-1 and

IRS-2, reduced binding of the p85 subunit of PI3-K to IRS-1 and IRS-2, and inhibition of insulin-stimulated PI3-K activity with the use of a mixture of 20 amino acids (Patti *et al.*, 1998). In this regard, it has been shown that certain amino acids inhibit the insulin-induced activation of PI3-K and glucose transport by increasing serine/threonine phosphorylation of IRS-1 through activation of the mTOR/p70 S6 kinase pathway without affecting IRS-1 tyrosine phosphorylation (Tremblay and Marette, 2001).

To further explore the cellular mechanisms behind the effect of protein on skeletal muscle insulin sensitivity, rats fed a standard chow diet or a high-fat diet in which the protein source was casein, soy, or cod proteins for 4 weeks were studied (Tremblay *et al.*, 2003). Interestingly, it was observed that soy protein did not completely prevent the deleterious effect on insulin-stimulated PI3-K activity caused by fat feeding. In addition, activation of the downstream kinase Akt/PKB by insulin, assessed by *in vitro* kinase assay, and phosphorylation of GSK-3 were impaired in muscle of high-fat-fed rats consuming casein or soy protein, but these defects were also fully prevented by dietary cod protein.

In summary, this study provides convincing evidence that soy protein and isoflavones are important modulators of insulin signaling and action in skeletal muscle and, thus, they may be a useful dietary intervention for the prevention of peripheral insulin resistance, an important feature of type 2 diabetes.

TECHNOLOGICAL ISSUES

There is an increasing trend to integrate soybean into conventional foods, as either a complete or a partial replacement. Soy flours have been tested in baked products as both functional ingredients and protein fortifiers. However, soy flours have both positive and negative characteristics in food products. In baked goods, the creamy color and beany or nutty flavor can contribute to the sensory quality. When used in larger quantities, soy flours have an astringent aftertaste and/or a chalky mouthfeel. The flatulence problem remains because soy flours contain most of the original carbohydrates. In bakery products, soy-based ingredients have been used for a variety of functional reasons. For example, soybean protein products improve crust color, crumb body, resilience, and toasting characteristics in bread.

SUMMARY POINTS

- Human clinical trials have shown antiobesity and antidiabetic effects of soy protein and isoflavones.
- In rats, soybean flour diet activates the β-cell cAMP/PKA pathway, increasing insulin secretion in response to glucose.
- Soybean isoflavones alter food intake by interfering with the leptin/insulin-NPY axis. Results are controversial.
- In rats, soybean flour diet decreases relative weight of fat deposits and even reduces the energetic expenditure without alterations in food intake.
- Soybean diet interferes in various steps of the insulin signaling pathway.
- In rats, soybean diet decreases hepatic fat synthesis.
- Soybean diet increases insulin sensitivity in skeletal muscle.
- Muscle insulin-reduced phosphorylation of GSK-3 is improved by soy proteins and isoflavones, partially preventing deleterious effects of fat feeding.

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CHAPTER 46

Metabolic Effects of Bread Fortified with Wheat Sprouts and Bioavailability of Ferulic Acid from Wheat Bran

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

AXOS Arabinoxylan oligosaccharides
FFA Free fatty acids
OGTT Oral glucose tolerance test

INTRODUCTION

Among the plant foods, cereals occupy the majority of the world's planted area. Cereals contribute more than 60% of the world food production and provide the bulk of energy, dietary proteins, vitamins, and minerals to the world population (Betschart, 1982). With increasing dependence on cereal grains to provide the energy and micronutrient requirements of humans in developing countries, the need for raising the overall nutritional status

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of cereal grains has become increasingly important, and much effort has been made to improve the amount and quality of cereal grains. The methods that have been applied range from genetic improvement to different processing technologies, such as milling, preparation of protein concentrates, cooking, fermentation, and malting or sprouting. In East Asia, fermentation and sprouting are widely and routinely used to process grain legumes. However, these processing technologies are not commonly used for cereals. The sprouts of various cereals have been used for centuries in many traditional dishes in the Orient. This practice has recently become popular in the Western world. The sprouting of cereal grains for a limited period of time has been reported to improve their nutritional composition (Lorenz and D'Appolonia, 1980).

During germination, the reserves within the storage tissue of the seed are mobilized to support seedling growth. As germination leads to degradation of starch deposits, a change in composition and accessibility of nutrients occurs within the grain. Moreover, the subsequent development of the growing embryo causes the synthesis of additional bioactive molecules, such as metabolites, enzymes, co-enzymes, and cell wall compounds, resulting not only in a decrease in starch and sugar but also in an enrichment of bioactive compounds (Merx *et al.*, 1994; Plaza *et al.*, 2003). Contents of vitamins such as riboflavin, thiamine, biotin, pantothenic acid, tocopherols, and folates have been reported to amount to 1.5−3.8 times those in nongerminated seeds (Koehler *et al.*, 2007; Merx *et al.*, 1994; Plaza *et al.*, 2003). Furthermore, studies have shown that during wheat germination, the content of soluble and insoluble dietary fiber increases by 25% (Koehler *et al.*, 2007).

Wheat is the principal cereal used in the preparation of a variety of bakery products. Utilization of germinated wheat in bakery products can involve both field-sprouted and intentionally or artificially germinated wheat. Field sprouting is most common in Canada, Australia, and Europe. More than 15% of wheat produced suffers from field sprouting. The flours obtained from grains sprouted in the field have been shown to have detrimental effects on dough and bread properties and on pasta products (Huang and Varriano-Marston, 1980; Lemar and Swanson, 1976; Ranhotra *et al.*, 1977). The germinated wheat flour possesses excessively high α-amylase activity. Thus, the gluten from sprouted wheat has poor technological properties and low baking quality due to proteolytic hydrolysis of gluten proteins. Breads prepared from 100% flour of germinated wheat also exhibit very poor characteristics (Lemar and Swanson, 1976; Ranhotra *et al.*, 1977). Previous studies from our group showed that when using wheat grains germinated for 10 days for the preparation of a wheat bread at 30% w/w (based on dry matter), the resulting product shows a very poor baking quality and a strong silage flavor. However, by using wheat sprouts that had been germinated for only 4.25 days (102 h), an edible product with good baking and sensory qualities was obtained (Andersen *et al.*, 2008).

Several epidemiological studies show that the intake of whole grain cereals protects against the development of type 2 diabetes (Meyer *et al.*, 2000; Salmeron, Ascherio, *et al.*, 1997; Salmeron, Manson, *et al.*, 1997; Schulze *et al.*, 2004). These effects are thought to result mainly from an increased intake of dietary fiber. However, as mentioned previously, in addition to dietary fiber, whole grains contain a wide range of nutrients and bioactive compounds, such as vitamins, sterols, and polyphenols, which have also been suggested to contribute to the positive health effects. Knowledge about the contribution of nonfiber compounds to the bioactivity of whole grains is scarce, however. Furthermore, data on how those nonfiber compounds influence glucose and fatty acid metabolism in mammals are very limited. Therefore, a study was designed to test the influence of wheat bread containing germinated wheat sprouts compared to a wheat bread containing imbibed wheat kernels, both having the same amount of dietary fiber, on glucose homeostasis in healthy volunteers after 9 days of dietary intervention. This study used a longitudinal design. After an overnight fast (12 h), a standard oral glucose tolerance test (OGTT) was performed to determine the influence of the

two breads on glucose metabolism in the fasting as well as in the postprandial state. The participants consumed one portion of 300 g of the bread fortified with soaked wheat kernels per day for a total of 9 days. This was followed by a 10-day washout period. On the morning after the washout-period, a standard OGTT was performed again, and the bread fortified with wheat sprouts was given in portions of 300 g to the subjects. After this period, an oral glucose tolerance test was performed again.

IMPACT OF WHEAT SPROUTS ON GLUCOSE AND INSULIN METABOLISM

In an animal model, the intake of pregerminated brown rice was shown to produce lower postprandial glucose and insulin levels compared to white rice, and the authors ascribed this effect to the higher dietary fiber content of the pregerminated rice (Seki *et al.*, 2005). The impact of dietary fiber on glucose and insulin metabolism is relatively well investigated. Intake of soluble fiber increases the viscosity of stomach and small intestine contents, thereby hindering carbohydrate digestion and absorption (Leclere *et al.*, 1994). However, in population studies, intake of insoluble fiber, but not viscous fiber, has been associated with a decreased risk for type 2 diabetes or cardiovascular disease (Jenkins *et al.*, 2000; Salmeron, Manson, *et al.* 1997). The mode of action of insoluble fiber in this connection remains unclear. As possible mechanisms of the positive effects of dietary fiber on hepatic glucose production, fermentation processes in the colon leading to the production of short-chain fatty acids and a decreased hepatic glucose output have been discussed (Thorburn *et al.*, 1993).

In our study, consumption of a bread fortified with wheat sprouts revealed a glucose-lowering effect in the fasting as well as in the postprandial stage. This effect could not be observed after the intake of the bread fortified with imbibed wheat kernels. In contrast, plasma insulin levels of the subjects remained unchanged after intake of the bread fortified with wheat sprouts. The glucose-lowering effect is therefore likely to result from an improved peripheral insulin sensitivity, which may cause the improved glucose handling after a 9-day intake of the wheat bread fortified with wheat sprouts. This was confirmed by calculation of the homeostatic model assessment for insulin resistance in order to estimate the degree of insulin resistance. This calculation showed an increased insulin sensitivity after the intake of the bread fortified with wheat sprouts compared to the bread fortified with imbibed wheat kernels (Table 46.1).

Interestingly, Kim *et al.* (2006) showed that the naphthalenemethyl ester derivative of the phenolic compound dihydroxyhydrocinnamic acid was able to decrease blood glucose levels in streptozotocin-induced diabetic C57BL/6 mice and spontaneously diabetic ob/ob mice to near normoglycemia. In addition, this phenolic compound increased glucose uptake and enhanced phosphorylation of the insulin receptor-β subunit and insulin receptor substrate 1 in adipocytes *in vitro* and *in vivo*, which led to an increase in insulin receptor signaling. The hydroxycinnamic acid caffeic acid enhanced the glucose uptake into isolated adipocytes in a concentration-dependent manner (Hsu *et al.*, 2000). This may in turn lead to an enhanced glucose utilization. Ferulic acid effectively suppressed blood glucose levels in streptozotocin-induced diabetic mice and in KK-Ay mice (Ohnishi *et al.*, 2004).

In order to test whether ferulic acid is able to increase glucose uptake in insulin-sensitive adipocytes, murine 3T3-L1 adipocytes were incubated with or without insulin and/or ferulic acid. These experiments showed that ferulic acid positively affects glucose uptake in adipocytes *in vitro* (Figure 46.1).

In consideration of these results, it seems reasonable that the phenolic compounds in general and/or ferulic acid in particular in the wheat sprouts administered to humans in the

TABLE 46.1 Impact of a Bread Fortified with Wheat Sprouts or Imbibed Wheat Kernels on Plasma Glucose, Insulin, and FFA Levels in Healthy Volunteers[a]

	0 min	30 min	60 min	120 min	180 min
Plasma glucose (mmol/l)					
Before control bread period	4.6 ± 0.08	7.2 ± 0.31	5.0 ± 0.20	4.5 ± 0.17	4.5 ± 0.17
After control bread period	4.4 ± 0.09	7.5 ± 0.17	5.0 ± 0.22	4.5 ± 0.17	4.4 ± 0.09
Before experimental bread period	4.8 ± 0.50	7.5 ± 1.12	5.4 ± 0.74	4.3 ± 0.74	4.4 ± 0.60
After experimental bread period	4.3 ± 0.66**	6.5 ± 0.07**	4.7 ± 0.69**	4.2 ± 0.61	4.2 ± 0.38
Plasma insulin (μU/ml)					
Before control bread period	4.7 ± 0.47	31.6 ± 3.25	19.7 ± 2.32	11.7 ± 2.37	4.0 ± 0.85
After control bread period	1.9 ± 0.20***	20.5 ± 2.50**	17.2 ± 2.50	6.5 ± 1.46	1.6 ± 0.24**
Before experimental bread period	2.3 ± 0.33	21.0 ± 1.95	18.3 ± 2.23	4.8 ± 1.32	2.3 ± 0.76
After experimental bread period	1.6 ± 0.31	15.0 ± 2.41	15.5 ± 2.75	4.8 ± 1.22	2.0 ± 0.45
Plasma FFA (mmol/l)					
Before control bread period	0.31 ± 0.031	0.18 ± 0.024	0.07 ± 0.015	0.08 ± 0.020	0.29 ± 0.048
After control bread period	0.20 ± 0.028***	0.13 ± 0.015	0.03 ± 0.009*	0.02 ± 0.007*	0.24 ± 0.046
Before experimental bread period	0.19 ± 0.023	0.14 ± 0.017	0.03 ± 0.009	0.04 ± 0.07	0.21 ± 0.037
After experimental bread period	0.18 ± 0.028	0.08 ± 0.012**	0.05 ± 0.009	0.04 ± 0.07	0.16 ± 0.037
HOMA-IR					
Before control bread period	1.1 ± 0.13				
After control bread period	0.4 ± 0.05***				
Before experimental bread period	0.6 ± 0.08				
After experimental bread period	0.3 ± 0.06				

FFA, free fatty acid; HOMA-IR, homeostatic model assessment for insulin resistance.
Source: Data from Andersen *et al*. (2008).
[a]*Mean fasting and postprandial plasma glucose, insulin, and FFA levels as well as mean HOMA-IR of the subjects before and after a daily intake of 300 g of a bread fortified with imbibed wheat kernels (control bread) for 9 days and before and after a daily intake of 300 g of a bread fortified with wheat sprouts (experimental bread). Data are given as mean ± SEM. Asterisks indicate a significant difference of the values before and after the respective diet (*$p \leq 0.05$, **$p \leq 0.01$; ***$p \leq 0.001$).*

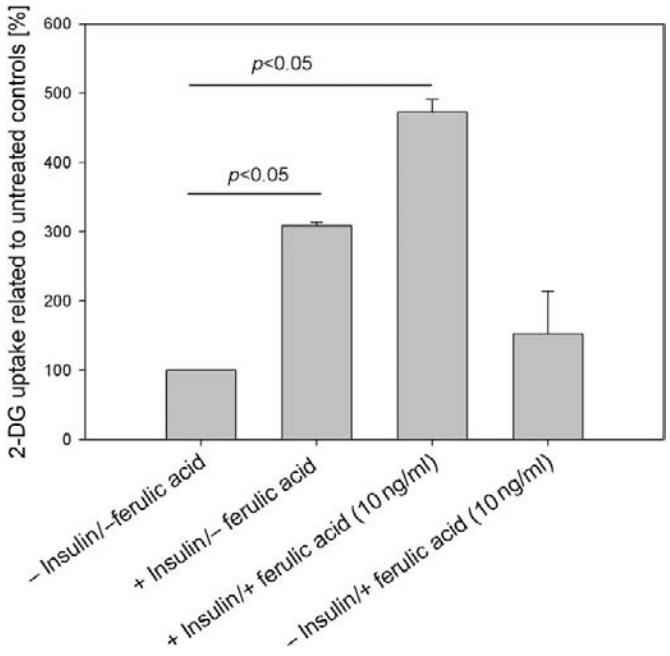

FIGURE 46.1
Ferulic acid affects glucose uptake in adipocytes. Uptake of the glucose analog 2-deoxyglucose in murine 3T3-L1 adipocytes after incubation with or without insulin (100 μg/ml) and/or ferulic acid (10 ng/ml). Data are given as percentage related to the untreated control (without insulin, without ferulic acid) ± SEM. Statistically significant differences are indicated.

intervention trial presented here are responsible for the glucose-lowering effect through enhancement of glucose uptake by insulin-sensitive tissues. However, the exact biochemical mechanisms of this health benefit remain speculative.

IMPACT OF WHEAT SPROUTS ON FATTY ACID METABOLISM

Because during germination the kernel undergoes severe compositional and structural changes, the intake of bread fortified with wheat seedlings may lead to a higher bioavailability of the fiber compared to the swollen wheat kernels in the control bread. This higher bioavailability might have been associated with a higher fermentation rate in the colon of the subjects. Such fermentation processes result in the formation of short-chain fatty acids (acetate, butyrate, and propionate), which have been shown to decrease hepatic glucose production in humans (Thorburn *et al.*, 1993). In addition to affecting hepatic glucose production, short-chain fatty acids have been reported to influence lipid metabolism. In humans, there is evidence that propionate (Venter *et al.*, 1990) and acetate (Wolever *et al.*, 1991) likely decrease free fatty acid (FFA) levels. Increased suppression of FFA plasma concentrations could indicate greater uptake of glucose by insulin-sensitive tissues and, therefore, improve postprandial glycemia. However, in the human intervention trial presented here, we observed a severe suppression of FFA levels after intake of the bread fortified with imbibed wheat kernels and a less pronounced effect after a 9-day intake of the bread fortified with wheat sprouts (see Table 46.1), making it unlikely that in our study a higher fermentation rate during the experimental bread period was responsible for the lower FFA levels and the lower glucose levels. It rather seems plausible that the higher dietary fiber intake of the subjects *per se* led to lower FFA levels during the control bread period, without affecting the fasting as well as the postprandial glucose levels. Because germination is accompanied by an increase in a multitude of compounds, it also seems feasible that other ingredients besides fiber are responsible for the glucose-lowering effect.

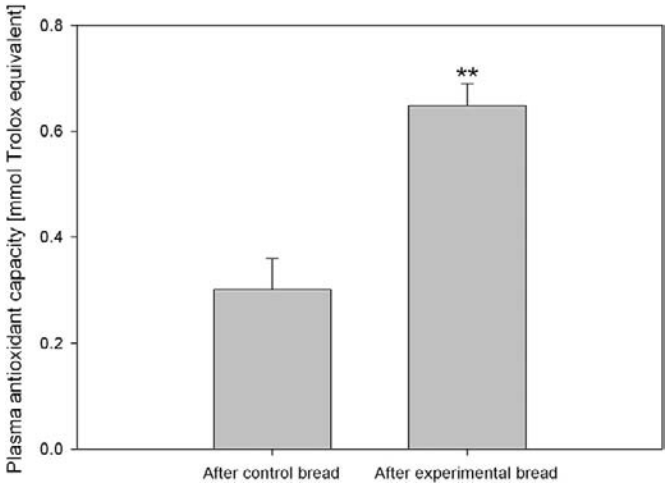

FIGURE 46.2
The intake of a bread fortified with wheat sprouts enhances plasma antioxidant capacity in healthy volunteers. Mean fasting plasma antioxidant capacity of the subjects after the intake of a bread fortified with imbibed wheat kernels (control bread) and a bread fortified with wheat sprouts (experimental bread). Data are given as mean ± SEM. Asterisks indicate a significant difference of the values before and after the respective diet (** $p \leq 0.01$).

IMPACT OF WHEAT SPROUTS ON ANTIOXIDANT STATUS OF HUMAN PLASMA

Grains are thought to be particularly rich sources of phenolic acids, which are located in the bran layer. The most abundant phenolic compounds in cereals belong to the chemical class of hydroxycinnamic acids. The main phenolic compound is ferulic acid, followed by diferulic acids and sinapic acid, *p*-coumaric acid, and caffeic acid. Germination of grains leads to an increase of these phenolic compounds by up to 150% (Koehler *et al.*, 2007; Liukkonen *et al.*, 2003). In our study, we demonstrated that the content of phenolic compounds increased by 40% after 4 days of germination (Andersen *et al.*, 2008). It is well-known that free hydroxycinnamic acids are effective antioxidants. However, approximately 95% of grain phenolic compounds are covalently linked to cell wall polysaccharides. Ferulic acid is bound to the arabinoxylans via an ester bond with the primary hydroxyl group at the C5 position of α-L-arabinofuranosyl residues. However, the antioxidant capacity of these bound phenolic compounds is still being discussed (Perez-Jiminez and Saura-Calixto, 2005). Interestingly, after a 9-day intake of a wheat bread fortified with wheat sprouts, the higher intake of phenolic compounds from the sprouts led to a higher antioxidant capacity of the subjects' plasma, indicating a higher bioavailability of the present phenolic compounds in wheat sprouts (Figure 46.2). However, the content of phenolic compounds in general in the plasma of the subjects was not enhanced after the experimental bread period (Andersen *et al.*, 2008). The content of ferulic acid in particular was slightly enhanced, but this difference did not reach statistical significance (Figure 46.3). Because the peak time for maximal urinary excretion has been shown to be approximately 7 h (Yang *et al.*, 2007), this result might be explained by the fact that our subjects underwent an overnight fast before blood drawing.

BIOAVAILABILITY OF FERULIC ACID

It is known that esterified hydroxycinnamates cannot be absorbed as part of complex molecules, but esterases present in the small intestine of humans have been shown to be able to cleave these ester bonds. This may result in the release of the free hydroxycinnamic acids into the lumen, where they can be absorbed (Andreasen *et al.*, 2001). It was shown that

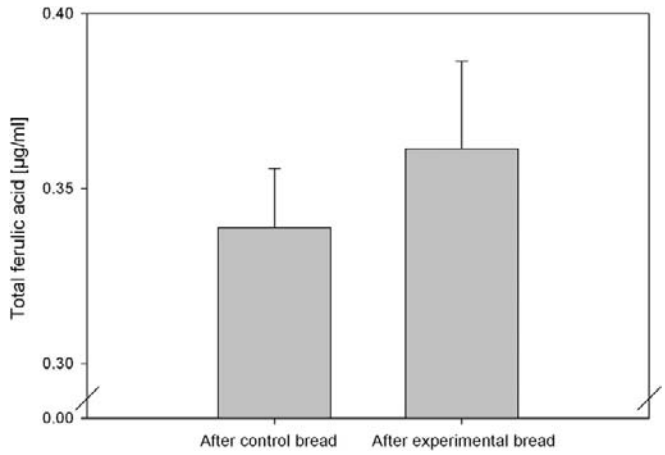

FIGURE 46.3
The plasma content of ferulic acid in healthy volunteers is not enhanced after intake of a bread fortified with wheat sprouts. Mean fasting plasma content of total ferulic acid of the subjects after the intake of a bread fortified with imbibed wheat kernels (control bread) and a bread fortified with wheat sprouts (experimental bread). Data are given as mean ± SEM.

ferulic acid was taken up in the plasma of six volunteers after a single meal of wheat bran cereals, reaching maximum concentrations of 150−210 nM between 1 and 3 h after ingestion of the cereals and decreasing rapidly after 3 and 6 h (Kern *et al.*, 2003). During germination, cell wall components are synthesized, and this leads to a higher content of phenolic compounds but does not necessarily imply a higher bioavailability of the respective compounds.

ENHANCEMENT OF THE BIOAVAILABILITY OF FERULIC ACID FROM WHEAT BRAN

Different bioprocessing techniques involving fermentation or enzymatic and fermentation treatments of wheat bran have been developed with the aim to improve the bioaccessibility of phenolic compounds in bran-containing breads (Anson *et al.*, 2009). Anson *et al.* assessed the bioaccessibility of ferulic acid, *p*-coumaric acid, and sinapic acid with an *in vitro* model of the upper gastrointestinal tract. Colonic metabolism of the phenolic compounds in the nonbioaccessible fraction of the breads was studied with an *in vitro* model of the human colon. The most effective treatment was the combination of enzymes and fermentation that increased the bioaccessibility of ferulic acid from 1.1 to 5.5%. Because germination leads to the synthesis of proteases as well as enzymes that cleave carbohydrates, it should be possible to enhance the bioavailability of phenolic compounds by treatment of cereal products with an extract from wheat sprouts. In order to test this hypothesis, a wheat bran extract (containing approximately 80% arabinoxylan oligosaccharides (AXOS) and approximately 1.5% ferulic acid ester linked to AXOS) was treated with an extract from wheat sprouts and given to healthy volunteers. The nontreated wheat bran extract was used as a control. Blood and urine samples were taken, and the content of ferulic acid and its metabolites was determined by liquid chromatography−mass spectrometry (MS)/MS via stable isotope dilution assay using d_3-ferulic acid as internal standard. Enzymatic hydrolysis of the ferulic acid metabolites was performed according to Zhao *et al.* (2003).

Ferulic acid provided in the form of feruolyated AXOS present in wheat bran extract was rapidly taken up, metabolized, and reached its maximum concentration in plasma 2 h after ingestion (Figure 46.4A). The main metabolite is the sulfated form of ferulic acid (Figure 46.4B). This has already been shown for rats after ingestion of free ferulic acid (Rondini *et al.*, 2002). Interestingly, whereas the glucuronidated form rapidly decreases from the plasma

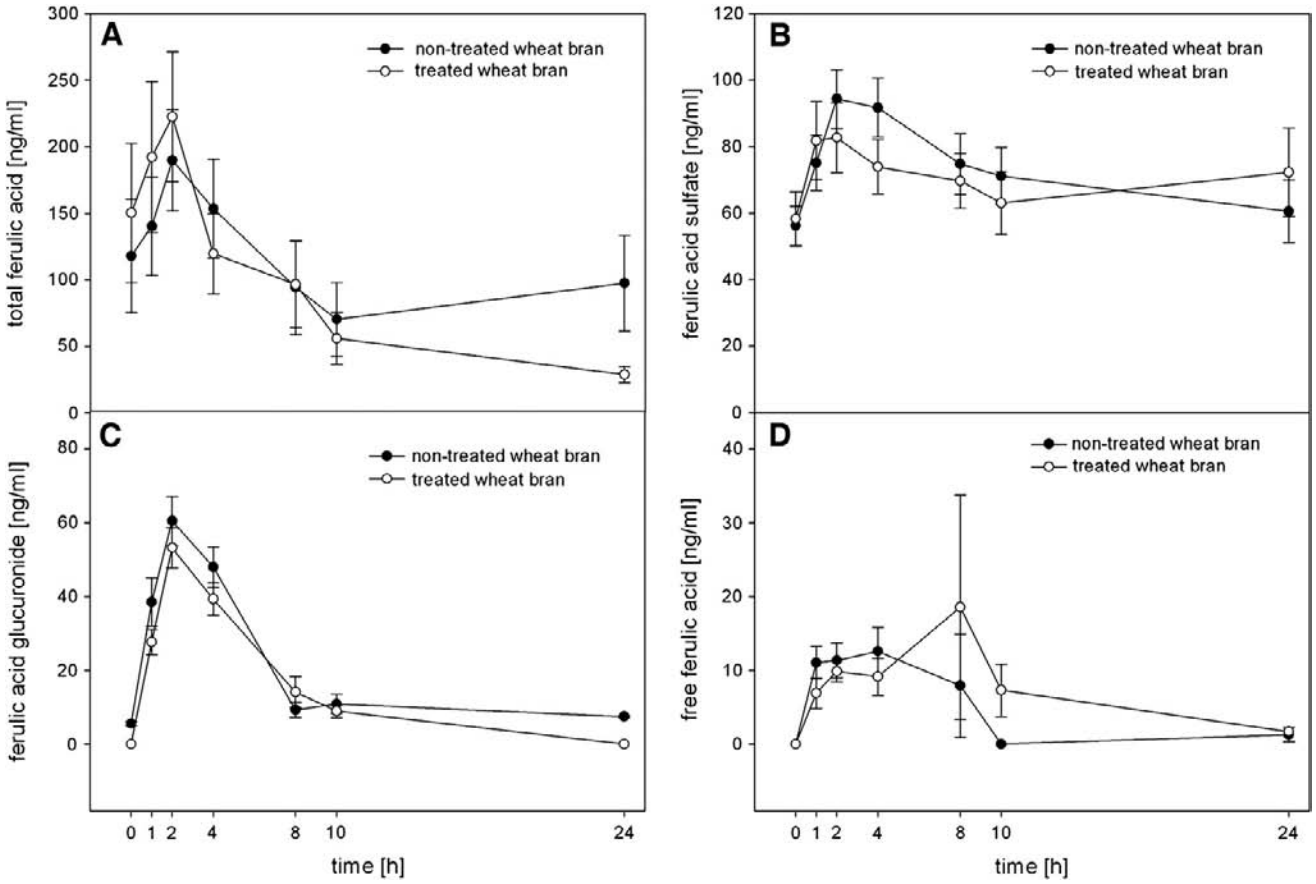

FIGURE 46.4

Impact of treating an extract from wheat bran with an extract from wheat sprouts on plasma levels of ferulic acid as well as its metabolites. Mean concentrations of total ferulic acid (A), ferulic acid sulfate (B), ferulic acid glucuronide (C), and free ferulic acid (D) in subjects' plasma after the intake of wheat bran extract (●) and after the intake of wheat bran extract treated with an extract from wheat sprouts (○). Data are given as mean ± SEM.

(Figure 46.4C), the sulfated form is found in nearly constant concentrations for approximately 10 h in the subjects' plasma (Figure 46.4B). Compared to its metabolites, free ferulic acid concentrations were lowest (Figure 46.4D).

In urine, the free ferulic acid as well as the metabolites reach their maximum levels at 3–6 h (Figure 46.5), with free ferulic acid showing the highest concentration in urine (Figure 46.5D).

However, administration of a wheat bran extract treated with an extract from wheat sprouts does not lead to higher ferulic acid concentrations in plasma and urine of healthy subjects (see Figures 46.4 and 46.5). Therefore, other strategies should be applied to enhance the bioavailability of ferulic acid from cereals.

CONCLUSION

The beneficial health effects of wheat sprouts may be due to not only the fiber content but also their high amount of phenolic compounds. The health-promoting effects range from antioxidant functions to glucose-lowering effects. However, because the bioavailability of phenolic compounds from grains is very low, strategies have to be developed to enhance their natural bioavailability.

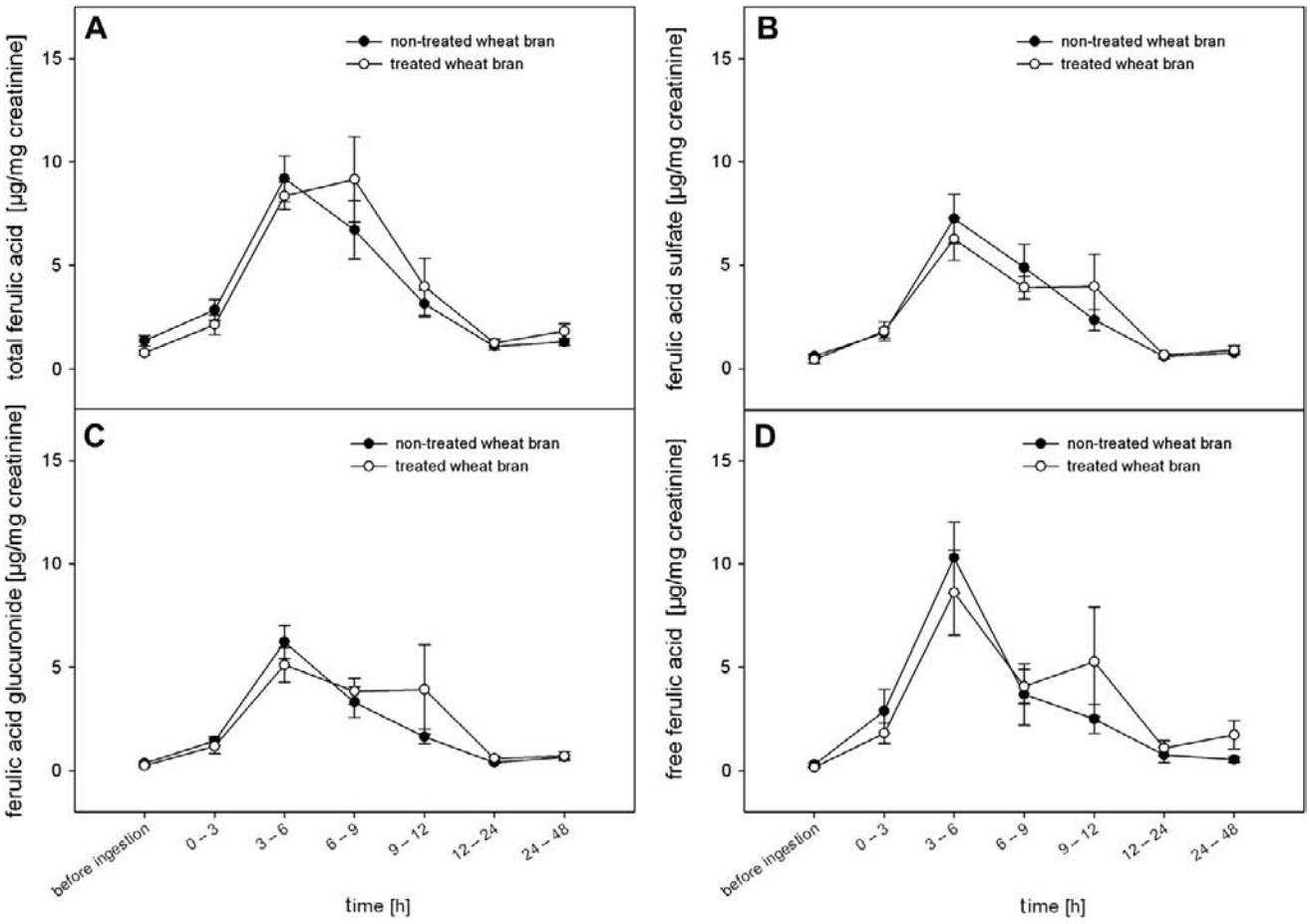

FIGURE 46.5

Impact of treating an extract from wheat bran with an extract from wheat sprouts on urine levels of ferulic acid as well as its metabolites. Mean concentrations of total ferulic acid (A), ferulic acid sulfate (B), ferulic acid glucuronide (C), and free ferulic acid (D) in subjects' urine after the intake of wheat bran extract (●) and after the intake of wheat bran extract treated with an extract from wheat sprouts (○). Data are given as mean ± SEM.

SUMMARY POINTS

- Germination causes an increase in bioactive compounds.
- The intake of wheat sprouts leads to higher plasma antioxidant capacity in humans.
- The intake of wheat sprouts leads to an insulin-sensitizing effect in humans.
- These effects cannot be attributed to a higher intake of dietary fiber; therefore, other compounds must be responsible for the glucose-lowering effect.
- It is feasible that phenolic compounds exhibit an insulin-sensitizing effect.
- Suitable strategies will have to be developed to increase the low bioavailability of phenolic compounds from cereals.

Acknowledgment

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